Phylogeny and evolution of a highly diversified catfish subfamily : the Loricariinae (Siluriformes, Loricariidae)

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N° d'ordre 146-2011 Année 2011

THESE DE L'UNIVERSITE DE LYON

délivrée par

L'UNIVERSITE CLAUDE BERNARD LYON 1

et préparée en cotutelle avec

L'UNIVERSITE DE GENEVE

ECOLE DOCTORALE

E2M2- Évolution, Écosystèmes, Microbiologie, Modélisation.

DIPLOME DE DOCTORAT

(arrêté du 7 août 2006 / arrêté du 6 janvier 2005)

soutenue publiquement le 15 septembre 2011

par

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Je n'ai jamais cherché le pourquoi du comment (Smet, 1992)

Résumé

Phylogénie et évolution d'une sous-famille très diversifiée de poissons-chats: les Loricariinae (Siluriformes, Loricariidae).

Les Loricariinae appartiennent à la famille des poissons-chats néotropicaux cuirassés Loricariidae, la famille de poissons-chats la plus riche en espèce au monde, et se caractérisent par un pédoncule caudal long et aplati et par l'absence de nageoire adipeuse. Préalablement aux études évolutives réalisées, une phylogénie exhaustive et robuste a été établie sur la base de données mitochondriales et nucléaires. Cette phylogénie a ensuite été utilisée dans des analyses multivariées et multi-tableaux afin de révéler les principales tendances évolutives de la sous-famille. La phylogénie obtenue indique que la tribu Harttiini forme un groupe paraphylétique et est restreinte à trois genres, et que dans la tribu Loricariini, deux sous-tribus sœurs se distinguent, les Farlowellina et les Loricariina, chacune présentant des patterns évolutifs complexes. Plusieurs nouveaux taxa ont aussi été mis en évidence et décrits. En utilisant la phylogénie comme outil exploratoire, nous avons démontré : (1) avec l'analyse de co-inertie que les caractères diagnostiques fournis pour définir les différents genres étaient sous dépendance phylogénétique ; (2) avec l'analyse de co-inertie multiple que les forces évolutives sous-jacentes dirigeant leur diversification incluaient des composantes intraphénotypiques (morphologie et génétique) et extraphénotypique (écologie et distribution) ; (3) avec l'analyse RLQ que des évènements de co-dispersion entre espèces co-distribuées avaient eu lieu et étaient responsables de la distribution actuelle des espèces ; et (4) avec l'analyse de patterns multi-échelles que la co-évolution des traits liés aux caractéristiques de la bouche était liée à des fonctions reproductrices responsables d'une évolution tertiaire de cet organe.

Mots clés: phylogénie moléculaire, analyses multivariées, analyses multi-tableaux, co-évolution, co-dispersion, contraintes évolutives.

Abstract

Phylogeny and evolution of a highly diversified catfish subfamily: the Loricariinae (Siluriformes, Loricariidae).

The Loricariinae belong to the Neotropical mailed catfish family Loricariidae, the most speciose catfish family in the world, and are united by a long and flattened caudal peduncle and the absence of an adipose fin. Despite numerous works conducted on this group, no phylogeny is presently available. Prior to conduct evolutionary studies, an exhaustive and robust phylogeny was reconstructed using mitochondrial and nuclear data. Then, this phylogeny was used in multivariate and multi-table analyses to reveal the main evolutionary trends of the subfamily. The resulting phylogeny indicated that the Harttiini tribe, as classically defined, formed a paraphyletic assemblage and was restricted to three genera, and within the Loricariini tribe, two sister subtribes were distinguished, Farlowellina and Loricariina, both displaying complex evolutionary patterns. In addition several new taxa were highlighted and described. Subsequently using this phylogeny as exploratory tool, we demonstrated: (1) using co-inertia analysis that the diagnostic features provided to define the different genera were phylogenetically dependent; (2) using multiple co-inertia analysis that the underlying evolutionary forces shaping their diversification included intraphenotypic (morphology and genetics) and extraphenotypic (ecology and distribution) components; (3) using the RLQ analysis that co-dispersion events occurred between co-distributed species responsible for the current fish distribution; and (4) using the multi-scale pattern analysis that the co-evolution in traits related to the mouth characteristics was linked to reproductive functions responsible for a tertiary evolution of this organ.

Keywords: molecular phylogeny, multivariate analyses, multi-table analyses, co-evolution, co-dispersion, evolutionary constraints.

Remerciements

Mes premiers remerciements vont tout naturellement à mes directeurs de thèse : Sonia Fisch-Muller au Muséum d'histoire naturelle de la Ville de Genève, Denis Duboule à l'Université de Genève et Stéphane Dray à l'Université de Lyon sans qui ce travail n'aurait jamais pu être réalisé. Je les remercie chaleureusement pour la confiance qu'ils m'ont accordée tout au long de ces années, pour leur patience et encouragements, et pour avoir tout simplement cru en moi.

Je tenais également à remercier Danielle Decrouez, directrice du Muséum d'histoire naturelle ainsi que le Département de la Culture et plus généralement la Ville de Genève pour avoir soutenu ce projet et permis son aboutissement dans de bonnes conditions.

Ce travail n'aurait pas non plus pu être initié sans la complicité de Volker Mahnert, ancien directeur du Muséum, qui a pris le temps de préparer sa succession avant de jouir d'une retraite bien méritée. Il a malgré tout continué à suivre avec intérêt la progression de mes travaux, venant régulièrement nous narguer au bureau paré d'un bronzage toujours impeccable.

Mes remerciements s'adressent également à Pierre Pontarotti, Université de Provence ; à John Lundberg, Academy of Natural Sciences of Philadelphia ; à Sylvain Dolédec, Université de Lyon ; et à Juan Montoya-Burgos, Université de Genève pour avoir accepté la tache ô combien ingrate d'évaluer ce travail.

Je suis infiniment reconnaissant à tous mes collègues qui par leurs aides multiples à différents niveaux comme lors de visites dans les institutions, par l'envoi de matériel ou pour leur soutien logistique lors de la collecte de spécimens sur le terrain, ont fourni tout le matériel nécessaire à la réalisation de ce projet ou ont facilité l'aboutissement de ce dernier d'une autre façon, en particulier et en essayant de n'oublier personne : C. Oliveira, M. Chiachio et G. Costa Silva, LBP; M. Sabaj Perez et J. G. Lundberg, ANSP ; E. Bermingham et G. R. Reina, STRI ; M. Lucena, MCP ; J. Armbruster, AUM ; O. T. Oyakawa, MZUSP ; J. Casciota et A. Almirón, MLP ; Y.P. Cardoso, AUGM-UNLP ; M. S. Rodriguez, LIRP ; P.

Keith, R. Causse et P. Pruvost, MNHN ; M. van Oijen et K. van Egmond, RMNH ; R. Vonk et H. Praagman, ZMA ; J. Maclaine, BMNH ; H. Wellendorf, NMW ; S. Schaefer, AMNH ; M. A. Rogers, FMNH ; D. Catania, CAS ; S. Kullander et E. Åhlander, NRM ; G. M. Gonzo, UNSA ; L. Rapp Py-Daniel et M. Rocha, INPA ; H. Ortega, M. Hidalgo et V. Meza Vargas, MUSM ; T. Pequeño et collaborateurs, CIMA ; L. Rodriguez (Conservación de Recursos Naturales) ; P. Gaucher, CNRS Guyane ; R. Vigouroux et P. Cerdan, Hydreco Guyane ; O. Tostain, Ecobios Cayenne ; B. de Mérona, IRD ; F. Meunier, MNHN ; J. Mol et K. Wan Tong You, Celos ; P. Ouboter, NZCS ; C. Weber, A. et S. de Chambrier, J. A. Hollier, P. Wagneur, F. Marteau et A. Merguin, MHNG ; J. Montoya-Burgos, J. Pawlowski et J. Fahrni, unige ; P.-Y. Le Bail, INRA ; M. Dewynter, ONF Guyane ; F. Melki, Biotope France ; C. Bernard, CSBD ; P. de Rham, Y. Fulliquet et T. Blasco, Lausanne ; C. Dlhouy, San Lorenzo ; S. Rubin et L. Moissonnier, France ; H. Bleher, Italie ; V. Piron et C. Niel, Parc de la Guyane.

Pour leur soutien financier je remercie la Fondation G. et A. Claraz pour leur contribution aux missions de terrain au Suriname en 2005, 2007 et 2008, et en Guyane française en 2006 ; l'Académie Suisse des Sciences Naturelles (ScNat) pour les missions au Guyana en 2004 et en Guyane française en 2006; le Fonds C. Topali pour avoir soutenu l'achat d'une machine PCR 96 puits en 2009; le All Catfish Species Inventory (NSF-DEB 0315963) pour les déplacements ayant permis la consultation des collections du NMW, Vienne, et de l'ANSP, Philadelphie (09/2007 et 12/2007), et pour avoir financé l'accès ouvert à l'article publié dans Zootaxa (fourni en Annexe 2); la Société Académique de Genève pour avoir permis la présentation de résultats de recherche au 13th Congrès de Biologie Évolutive à Marseille en 2009 et le Fonds A. Lombard pour l'acquisition de données en 2010. Je suis également reconnaissant à A. Huser, Sallmann-Fehr AG pour le don de filets de pêche pour les missions au Suriname en 2005 et 2007.

Merci également à tous ceux qui ont réussi à me supporter pendant ces années : Pedro, Alex, Nagwa, Jean-Louc (x2), Alain, Andréas, Luc, Rachid, Saïd, Patrick, Nicole, Yamila, Anouchka, Sophie (x 2), Yann, Aurélia, Cécile, Ilham , Slim, Frédéric, Béa, Loïc « siponcle d'argent », Jean « alors, qu'est-ce que c'est ?», Kenneth et à tous mes collègues que j'enquiquine régulièrement à l'administration, à la loge, à la déco, aux dessins-photos-informatique-impression-reliure, à la menuiserie, à la serrurerie, à l'animalerie (qu'est ce t'as encore fait ?), aux ampoules, à la surveillance, au nettoyage… bref merci à tous ces métiers

toujours présents, toujours serviables et qui nous rendent la vie au quotidien tellement plus facile et agréable (et qu'on oublie trop souvent).

Une pensée toute particulière pour mes collègues et amis : Claude Weber, personnage farfelu mais ô combien attachant, inventeur émérite, entre autre, du rapporteur à museau, de la loupe à bavette et du pied à coulisse revolver, Juan Montoya pour les discussions toujours enrichissantes « au bureau à sciences » et pour toutes nos aventures Guyanaises «J'ai eu peur, j'ai crié, j'ai couru et j'ai sauté dans la rivière lorsque j'ai vu le tigre sauter au dessus de la mer !» et Jan Mol pour nos aventures surinamaises et hollandaises et pour nos longues discussions sur l'art de vivre et la barbarie culinaire autour de petits déjeuners à l'omelette à la saucisse et de déjeuners aux sandwichs à la confiture.

Enfin, bien sûr je remercie mes proches et ma famille pour avoir réussi à subir toutes ces années de week-ends passés derrière le PC, de repas après 21h00 et pour les dernières vacances où je ne suis même pas venu. Merci à Frédérique, ma compagne depuis maintenant 20 années pour avoir eu la force de tenir jusque là et pour m'avoir supporté dans ces périodes ingrates, merci à mes enfants Grégoire et Aurèle pour leur affection et amour de tous les jours et pour faire semblant d'apprécier les blagues pourries de leur papa. Merci également à mes parents et grands parents pour leur soutien moral, et à mes frères Quentin et Adrien. Avec une pensée très émue pour Adrien qui nous a brutalement et tragiquement quittés. Ce coup de téléphone de mes parents reçu ce samedi 30 avril 2011 à 14h34 nous a littéralement foudroyé alors que j'écrivais les dernières lignes de ce manuscrit.

Enfin parce que l'accomplissement d'un cursus dépend parfois de décisions personnelles je remercie encore tout particulièrement Pascal Fontaine, Claude Amoros, Pierre Spierer et Denis Duboule pour m'avoir offert la possibilité d'intégrer différentes formations ayant permis la finalisation de mon cursus universitaire.

Maintenant je ne sais pas ce que je vais bien pouvoir faire… peut-être cultiver mon jardin.

Résumé étendu

Phylogénie et évolution d'une sous-famille très diversifiée de poissons-chats: les Loricariinae (Siluriformes, Loricariidae).

Dans une étude préliminaire réalisée lors de mon travail de Master et portant sur les Loricariinae, j'ai eu la possibilité de proposer une première vue d'ensemble des caractéristiques morphologiques de cette sous-famille afin d'établir une clé d'identification et de développer les principales caractéristiques des différents genres. A cette occasion, lors d'une première tentative de compréhension de l'évolution morphologique des Loricariinae, j'ai établi une phylogénie restreinte et tenté d'évaluer la pertinence des caractères morphologiques utilisés dans la clé afin de répondre à la question suivante : « les regroupements obtenus dans la clé sont-ils naturels, c'est-à-dire suivant la phylogénie, ou artificiels sur la base de ces caractères morphologiques ? ». Si la question biologique peut apparaître relativement simple, tenter d'y apporter une réponse devint rapidement un véritable défi d'un point de vue méthodologique. La principale difficulté a consisté à réconcilier les différents objets statistiques spécifiques de chaque approche (c.a.d. analyses multivariées pour les données morphologiques et arbre phylogénétiques pour les données moléculaires) afin de permettre leur comparaison. Partant de ce constat, j'ai remarqué que cette difficulté représentait un des principaux problèmes des études écologiques, et que différentes méthodes avaient été développées pour en tenir compte à l'Université de Lyon au travers du logiciel ADE 4 (Thioulouse *et al*., 1997). La principale approche de cette thèse a donc consisté à essayer de bénéficier des développements méthodologiques en écologie pour les intégrer dans une problématique évolutive. Ce manuscrit se place ainsi à l'interface de différentes disciplines (*e.g*. morphologie, systématique, phylogénie, analyses statistiques) et essaie de promouvoir une approche multidisciplinaire mélangeant les spécialités de trois institutions pour une étude évolutive exhaustive de la sous-famille des Loricariinae.

Les eaux douces néotropicales abritent environ un huitième de la biodiversité des vertébrés et un cinquième de toutes les espèces de poissons avec environ 6 000 espèces sur une estimation de 32 000 (Lévêque *et al*., 2008). Les Loricariidae, ou poissons-chats

cuirassés, sont endémiques de l'Amérique latine où ils forment la plus riche, la plus diversifiée et la plus spécialisée des familles de Siluriformes, comprenant 716 espèces valides et quelques 300 non décrites distribuées en 96 genres (Reis *et al*., 2003; Ferraris, 2007). Les Loricariidae sont caractérisés par un corps déprimé, la présence de plaques osseuses garnies d'odontodes recouvrant la tête et le corps, exception faite parfois de la région abdominale, la présence d'une unique paire de barbillons maxillaires et la modification importante de la bouche en une ventouse. Leur morphologie très spécialisée a fait des Loricariidae un groupe reconnu comme monophylétique dès les premières classifications parmi les Siluriformes (de Pinna, 1998). Ils ont connu, à l'instar d'autres groupes, une véritable radiation évolutive sur l'ensemble du sous-continent américain, du Costa Rica jusqu'en Argentine, tant sur le versant pacifique que sur le versant atlantique de la Cordillère des Andes. Schaefer et Stewart (1993) comparent cette radiation à celle des Cichlidae des grands lacs du Rift africain. Parmi les six sous-familles de Loricariidae, les Loricariinae se caractérisent par un pédoncule caudal long et déprimé et par l'absence de nageoire adipeuse. Ils vivent en relation étroite avec le substrat et présentent, en conséquence, de remarquables différenciations morphologiques liées au nombre important de milieux conquis, aussi bien lotiques que lentiques, sur des substrats inorganiques (rocheux, graveleux, sableux, vaseux…) ou organiques (bois mort, débris végétaux…). Outre les modifications générales de forme qui font que certains ressembleront, par mimétisme, aux branches mortes qui jonchent le lit des cours d'eaux, ou que d'autres seront très aplatis pour leur permettre de s'enfouir dans les substrats meubles, les Loricariinae possèdent également les plus fortes modifications des structures de la bouche, qui reste l'organe le plus spécialisé chez les Loricariidae. Une diversité très importante dans la structure des lèvres, qui peuvent être fortement papilleuses, filamenteuses ou lisses est aussi observée. Certains groupes possèdent des dents nombreuses, pédonculées et organisées en peignes, caractéristiques des espèces qui broutent le tapis algaire riche en épibenthos. D'autres possèdent au contraire peu de dents, voire aucune dent sur les prémaxillaires. Ces dents sont souvent fortement différenciées, bicuspides, droites et épaisses, en cuillère, de dimension réduite ou très longues. Le dimorphisme sexuel est extrêmement marqué et consiste, le plus souvent, en un développement en brosse des odontodes de la marge du museau et des épines et rayons pectoraux du mâle mature. Dans certains cas, il existe également un dimorphisme sexuel dans la structure des dents et des lèvres. Bien que très différenciés morphologiquement, la systématique des Loricariinae reste confuse et sujette à controverses car reposant essentiellement sur les opinions personnelles des différents auteurs, sans réel fondement objectif.

Isbrücker (1979) classe les Loricariinae sur des bases morphologiques en quatre tribus et huit sous-tribus : les Loricariini divisés en six sous-tribus : Loricariina, Planiloricariina, Reganellina, Rineloricariina, Loricariichthyina et Hemiodontichthyina, les Harttiini subdivisés en deux sous-tribus : Harttiina et Metaloricariina, les Farlowellini et les Acestridiini. Le même auteur (1981a: p. VI, 71) émet des doutes au sujet du placement des Acestridiini au sein des Loricariinae, remarquant que: "The exposed cleithrum and coracoid, together with the peculiar odontodes on the unbranched pelvic fin ray ('spine') are characters otherwise occurring typically only in various members of the subfamily Hypoptopomatinae."; néanmoins il les maintient en tant que membre des Loricariinae. Dans le même travail il décrit deux nouvelles sous-tribus, Ricolina et Pseudoloricariina, développe les caractéristiques principales de chaque rang: sous-famille, tribu, sous-tribu, et genre, et établit une clé provisoire des genres de Loricariidae. Rapp Py-Daniel (1981) décrit un nouveau genre, *Furcodontichthys*, et le place au sein des Loricariini, sous-tribu Loricariina. Martín Salazar *et al.* (1982) décrivent *Dentectus* en tant que membre des Loricariini, sous-tribu Planiloricariina. Dans cet article, ils complètent la diagnose des Planiloricariina, parmi lesquels ils transfèrent les genres *Rhadinoloricaria*, *Crossoloricaria*, et *Pseudohemiodon*. Isbrücker *et al.* (1983) décrivent *Aposturisoma* en tant que membre des Farlowellini. Isbrücker et Nijssen (1984, 1986) décrivent *Pyxiloricaria* puis *Apistoloricaria*, et les placent au sein des Loricariini, sous-tribu Planiloricariina. En utilisant des méthodes cladistiques, Schaefer (1986, 1987) établit la monophylie des Loricariinae sur la base de caractères ostéologiques. Nijssen et Isbrücker (1987) suggèrent que les Acestridiini soient considérés en tant que représentants de la sous-famille des Hypoptopomatinae. Schaefer (1991) propose une phylogénie de la sous-famille des Hypoptopomatinae et confirme cette position. Dans sa thèse de doctorat, Rapp Py-Daniel (1997) propose une phylogénie de la sous-famille réalisée à partir de 175 caractères ostéologiques et 17 caractères de morphologie externe et portant sur 21 genres regroupant 61 espèces. Elle confirme la monophylie des Loricariinae et reconnaît deux des trois tribus *sensu* Isbrücker (1979), les Harttiini et les Loricariini, les Farlowellini devenant représentants des Harttiini. Montoya-Burgos *et al.* (1998) proposent la première phylogénie moléculaire de la famille des Loricariidae, basée sur les marqueurs mitochondriaux 12S et 16S, portant essentiellement sur la sous-famille Hypostominae. Ils incluent dans cette analyse neuf Loricariinae correspondant à huit genres. Ils retrouvent les Farlowellini en tant que groupe frère des Loricariini et fournissent une première évidence de la paraphylie des Harttiini tels que définis par Isbrücker (1979) et Rapp Py-Daniel (1997), *Harttia*, genre nominal de la tribu Harttiini, formant le groupe frère des autres membres de la sous-famille. Isbrücker et

Isbrücker et Michels (dans Isbrücker *et al*., 2001) décrivent quatre nouveaux genres, *Fonchiiichthys*, *Leliella*, *Quiritixys* et *Proloricaria*, et revalident *Hemiloricaria* Bleeker, 1862 sur la base d'un nombre très succinct de caractères de validité douteuse car essentiellement basés sur le dimorphisme sexuel. Rapp Py-Daniel et Oliveira (2001) mettent *Cteniloricaria* en synonymie de *Harttia*. Ferraris (2003) maintient la validité de *Cteniloricaria*, met en synonymie tous les genres décrits par Isbrücker et par Isbrücker et Michels (dans Isbrücker *et al*., 2001) et liste 197 espèces de Loricariinae répartis en 31 genres: *Apistoloricaria* (4 espèces), *Aposturisoma* (1 espèce), *Brochiloricaria* (2 espèces), *Crossoloricaria* (5 espèces), *Cteniloricaria* (3 espèces), *Dasyloricaria* (5 espèces), *Dentectus* (1 espèce), *Farlowella* (25 espèces), *Furcodontichthys* (1 espèce), *Harttia* (18 espèces), *Harttiella* (1 espèce), *Hemiodontichthys* (1 espèce), *Ixinandria* (2 espèces), *Lamontichthys* (4 espèces), *Limatulichthys* (1 espèce), *Loricaria* (11 espèces), *Loricariichthys* (17 espèces), *Metaloricaria* (2 espèces), *Paraloricaria* (3 espèces), *Planiloricaria* (1 espèce), *Pseudohemiodon* (7 espèces), *Pseudoloricaria* (1 espèce), *Pterosturisoma* (1 espèce), *Pyxiloricaria* (1 espèce), *Reganella* (1 espèce), *Rhadinoloricaria* (1 espèce), *Ricola* (1 espèce), *Rineloricaria* (47 espèces), *Spatuloricaria* (11 espèces), *Sturisoma* (14 espèces) et *Sturisomatichthys* (4 espèces). Provenzano *et al*. (2005) et Covain *et al*. (2006) (voir Annexe 1) maintiennent la synonymie entre *Cteniloricaria* et *Harttia*. En ajoutant les travaux de Retzer (2006) décrivant une nouvelle espèce de *Farlowella*, Provenzano *et al*. (2005) avec une nouvelle espèce de *Harttia*, Rodriguez and Miquelarena (2003) avec un nouveau *Loricaria*, Knaack (2003) et Rodriguez and Miquelarena (2005) avec chacun une nouvelle espèce de *Rineloricaria*, et Ghazzi (2005) avec un nouveau *Sturisoma*, Covain et Fisch-Muller (2007) (voir Annexe 2) dans une revue de la sous-famille reconnaissent 203 espèces valides réparties en 30 genres et proposent une clé de détermination des genres réalisée à partir des caractères diagnostiques classiquement utilisés pour les définir. Les analyses réalisées retrouvent en partie la subdivision en deux tribus, les Harttiini et les Loricariini, et quatre groupes morphologiques sont créés au sein des Loricariini : (1) le groupe *Pseudohemiodon* possédant des lèvres filamenteuses, un aplatissement dorso-ventral prononcé, une ouverture de bouche trapézoïdale et des carènes prédorsales modérées ; (2) le groupe *Loricaria* défini par des lèvres filamenteuses, par de fortes carènes prédorsales et par un aplatissement dorso-ventral généralement modéré ; (3) le groupe *Rineloricaria* caractérisé par une surface des lèvres papilleuse et par des barbillons marginaux de la lèvre inférieure absents ou faiblement développés ; et (4) le groupe *Loricariichthys* défini par une lèvre inférieure présentant un important sillon médian, par une surface de cette lèvre plus ou moins

lisse ou faiblement papilleuse et par la présence d'une double carène abdominale. Ferraris (2007) revient sur sa dernière classification et reconnaît *Fonchiiichthys* (2 espèces), *Hemiloricaria* (25 espèces) et *Proloricaria* (2 espèces). De plus, Ghazzi (2008) décrit neuf nouvelles espèces de *Rineloricaria*; Ingenito *et al*. (2008) décrivent deux nouveaux *Rineloricaria*; Fichberg et Chamon (2008) décrivent un nouveau *Rineloricaria*; Rapp Py-Daniel et Fichberg (2008) décrivent un nouveau *Rineloricaria*; Rodriguez et Miquelarena (2008) décrivent un nouveau *Rineloricaria*; Rodriguez et Reis (2008) décrivent deux nouveaux *Rineloricaria* et reconnaissent deux groupes morphologiques, nommément le « groupe de sable » comprenant les représentant plus graciles et le « groupe de roche » correspondant aux formes plus massives; Rodriguez *et al*. (2008) revoient la taxinomie d'*Ixinandria* et considèrent *I. montebelloi* comme synonyme junior de *I. steinbachi*; Thomas et Rapp Py-Daniel (2008) décrivent trois nouveaux *Loricaria*; de Carvalho Paixão et Toledo-Piza (2009) revoient *Lamontichthys* et décrivent deux nouvelles espèces ; et Thomas et Sabaj Pérez (2010) décrivent un nouveau *Loricaria*. En conséquence, les Loricariinae incluent 220 espèces valides réparties en 30 à 34 genres selon les auteurs. Parmi tous ces genres, 12 à 14 sont monotypiques et très peu parmi les plus riches en espèces ont été revus. *Loricaria* a été revu par Isbrücker (1981b), *Metaloricaria* par Isbrücker et Nijssen (1982), *Apistoloricaria* par Nijssen et Isbrücker (1988), *Farlowella* par Retzer et Page (1997), *Ixinandria* par Rodriguez *et al*. (2008) et *Lamontichthys* par de Carvalho Paixão et Toledo-Piza (2009).

Extrêmement riche en espèces, très dispersée en Amérique du Sud et très différenciée morphologiquement, la sous-famille des Loricariinae fournit ainsi un cadre idéal pour l'étude de l'évolution morphologique chez les vertébrés. Pourtant, paradoxalement au nombre d'études portant sur les Loricariinae, aucune phylogénie n'a, à ce jour, été publiée. Cette étape s'avère donc un pré-requis indispensable à toute étude évolutive. Ainsi, pour comprendre l'évolution de ce groupe et estimer si toutes ces modifications sont avant tout liées à leur histoire évolutive ou phylogénie (c'est-à-dire héritées d'un ancêtre commun), ou correspondent au contraire plutôt à des adaptations locales liées à leur écologie (plasticité phénotypique), une phylogénie moléculaire exhaustive et robuste de la sous-famille a été établie. Pour ce faire, nous nous sommes basés sur l'analyse des séquences d'ADN mitochondrial matrice des ARN ribosomiques 12S et 16S et du gène nucléaire *fish-reticulon4* pour rechercher le signal phylogénétique lié à l'évolution de ces marqueurs. Les études de diversité spécifique ont été réalisées, quant à elles, avec la séquence code-barres standard de la première sous-unité de la cytochrome c oxydase (COI) proposée par le Barcoding Of Life Initiative (BOLI) (Hebert *et al*., 2003). Les gènes 12S et 16S codent pour les deux sous-unités

du ribosome mitochondrial et sont classiquement utilisés comme marqueurs de rang familial dans les études phylogénétiques chez les poissons (voir par exemple : Ortí *et al*., 1996; Montoya-Burgos *et al.*, 1998; Murphy *et al*., 1999; Wilson *et al*., 2001; Rüber *et al*., 2006; Shimabukuro-Dias *et al.*, 2004; Campo *et al*., 2007; Hrbek *et al*., 2007; Almada *et al*., 2009; Cowman *et al*., 2009; Fernández et Schaefer, 2009; James Cooper *et al*., 2009; Javonillo *et al*., 2010; Straube *et al*., 2010). Le *fish reticulon4* (*f-rtn4*) est un nouveau marqueur nucléaire à évolution rapide en cours de développement. Les réticulons sont des protéines liées à la membrane du réticulum endoplasmique lisse (van de Velde *et al*., 1994). Les gènes *rtns* codent pour une large famille de protéines RTN présentes chez tous les eucaryotes. Parmi ces gènes, le *rtn4* (également appelé *nogo*) code pour une protéine potentiellement impliquée dans les processus de régénérations axoniques lors de lésion du système nerveux central. La RTN4 a particulièrement été étudié chez les mammifères car ces derniers, contrairement aux poissons, possèdent des capacités régénératrices limitées (Oertle *et al*., 2003 ; Diekmann *et al*., 2005). Les gènes *rtns* possèdent de nombreux et longs introns caractérisés par une plus grande variabilité mutationnelle que les exons, fournissant ainsi de l'information à des niveaux hiérarchiques relativement fins (inter-espèces). La variabilité du premier intron a été caractérisée chez les Loricariidae par Fisch-Muller *et al.* (sous presse) (Annexe 3) lors d'une étude comparative avec la région code-barres. Un fragment du gène *f-rtn4* a été utilisé avec succès pour la reconstruction d'une phylogénie de la sous-famille de Loricariidae des Hypoptopomatinae (Chiachio *et al*., 2008) et dans une étude populationnelle de *Guyanancistrus brevispinis* dans les Guyanes en utilisant le premier intron (Cardoso et Montoya-Burgos, 2009).

Le gène COI code une partie d'un large complexe enzymatique de la chaîne respiratoire mitochondriale. La région code-barres de ce gène, due à la nature dégénérée du code génétique, possède un taux de mutation très élevé en première et surtout troisième position des codons, et ce malgré un relatif conservatisme des acides aminés (Ward et Holmes, 2007). Ces taux de mutation élevés permettent ainsi l'accumulation rapide de mutations entre séquences et forment la base du système des codes-barres ADN. Ces différences accumulées entre séquences sont attendues faibles au sein d'une même espèce à cause de l'échange constant des mitochondries et élevées entre espèces à cause de l'arrêt de la transmission des mitochondries. Le système des codes-barres COI a été efficacement utilisé pour quantifier et qualifier la diversité de poissons (Ward *et al*., 2005; Hubert *et al.,* 2008; Ward *et al.,* 2009; Valdez-Moreno *et al*., 2009; Lara *et al*., 2010), et a permis la mise en

évidence d'espèces cryptiques (*e.g*. Ward *et al*., 2008a; Ward *et al*., 2008b; Lara *et al.*, 2010; Fisch-Muller *et al.* (sous presse) (Annexe 3)).

Parallèlement aux reconstructions phylogénétiques, différents types de données ont été rassemblés. Le premier type d'informations collectées repose sur l'étude de la morphologie. En systématique, l'approche typologique repose essentiellement sur les spécimens, et un des moyens les plus simples de délimiter les espèces consiste en l'évaluation des caractères morphologiques. On notera toutefois qu'utiliser des caractères morphologiques pour délimiter les espèces est assez éloigné de la définition usuelle de l'espèce biologique proposée par Mayr (1963): un complexe d'individus interféconds co-existant à un moment donné et génétiquement isolé d'autres complexes équivalents (pour des revues concernant les différents concepts d'espèce, voir Kottelat, 1997; Bock, 2004). Néanmoins la morphologie reste un des seuls moyens de caractériser et de décrire les espèces, et le systématicien doit souvent s'accommoder d'un concept morphologique de l'espèce. Le Code International de Nomenclature Zoologique (1999) fournit ainsi le cadre légal pour l'établissement de nouveaux noms, assurant la stabilité et l'universalité de la nomenclature. L'approche principale consiste donc à définir les espèces sur la base de similitudes ou différences observées. Cette étape peut s'avérer très subjective et différentes méthodes ont été développées pour tenter de fournir des critères objectifs pour la délimitation des limites inter-spécifiques reposant sur des données morphologiques (voir l'approche de taxinomie numérique). Cette approche assume donc *a priori* une corrélation entre l'évolution morphologique et l'évolution génétique ayant abouti à l'isolement reproductif, chacune étant liée à plus large échelle à l'évolution du génome. De plus, bien qu'apparemment étroitement apparentés, il convient de faire une différence entre le fait de rechercher des caractères distinctifs entre espèces et la méthode cladistique. Cette dernière tente de classifier les espèces sur la base de caractères dérivés partagés appelés synapomorphies, alors que le taxinomiste recherchera plutôt des caractères uniques non partagés, appelés autapomorphies, ces derniers étant par définition non informatifs d'un point de vue cladistique. Le type de données générées par cette approche est le plus souvent qualitatif et codé de manière binaire ou par modalités, les rendant facilement analysables par les méthodes multivariées usuelles comme l'Analyse des Correspondances (AC) ou l'Analyse des Correspondances Multiples (ACM). D'autres techniques reposant sur une approche quantitative ont été développées pour l'étude de la forme des spécimens. Ces méthodes appartiennent au domaine de la morphométrie qui est l'étude de l'apparence en tant que variations de forme et taille (Richtsmeier *et al*., 2002). On remarquera que, contrairement aux francophones, les anglo-saxons disposent de deux

mots pour qualifier la notion de forme : form et shape. Cette lacune explique probablement la difficulté à définir correctement cette notion de forme. C'est pour marquer ce distinguo que je viens d'utiliser le terme d'apparence en tant que qualificatif pour une notion de forme globale (form). La morphométrie étudie donc les variations de taille et de forme et leur covariation, ainsi que leurs covariations avec d'autres variables (Claude, 2008). Pour ce faire, deux approches ont été développées, chacune reposant sur un type propre de données : la morphométrie traditionnelle qui analyse les mesures, comptes, angles, rapports, et la morphométrie géométrique qui analyse les coordonnées de points homologues entre objets comparés. Chaque approche possède ses forces et faiblesses mais dans l'ensemble les résultats restent comparables (*e.g*. Parson *et al*., 2003; Maderbacher *et al*., 2008; Sidlauskas *et al*., 2011). Les données de morphométrie traditionnelle sont facilement analysées par l'Analyse en Composantes Principales (ACP) ou par l'Analyse Discriminante (AD).

En parallèle de la collecte d'informations morphologiques, j'ai eu la possibilité de mener ou de participer à plusieurs missions de terrain en Amérique latine. Différentes missions ont ainsi été conduites en Guyane, au Suriname, au Guyana et au Pérou, complémentant les données déjà acquises au Brésil, Pérou, Paraguay ou Panama. Ces missions qui permettent la découverte régulière d'espèces nouvelles, sont indispensables pour l'obtention de matériel permettant les études moléculaires et fournissent des données essentielles sur les biotopes fréquentés par ces espèces. En complément de la collecte de spécimens et de la prise d'échantillons, des observations de terrain sont ainsi également conduites. Après capture, les spécimens sont photographiés, identifiés individuellement par un numéro de terrain, des échantillons de nageoire sont prélevés pour les analyses ADN et identifiés par le même numéro, puis les spécimens sont fixés pour une conservation à long terme. Ce faisant, les lieux de capture sont géoréférencés par Global Positioning System avec prise de la latitude, de la longitude et de l'altitude. Les paramètres de l'eau tels que pH, conductivité, température et plus récemment turbidité et concentration en oxygène sont relevés. Des données qualitatives sur les biotopes sont notées, telles que le type de substrat, la vitesse du courant, ou le type de rivière. Ces données représentent une information de bonne qualité pour la caractérisation de l'environnement des poissons et sont classiquement utilisées dans les études des relations poissons-habitats (pour la Guyane française, voir *e.g*. Mérigoux *et al*., 1998; Mérigoux *et al*., 2001; revu par de Mérona *et al*, sous presse).

Toutefois, chacune des approches précédemment énoncées fournit son propre type de données et ne répond qu'à une question, bien que toutes soient généralement centrées sur la même question biologique générale. Unifier ces différentes sources d'information, qui

peuvent être de différentes natures statistiques, dans le même cadre exploratoire ou descriptif, demeure une tache complexe. Ces différentes données, organisées en différents tableaux, reposent souvent sur les mêmes entités statistiques (*e.g.* les individus, les stations…), établissant ainsi un lien entre tableaux, et peuvent êtres décrites par de nombreuses variables (*e.g.* données génétiques, morphométriques, environnementales…). Un des moyens d'unifier ces différentes observations consiste donc soit à réaliser une analyse des tableaux accolés et de rechercher un moyen de se libérer de la contrainte liée à la nature des données (quantitatives et qualitatives) pour les rendre compatibles, soit à réaliser des analyses préliminaires en fonction du type de données des tableaux séparés et de rechercher les structures communes des différentes analyses pour les inclure dans la même analyse globale.

La première approche rejoint celle de Hill et Smith (1976) qui ont développé une méthode pour réaliser une ACP sur un tableau mélangeant données quantitatives et qualitatives. Dans l'Analyse de Hill et Smith (AHS), les données quantitatives sont préalablement soumises à une ACP, alors que les données qualitatives sont soumises à une ACM. Les deux types d'information sont ensuite rendus compatibles par un système de repondération des colonnes du tableau afin de donner la même importance à chacune des variables de l'analyse même lorsque les données qualitatives sont multi-modales. Alors que l'ACP recherche des axes qui maximisent le carré des corrélations des variables quantitatives, que l'ACM recherche des axes qui maximisent la somme des rapports de corrélation des variables qualitatives, l'AHS établit un compromis entre les deux analyses préliminaires en recherchant des axes qui maximisent la moyenne des carrés des corrélations (variables quantitatives) et des rapports de corrélations (variables qualitatives). Cette idée de compromis représente la clé de voûte des approches multi-tableaux, et en ce sens, l'AHS représente un premier pas dans l'analyse simultanée de différentes sources de données. Cette analyse a été utilisée avec succès par Covain et Fisch-Muller (2007) (voir Annexe 2) pour mettre en évidence les caractéristiques morphologiques quantitatives et qualitatives classiquement utilisées comme caractères diagnostiques dans la réalisation d'une clé d'identification des différents genres de Loricariinae.

La seconde approche est atteinte par ce qu'on appelle les analyses multi-tableaux. Ces analyses recherchent les structures communes présentes dans les différents jeux de données et les retranscrivent dans le même cadre descriptif. Initialement dédiées à l'étude des structures écologiques comme les relations espèces-habitat, les analyses de co-structures essaient d'extraire l'information commune des différents jeux de données, par exemple, la composition spécifique et les paramètres environnementaux relevés dans les mêmes stations.

Cet aspect a été unifié par Dolédec et Chessel (1994) lorsqu'ils ont développé l'Analyse de Co-Inertie (ACI). L'ACI a pour but d'extraire la structure commune de deux tableaux reposant sur les mêmes entités statistiques. Le modèle mathématique de l'ACI est fourni dans Dolédec et Chessel (1994) et dans Dray *et al*. (2003b). Chacun des tableaux analysés (*e.g*. occurrences d'espèces et paramètres environnementaux pour plusieurs localités) est préalablement traité par une analyse préliminaire (*e.g*. ACP, ACM, AC, AHS) puis soumis à l'ACI afin de décrire la structure commune des deux tableaux. Le résultat de l'ACI consiste en deux nouveaux jeux de scores de covariance maximale. L'ACI maximise en effet un compromis entre la structure du premier tableau (*e.g*. un tableau d'occurrences d'espèces pour différentes localités), la structure du second tableau (*e.g*. un tableau de paramètres environnementaux pour ces mêmes localités) et leur lien.

Dolédec *et al*. (1996) ont ensuite étendu le concept de co-inertie à trois tableaux, et ont développé l'analyse RLQ. L'analyse RLQ a pour but de rechercher les relations entre un tableau R (*e.g*. un tableau de traits pour différentes espèces fournissant un lien externe sur les lignes) et un tableau Q (*e.g*. un tableau de variables environnementales pour différents sites fournissant un lien externe sur les colonnes), unifiés par un tableau L (*e.g*. un tableau croisé d'espèces par site), et établit un compromis en extrayant leurs structures communes. La RLQ diffère de l'ACI en ce que la relation entre les tableaux R et Q est fournie par le troisième tableau L, alors que dans l'ACI cette relation est donnée directement par les lignes (*i.e*. les mêmes entités statistiques) des deux tableaux analysés. Le modèle mathématique de la RLQ est décrit dans Dolédec *et al*. (1996) avec des adaptations dans Dray *et al*. (2002) et Dray et Legendre (2008). Les résultats de la RLQ consistent en deux nouveaux jeux de scores des deux tableaux R et Q de covariance maximale lorsque le tableau de lien est traité par une AC (Dolédec *et al*., 1996). Finalement, le critère de co-inertie a été étendu à K tableaux par Chessel et Hanafi (1996) qui ont développé l'Analyse de Co-Inertie Multiple (ACIM). L'ACIM identifie les structures communes présentes dans des jeux de données multiples ($n = k > 2$) reposant sur les mêmes entités statistiques en fournissant une typologie consensuelle (le compromis) maximisant le lien entre tous les tableaux simultanément. Ce lien est exprimé par la somme des carrés de covariances entre les combinaisons linéaires des variables de chaque tableau et le compromis.

Ce manuscrit s'articule autour de la question centrale de l'évolution de ce groupe très diversifié que sont les Loricariinae. Pour cela, j'ai voulu tirer profit de la spécificité de mes trois établissements d'affiliation que sont le Muséum d'histoire naturelle de la Ville de

Genève (MHNG), le Laboratoire de Biométrie et de Biologie Évolutive (LBBE) de l'Université de Lyon, et le département de Génétique et Évolution (GenEv) de l'Université de Genève, afin d'investiguer les patterns évolutifs de cette sous-famille. Tout particulièrement, j'ai essayé de tirer parti des développements récents en analyses multi-tableaux (et également au niveau logiciel au travers du logiciel libre R) ainsi que de la détection de nouveaux marqueurs phylogénétiques à évolution rapide, pour réaliser une étude exhaustive de différents jeux de données bien documentés obtenus à partir des spécimens, des échantillons de tissus pour les analyses ADN et des observations de terrain. Étant donné que les arbres phylogénétiques représentent des objets qualitatifs qui peuvent être facilement convertis en objets quantitatifs en utilisant, par exemple, les distances patristiques pour reconstruire une matrice de distances, l'information phylogénétique peut être intégrée dans un premier tableau et être analysé par une Analyse en Coordonnées Principales (Gower, 1966). Cette analyse représente ainsi la première étape pour l'exploration de tableaux multiples obtenus à partir des mêmes individus. L'utilisation des méthodes multi-tableaux fournit ainsi le cadre descriptif unificateur nécessaire à la réalisation des différents objectifs de cette thèse. L'adaptation des méthodes multi-tableaux à un contexte phylogénétique permet, en effet, d'explorer différents types de données simultanément et renforce ainsi considérablement notre connaissance du groupe dans des perspectives évolutives et de taxinomie intégrative. Lorsqu'un des jeux de données représente la phylogénie, toutes les structures présentes dans les autres tableaux peuvent être liées ensemble et à la phylogénie et ainsi être interprétées d'un point de vue évolutif. Cette approche permet d'explorer les relations entre la phylogénie et des données morphologiques, morphométriques, écologiques, distributionnelles et éthologiques, et de révéler ainsi les tendances évolutives acquise au cours du temps chez les Loricariinae. De plus, établir une relation entre une phylogénie et différents types de données, implique que ces données sont sous dépendance phylogénétique. Ce concept central en biologie comparative stipule qu'à cause de l'héritabilité des traits biologiques à partir d'ancêtres communs, les observations faites entre espèces ne sont pas indépendantes (voir Harvey et Pagel, 1991). Différentes méthodes ont été développées pour détecter la dépendance phylogénétique dans les données comparatives, une des dernières étant l'orthograme (Ollier *et al.*, 2006). L'orthograme décompose la variance des traits le long d'une phylogénie représentée par une base orthogonale. Néanmoins, sous sa forme originale, l'orthograme ne peut traiter que des données qualitatives. L'extension de l'orthograme aux données qualitatives et multivariées a donc été un pré-requis pour une étude d'ensemble de l'évolution des traits biologiques chez les Loricariinae. Cette structure unificatrice, rendant chaque orthograme directement

comparable a permis en conséquence le développement d'une nouvelle méthode multivariée pour l'exploration des patterns de co-évolution entre traits le long d'une phylogénie. Cette nouvelle approche adapte la technique de l'Analyse des Patterns Multi-Echelles (APME) développée pour l'analyse de données spatialisées (Jombart *et al*., 2009) dans un contexte phylogénétique. Cette nouvelle analyse diffère de l'approche multi-tableaux usuelle par la façon de décrire la phylogénie qui est directement utilisée en tant que variable exploratoire.

La reconstruction progressive de phylogénies robustes et exhaustives a permis une révision de la systématique des Loricariinae, ainsi que la description des nouveaux taxa mis en évidence par les analyses combinées. Ce manuscrit est organisé en chapitres centrés sur la problématique principale évolutive et répondant aux objectifs suivants :

(1) une évaluation de l'approche multi-tableaux a été réalisée en utilisant l'ACI pour explorer la co-structure entre une phylogénie reconstruite sur la base de marqueurs mitochondriaux et le jeu de données morphologiques publié par Covain et Fisch-Muller (2007) (Annexe 2). La systématique des Loricariinae a été revue, et la dépendance phylogénétique des caractères morphologiques utilisés de manière classique pour définir les différents genres a été évaluée.

(2) sur la base de spécimens déjà connus en collection (un seul au MHNG) et de matériel complémentaire fraîchement collecté au Pérou, un nouveau genre et nouvelle espèce a été décrit pour clarifier la systématique du groupe. De plus, une évaluation de l'alignement, en particulier dans les régions introniques du nouveau marqueur *f-rtn4* a été réalisée préalablement à la première phylogénie des Loricariinae reconstruite en combinant l'information nucléaire et mitochondriale (pour la caractérisation du premier intron de *f-rtn4* voir Fisch-Muller *et al*., sous presse; Annexe 3).

(3) l'ACIM a été utilisée pour une évaluation globale de la diversité d'une tribu de Loricariinae, les Harttiini, dans les Guyanes. Faisant suite à une première étude restreinte à un seul genre de cette tribu dans un seul pays (Covain *et al*., 2006; Annexe 1), l'information génétique, morphométrique, et écologique-distributionnelle a été unifiée dans le même cadre descriptif afin de révéler les forces évolutives ayant favorisé leur diversification au travers des Guyanes. De plus, les différent nouveaux taxa mis en évidence ont été décrits.

(4) l'analyse RLQ a été évaluée pour détecter les évènements de co-dispersion chez deux groupes de Loricariidae ayant une distribution commune : les Harttiini et le genre *Hypostomus*. La détection de co-structures dans chaque phylogénie étant potentiellement liée à des évènements de co-dispersion, les dates fournies pour la dispersion des espèces d'*Hypostomus* ont été appliquées à la phylogénie des Harttiini afin de proposer une hypothèse phylogéographique quant à leur diversification à l'échelle du sous-continent.

(5) une phylogénie exhaustive a été reconstruite (350 Unités Taxinomiques Opérationnelles), les orthogrames ont été généralisés et l'APME a été évaluée sur un jeu de données étendu mélangeant des données quantitatives (discrètes et continues), qualitatives (binaires, multi-modales et ordinales), intraphénotypiques (morphologie, éthologie) et extraphénotypiques (paramètres environnementaux) afin de détecter des patterns de co-évolution entre traits multiples le long de la phylogénie, et ainsi de révéler les variables impliquées dans les principales innovations chez les Loricariinae. De plus, les patterns évolutifs de ces innovations ont été mis en évidence et une datation pour l'apparition de ces structures a été proposée.

Les résultats obtenus dans les différents chapitres de cette thèse démontrent que la systématique de ce groupe n'était que partiellement connue. En particulier, la définition de la tribu Harttiini était erronée. Isbrücker (1979) définit les Harttiini par l'origine de la nageoire dorsale située pratiquement à l'aplomb de l'insertion des nageoires pelviennes, la nageoire caudale avec 12 (rarement 11) rayons branchus, l'absence d'encoche orbitaire et peu de variabilité dans la structure des lèvres et des dents. Il place *Sturisoma*, *Harttia*, *Lamontichthys*, *Harttiella*, *Pterosturisoma*, *Cteniloricaria*, *Sturisomatichthys* et *Metaloricaria* au sein de Harttiini. Sur la base de ces même caractères diagnostiques, Covain et Fisch-Muller (2007) (Annexe 2) ne retrouvent que partiellement ce regroupement en utilisant des méthodes de classification hiérarchique avec *Metaloricaria* et *Farlowella* se connectant en dehors des Harttiini à cause de caractères divergents. Néanmoins, dans un souci de faciliter l'identification des différents genres, ils maintiennent la classification d'Isbrücker (1979). Les phylogénies moléculaires reconstruites à l'aide de marqueurs mitochondriaux (chapitre 1) ou de jeux de données combinant des données nucléaires et mitochondriales (chapitres 2, 4 et 5) démontrent que ce groupe n'est pas naturel et que les Harttiini ne sont en fait restreints qu'à trois genres : *Harttia*, *Harttiella* et *Cteniloricaria* (chapitres 3 et 4). Les autres genres, hormis

Metaloricaria, appartiennent à une nouvelle sous-tribu des Loricariini nommée Farlowellina, et *Metaloricaria* forme le groupe frère de tous les Loricariina (chapitres 1, 2, 4 et 5). De plus, la phylogénie exhaustive présentée au chapitre 5 révèle des patterns évolutifs complexes au sein des Farlowellina avec *Farlowella*, *Sturisoma* et *Sturisomatichthys* retrouvés paraphylétiques malgré leur morphologie très dérivée les faisant ressembler à des morceaux de bois. Différentes synonymies (*Ixinandria* et *Apistoloricaria*) et revalidations génériques (*Proloricaria*) ont aussi été mises en évidence au sein des Loricariina. De plus, neuf nouvelles espèces (six *Harttiella*, deux *Harttia*, et une *Cteniloricaria*) et un nouveau genre et nouvelle espèce, *Fonchiiloricaria nanodon,* ont été mis en évidence et décrits (chapitres 2 et 3), augmentant le nombre d'espèces valides à 230 distribuées en 31 genres.

Ces modifications importantes de la structure de l'arbre phylogénétique sont la conséquence naturelle de caractères diagnostiques mal définis. Les caractères utilisés pour diagnostiquer les rangs tribaux et génériques ont donc été évalués au regard de la phylogénie. Dans le premier chapitre, nous démontrons que ces caractères sont en général suffisants pour définir de manière naturelle les rangs tribaux et sous-tribaux (incluant en partie les groupes morphologiques proposés par Covain et Fisch-Muller, 2007; Annexe 2) mais sont clairement insuffisants au niveau générique. Pour cela, nous avons utilisé l'ACI afin d'extraire la structure commune entre la phylogénie (préalablement convertie en matrice de distances) et un tableau de caractères morphologiques diagnostiques (quantitatifs et qualitatifs). Dans ce cas, l'ACI met en évidence les traits possédant la co-variation maximale avec la phylogénie ainsi que les associations phylogénétiques entre traits. Cette manière d'utiliser l'ACI représente donc une façon valable d'explorer un tableau de traits au regard d'une phylogénie, et ainsi de mettre en évidence la dépendance phylogénétique de traits multiples. Ce premier résultat s'avérant convaincant quant à la puissance de l'approche multi-tableaux en biologie comparative, son extension a été permise. Nous avons donc naturellement expérimenté les méthodes multi-tableaux pour différentes problématiques évolutives (*i.e*. au moins un des tableaux représente la phylogénie).

Dans une étude de diversité réalisée sur les Harttiini des Guyanes, l'ACIM a été utilisée pour unifier morphométrie, génétique et écologie-distribution des espèces dans la même analyse (chapitre 3). L'ACIM a révélé les liens existant entre ces trois types de données et a fourni des évidences flagrantes quant à la validité de trois genres de Harttiini, différant dans les combinaisons de ces différentes données. Cette analyse a aussi démontré que la diversité réelle était deux fois plus importante que précédemment reconnu. Cette diversité importante a été façonnée par (ou orientée vers) une composante intraphénotypique

correspondant aux adaptations morphologiques et à la divergence génétique, et une composante extraphénotypique correspondant à l'écologie et la distribution des espèces. Les adaptations morphologiques incluent des modifications importantes en taille et en forme en particulier au niveau du pédoncule caudal. Ces modifications morphologiques sont corrélées à la divergence génétique et à certains paramètres environnementaux tels que le type de biotope colonisé (crique forestière ou fleuve) et la température, ainsi qu'à des gradients distributionnels correspondant à l'altitude et à la longitude.

Dans le quatrième chapitre, nous avons évalué la capacité de l'analyse RLQ à détecter des co-structures dans deux phylogénies indépendantes contraintes par la distribution de leurs espèces. La force de la RLQ repose sur le tableau L fournissant l'hypothèse contraignant l'analyse. Les co-structures mises en évidences sont donc directement interprétables à la lumière de cette hypothèse, les autres co-structures apparentes étant potentiellement liées à des facteurs cachés. Les résultats du chapitre 3 démontrent en effet que l'évolution d'un groupe est par essence multifactorielle impliquant des paramètres intra et extra phénotypiques. L'interprétation visuelle de co-structures potentielles dans l'ordre des branchements d'arbres phylogénétiques est donc risquée et devrait être évitée autant que possible, d'autres contraintes évolutives indépendantes pouvant expliquer de tels patterns. En assumant donc l'hypothèse de co-dispersion des espèces, c.a.d. que les espèces actuelles peuvent être présentes dans le même bassin parce qu'elles ont colonisé simultanément ce bassin à cause des mêmes évènement historiques (*e.g*. capture de tête de bassin, contact secondaire des estuaires, fracture géologique), nous avons exploré la phylogénie des Harttiini et celle *d'Hypostomus* déjà publiée par Montoya-Burgos (2003). La RLQ détecte parfaitement une co-structure phylogénétique sous contrainte spatiale forte et significative dans les deux phylogénies impliquant une co-dispersion entre les espèces des bassins du Sao Francisco et de l'Amazone. Ce résultat est renforcé par les tests du quatrième coin développés par Legendre *et al.* (1997) et étendus par Dray et Legendre (2008) afin de pouvoir combiner différents modèles de tests dans la procédure générale, et par Dray (en préparation) pour tester le lien individuel entre chaque variable de R et Q (ici les coordonnées principales décrivant les phylogénies) et les axes de la RLQ (le compromis établi entre les phylogénies et la co-dispersion des espèces). La co-structure phylogénétique sous contrainte spatiale observée n'est donc pas due au hasard. La datation fournie pour cet évènement de co-dispersion chez *Hypostomus* a donc été appliquée naturellement à ce même évènement chez les Harttiini afin de révéler l'histoire de dispersion des espèces de cette tribu à l'échelle du sous-continent. Les datations suivantes obtenues pour la phylogénie des Harttiini corroborent parfaitement celles

obtenues pour *Hypostomus*, suggérant un contexte temporel commun de diversification. Cette diversification soudaine des Harttiini et des *Hypostomus* révèle un pattern explosif de radiation à la base de chaque lignée, chaque clade de chaque phylogénie apparaissant à la même période.

Les approches multi-tableaux utilisées dans les chapitres 1, 3 et 4 reposent sur une représentation d'une matrice de distances phylogénétiques utilisant les coordonnées principales (Gower, 1966), qui ne sont pas toujours les meilleurs descripteurs pour une phylogénie. Une méthode multivariée alternative a été proposée dans le chapitre 5, basée sur la représentation des propriétés topologiques de l'arbre phylogénétique via une base orthonormale. Pour cela, nous avons étendu les orthogrames développés par Ollier *et al*. (2006) afin de tester les variables qualitatives et les données multivariées incluant les tableaux complets mélangeant variables quantitatives et qualitatives, fournissant ainsi un nouveau test global de l'autocorrélation phylogénétique. Ces nouveaux outils mettent en avant la dépendance phylogénétique des données d'un tableau à différents niveaux (global ou local) en utilisant le même cadre statistique. Cette structure unificatrice a permis par la suite l'adaptation de l'APME dans le contexte phylogénétique afin d'explorer les patterns de co-évolution entre traits le long de la phylogénie. Dans cette approche, la phylogénie n'est plus décrite par des coordonnées principales mais par une autre base orthogonale. L'orthograme multivarié calculé sur le jeu de données mélangeant des données quantitatives (discrètes et continues), qualitatives (binaires, multi-modales et ordinales), intraphénotypiques (morphologiques et éthologiques) et extraphénotypiques (écologiques) révèle que ces données sont fortement autocorrélées phylogénétiquement et impliquent les nœuds les plus profonds de l'arbre dans l'explication de la distribution de la variance de ces traits. Les orthogrames univariés confirment ce résultat avec la plupart de la variation des traits liés aux caractéristiques de la bouche effectivement expliquée par les premiers vecteurs des orthogrames (nombre de dents sur les prémaxillaires et les dentaires, forme des dents et de la bouche, surface des lèvres, barbillons maxillaires et marginaux) ainsi que le nombre de rayons branchus dans la nageoire caudale, la présence ou l'absence de carènes pré-dorsales et d'encoche post-orbitale, confirmant les résultats du premier chapitre utilisant l'ACI (et donc des coordonnées principales). L'APME confirme également ces résultats et révèle des associations fortes entre les caractéristiques de la bouche et les nœuds profonds de la phylogénie, confirmant ainsi que toutes ces structures sont liées entre elles. Néanmoins, peu de corrélations ont été observées avec les variables écologiques et écomorphologiques, impliquant que la co-évolution observée entre les différentes caractéristiques buccales n'était

pas liée à l'écologie tel qu'envisagé au chapitre 1. L'APME a révélé que ces modifications de l'appareil buccal étaient en fait liées à des caractéristiques sexuelles que sont la stratégie de reproduction et le dimorphisme sexuel. La co-évolution entre les différentes caractéristiques de la bouche a donc été guidée par des contraintes comportementales suggérant un effet de sélection sexuelle. De la condition initiale liée aux fonctions de respiration et de nourrissage, la bouche des Loricariidae a évolué vers de nouvelles fonctions liées à l'adhérence au substrat et à la locomotion (voir Geerinckx *et al*., 2011). Chez les Loricariinae, depuis cette fonction secondaire, la bouche a continué d'évoluer vers une troisième fonction liée, elle, à la reproduction. De manière surprenante, ces innovations ont été concomitantes avec la perte d'un dimorphisme sexuel secondaire marqué par l'hypertrophie des odontodes. Cette hypertrophie des odontodes de la marge du museau, des nageoires pectorales et parfois de la région prédorsale (voire du corps entier) des mâles matures peut parfois être particulièrement importante chez les pondeurs sur substrat découvert ou caché. Cette caractéristique a disparu chez les incubateurs buccaux. L'hypertrophie des odontodes a donc pu résulter de la sélection exercée par les femelles sur les mâles, alors que leur perte a pu être sélectionnée naturellement par prédation (la mort du mâle impliquant immédiatement la perte du frai). La généralisation des orthogrames à tous types de données ainsi que l'APME ont permis de révéler non seulement les patterns de co-évolution entre traits, mais aussi de mettre en évidence les régions de l'arbre phylogénétique concernées par ces changements. Cette analyse puissante est ainsi capable de détecter parmi de nombreux traits de natures différentes, tous potentiellement sous dépendance phylogénétique, ceux qui ont connu des modifications évolutives similaires pour différents niveaux de la phylogénie. Cette analyse souligne donc l'importance des patterns évolutifs dans la comparaison entre de multiples traits, tous les traits phylogénétiquement contraints n'étant pas nécessairement liés au même niveau (on pourra faire un parallèle avec la précédente remarque sur l'interprétation visuelle de co-structures apparentes dans les arbres phylogénétiques et l'existence possible de paramètres cachés).

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General Introduction

During a preliminary study conducted on Loricariinae catfishes for my Master thesis, I proposed a first overview of the morphological characteristics of this subfamily to construct an identification key and developed the main characteristics of the different genera. In the same time, I reconstructed a small molecular phylogeny and tried to evaluate the relevance of the morphological characters used in the key in an evolutionary perspective to reply to the question: were the obtained groupings natural or artificial using these morphological features? However, if this biological question may appear quite simple, trying to provide a reply became quickly challenging in a methodological perspective. The main difficulty consisted in reconciling the different statistical objects specific for each approach (*i.e.* multivariate analyses for the morphological data and phylogenetic trees for the molecular data) to allow their comparison. Following this observation, I noticed that this difficulty was one of the main concerns in ecological studies, and that different methods were developed at the University of Lyon for the ADE 4 software (Thioulouse *et al*., 1997). This manuscript takes accordingly place at interface of different disciplines and tries to promote an interdisciplinary approach for an exhaustive evolutionary study of the Loricariinae subfamily.

1- General context

Scientific activities of the Museum of natural history of the City of Geneva (MHNG) are centred on three main objectives: conservation of biological collections, scientific research, and popularization of knowledge through exhibitions and other public activities. The research in museums is essentially specimen-based, and biological collections represent the main source of information for the discovery, characterization and valorisation of the biodiversity. Following the example of other museums in the world, the MHNG represents a real library devoted to natural sciences. The Department of herpetology and ichthyology houses a collection of about 120,000 lots or specimens of fish, amphibians and reptiles including 3,331 type specimens of fish (175 primary types) and 1,492 type specimens of

amphibians and reptiles (132 primary types). In addition an important collection of tissue samples for molecular analyses obtained from more than 10,000 fish specimens is also maintained and regularly increased. The specimens deposited in the ichthyological collection represent the basis of researches in fish systematics by the study of the morphology. The morphology is directly accessible through different techniques (morphometry, osteology, qualitative descriptions…). However, despite the high quality of the information provided by such characters, the study of the morphology remains often insufficient for a global comprehension of the group under study, and especially in the discovery of hidden diversity such as cryptic speciation. For these reasons, the classical morphological approach is now often coupled to molecular data (*e.g*. Fisch-Muller *et al*., 2001; Weber and Montoya-Burgos, 2002; Zawadski *et al*., 2002). Molecular data also provide the necessary evolutionary frame by reconstructing phylogenetic trees. These phylogenies represent a powerful exploratory tool allowing the reconstruction and tracking of evolutionary changes along the tree.

Reconstructing the phylogeny of species remains a challenging task. This aspect benefited from the collaboration between MHNG and the Department of Genetics and Evolution (GenEv) of the University of Geneva. The laboratory of molecular phylogeny and evolution in vertebrates studies the major evolutionary forces responsible for species diversification in a highly documented and investigated Neotropical region: the Guianas. For this, the laboratory developed fast evolving markers allowing investigations at a fine geographical scale. The use of such makers, coupled to more evolutionary constrained ones, allow the reconstruction of large phylogenies for different taxonomical levels (family, subfamily, tribe, genus, species, and population) [*e.g*. Chiachio *et al*., 2008; Cardoso and Montoya-Burgos, 2009; Fisch-Muller *et al*., in press (see Annex 3)].

Nevertheless, comparing biological data is not so easy due to the inherent complexity of the data, the wide range of data sources, the large amount of noise present in data sets related to individual and stochastic processes, and the different nature of the data under study. This complexity forces the use of different methods, relying on different assumptions rendering direct comparison of results impossible. Unifying different data sources within the same descriptive frame is one of the main objectives of the Laboratory of Biometry and Evolutionary Biology (LBBE) of the University of Lyon (France). This laboratory is amongst the pioneer and most innovative in the development of multivariate and multi-table methods for the free software R (R development core team, 2009) through different packages such as

ade4 (Dray and Dufour, 2007), ade4TkGUI (Thioulouse and Dray, 2007), adephylo (Jombart *et al.,* 2010), adegenet (Jombart, 2008) and adehabitat (Calenge, 2006). Initially mainly devoted to ecological studies (*e.g*. Chessel and Hanafi, 1996; Dolédec and Chessel, 1996; Dolédec *et al*., 1996; Dray *et al*., 2002; Dray *et al*., 2003a; Dray *et al*., 2003b; Bady *et al*., 2004; Dray and Legendre, 2008; Jombart *et al*., 2009) the multi-table methods benefit from recent developments in the description and analysis of spatially constrained data that subsequently allowed extrapolation to phylogenetically constrained data (*e.g*. Ollier *et al*., 2006; Pavoine *et al*., 2008; Pavoine *et al*., 2010; Jombart *et al*., 2010).

2- Scientific context

2.1- Group of interest

In ichthyology, research conducted in the MHNG mainly focus on the Neotropical fauna. Initiated around 30 years ago with the Characiformes, the study of South American fish is today mainly focussed on the Siluriformes, or catfish. The Neotropical freshwaters are home to one quarter of the total world ichthyodiversity, with a prediction of around 8,000 extant species out of a mean estimation of 32,000 (Lévêque *et al*., 2008). In Central and South America, the Ostariophysi are undoubtedly the largest represented group and among them, the Siluriformes exhibit the greatest diversity with around 1,647 described species (Reis *et al.* 2003) distributed in 16 families, one of which was discovered and described only recently (Rodiles-Hernández *et al.* 2005). Within the Siluriformes, the Loricariidae, or armoured catfish, represents the most species-rich family in the world with 716 valid species and around 300 undescribed species distributed in 96 genera (Reis *et al*., 2003; Ferraris, 2007). Loricariids are characterized by a depressed body covered by bony plates, a single pair of maxillary barbels, and above all, by the modification of the mouth into a sucker disk. This structural transformation enables these fishes to adhere to the substrate, even in particularly fast flowing waters. The mouth and teeth show strong adaptations to feeding by scraping submerged substrates to eat algae, small invertebrates, detritus, and even wood. Loricariids have undergone an evolutionary radiation on a subcontinental scale, from Costa Rica to Argentina, both on the Pacific and Atlantic slopes of the Andes. They have colonized nearly all freshwater habitats from the torrential waters flowing from the Andes to quiet brackish

waters of the estuaries, black and acidic waters of the Guiana Shield, and subterranean systems. Schaefer and Stewart (1993) compare this radiation to that of the Cichlidae of the Great Lakes of the Rift Valley in Africa. Extremely variable colour patterns and body shapes among loricariid taxa reflect their high degree of ecological specialization. Because of their highly specialized morphology loricariids have been recognized as a monophyletic assemblage in the earliest classifications of the Siluriformes (de Pinna, 1998). Within the Loricariidae, members of the subfamily Loricariinae are united by a long and depressed caudal peduncle and by the absence of an adipose fin, but they exhibit substantial variation in body shape, lip morphology and dentition. Even though members of this subfamily are morphologically well characterized, the systematics of the Loricariinae remains confused and controversial, relying mainly on different authors' personal opinions. Isbrücker (1979) listed twenty-seven genera of Loricariinae, described eight as new, and classified them into four tribes and eight subtribes on the basis of morphology, without phylogenetic inferences: the Loricariini, including six subtribes (Loricariina, Planiloricariina, Reganellina, Rineloricariina, Loricariichthyina and Hemiodontichthyina), the Harttiini, including two subtribes (Harttiina and Metaloricariina), the Farlowellini, and the Acestridiini. The same author (1981a: p. VI, 71) voiced doubts concerning the placement of Acestridiini among Loricariinae, noting that: "The exposed cleithrum and coracoid, together with the peculiar odontodes on the unbranched pelvic fin ray ('spine') are characters otherwise occurring typically only in various members of the subfamily Hypoptopomatinae."; nevertheless, he maintained them as members of Loricariinae. In the same work he also described two new subtribes, Ricolina and Pseudoloricariina, developed the main characteristics of each rank: subfamily, tribe, subtribe, and genera, and provided a provisional key to the genera of Loricariidae. Rapp Py-Daniel (1981) described a new genus, *Furcodontichthys*, and placed it in the Loricariini, subtribe Loricariina. Martín Salazar *et al.* (1982) described *Dentectus* as a representative of the tribe Loricariini, subtribe Planiloricariina. In this paper, he completed the diagnosis of Planiloricariina, in which he transferred the genera *Rhadinoloricaria*, *Crossoloricaria*, and *Pseudohemiodon*. Isbrücker *et al.* (1983) described *Aposturisoma* as a representative of the Farlowellini. Isbrücker and Nijssen (1984, 1986) described *Pyxiloricaria* and *Apistoloricaria,* respectively, and placed them in the Loricariini, subtribe Planiloricariina. Using phylogenetic methods, Schaefer (1986, 1987) established the monophyly of the Loricariinae on the basis of morphological data. Finally, Nijssen and Isbrücker (1987) suggested, referring to a Ferraris personal communication, that the Acestridiini were representatives of the subfamily Hypoptopomatinae. Schaefer (1991) confirmed this status and diagnosed the tribe

Hypoptopomatini including, among others, the Acestridiini. In her PhD thesis, Rapp Py-Daniel (1997) proposed a phylogeny of the Loricariinae based on a phylogenetic analysis of morphological characters. She confirmed the monophyly of the subfamily, and of two of the three remaining tribes *sensu* Isbrücker (1979), Harttiini and Loricariini; members of Farlowellini were placed within Harttiini. Montoya-Burgos *et al.* (1998) proposed the first molecular phylogeny of the family Loricariidae with emphasis on the subfamily Hypostominae. Although, their analysis included only nine representatives of the subfamily Loricariinae, they partially confirmed their subdivision into two main groups, with *Farlowella*, a representative of the Farlowellini, being the sister genus of *Sturisoma*, a representative of the Harttiini, and *Harttia* located at the base of the subfamily. Outside of *Harttia*, the two main groups supported were *Farlowella* and *Sturisoma* sister group of the remaining six genera corresponding to Loricariini. Isbrücker and Isbrücker and Michels (in Isbrücker *et al*., 2001) described four new genera: *Fonchiiichthys*, *Leliella*, *Quiritixys* and *Proloricaria*, and revalidated the genus *Hemiloricaria* Bleeker, 1862 on the basis of a very restricted number of characters of questionable validity because they focus mainly on sexual dimorphism. Rapp Py-Daniel and Oliveira (2001) put *Cteniloricaria* in the synonymy of *Harttia*. Ferraris (2003) maintained the validity of *Cteniloricaria*, put in synonymy all the genera described by Isbrücker and Isbrücker and Michels (in Isbrücker *et al*., 2001), and listed 197 species of Loricariinae distributed in 31 genera: *Apistoloricaria* (4 species), *Aposturisoma* (1 species), *Brochiloricaria* (2 species), *Crossoloricaria* (5 species), *Cteniloricaria* (3 species), *Dasyloricaria* (5 species), *Dentectus* (1 species), *Farlowella* (25 species), *Furcodontichthys* (1 species), *Harttia* (18 species), *Harttiella* (1 species), *Hemiodontichthys* (1 species), *Ixinandria* (2 species), *Lamontichthys* (4 species), *Limatulichthys* (1 species), *Loricaria* (11 species), *Loricariichthys* (17 species), *Metaloricaria* (2 species), *Paraloricaria* (3 species), *Planiloricaria* (1 species), *Pseudohemiodon* (7 species), *Pseudoloricaria* (1 species), *Pterosturisoma* (1 species), *Pyxiloricaria* (1 species), *Reganella* (1 species), *Rhadinoloricaria* (1 species), *Ricola* (1 species), *Rineloricaria* (47 species), *Spatuloricaria* (11 species), *Sturisoma* (14 species), and *Sturisomatichthys* (4 species). Provenzano *et al*. (2005) and Covain *et al*. (2006) (see Annex 1) maintained the synonymy of *Cteniloricaria* with *Harttia*. With addition of Retzer (2006) who described a new species of *Farlowella*, Provenzano *et al*. (2005) who described a new species of *Harttia*, Rodriguez and Miquelarena (2003) who described a new *Loricaria*, Knaack (2003) and Rodriguez and Miquelarena (2005) who respectively described a new species of *Rineloricaria*, and Ghazzi (2005) who described a new *Sturisoma*, Covain and Fisch-Muller

(2007) (see Annex 2) recognized 203 valid species distributed in 30 genera in a review of the subfamily including a generic identification key and a synopsis for each genus. Based on external morphological analyses, they partly confirmed the splitting of the subfamily into two tribes, the Harttiini and the Loricariini, and proposed four morphological groups within the Loricariini: (1) the *Pseudohemiodon* group, (2) the *Loricaria* group, (3) the *Rineloricaria* group, and (4) the *Loricariichthys* group. Ferraris (2007) revised partly his previous statement and considered *Fonchiiichthys* (2 species), *Hemiloricaria* (25 species), and *Proloricaria* (2 species) valid. In addition, Ghazzi (2008) described nine new species of *Rineloricaria*; Ingenito *et al*. (2008) described two new *Rineloricaria*; Fichberg and Chamon (2008) described one new *Rineloricaria*; Rapp Py-Daniel and Fichberg (2008) described one new *Rineloricaria*; Rodriguez and Miquelarena (2008) described one new *Rineloricaria*; Rodriguez and Reis (2008) described two new *Rineloricaria* and recognised two morphological groups, namely the sandy group comprising slender representatives of the genus, and the rocky group comprising stockier forms; Rodriguez *et al*. (2008) revised the taxonomy of *Ixinandria* and considered *I. montebelloi* as a junior synonym of *I. steinbachi*; Thomas and Rapp Py-Daniel (2008) described three new *Loricaria*; de Carvalho Paixão and Toledo-Piza (2009) revised *Lamontichthys* and described two new species; and Thomas and Sabaj Pérez (2010) described one new *Loricaria*. As a result, the Loricariinae comprise 220 valid species distributed in 30 to 34 genera according to the different authors. Among all these genera, 12 to 14 are monotypic and very few of the most speciose have been revised. *Loricaria* was revised by Isbrücker (1981b), *Metaloricaria* by Isbrücker and Nijssen (1982), *Apistoloricaria* by Nijssen and Isbrücker (1988), *Farlowella* by Retzer and Page (1997), *Ixinandria* by Rodriguez *et al*. (2008), and *Lamontichthys* by de Carvalho Paixão and Toledo-Piza (2009).

2.2- Methodological approach

2.2.1- Molecular approach

2.2.1.1- Choice of molecular markers

The genetic markers for phylogenetic reconstructions were selected in a way to provide sufficient variability to resolve generic and specific (and sometimes populational) interrelationships at the subfamilial rank. For this, we selected the mitochondrial non-protein coding genes 12S and 16S rRNA, and the fast evolving nuclear gene coding for the homolog of the zebrafish reticulon 4 receptor-like 2 a (*rtn4rl2a*, synonym NgRH1a) or *f-rtn4* (see Montoya-Burgos *et al*., 2010). For biodiversity assessments we used the standard mitochondrial 648-bp 5' target region of the cytochrome *c* oxidase I (COI) gene proposed by the Barcoding Of Life Initiative (BOLI) (Hebert *et al*., 2003).

The mitochondrial 12S and 16S ribosomal RNA gene sequences encode for the two subunits of the mitochondrial ribosome. The mitochondrial genome encodes for 37 genes, of which 13 form subunits of the respiratory chain complexes. Remaining genes for these complexes are encoded by the nuclear genome. Consequently an accurate coordination between nuclear and mitochondrial genes expression is necessary. Having its own genetic code different from the nuclear genetic code, mitochondria need their own protein biosynthesis system in the form of the mitochondrial ribosome built around 12S rRNA and 16S rRNA (Abhyankar *et al*., 2009). The structure of the mitochondrial ribosome consists in a succession of structurally highly constrained regions corresponding to stems, and more relaxed ones forming loops. The stems are responsible for the secondary structure of the ribosome by pairwise nucleotide matching. The secondary structure is maintained by compensatory mutations in paired nucleotides. When one mutation occurred in one site of a stem, it is compensated by a complementary mutation in its paired nucleotide site of the paired stem. These particularities in the structure of ribosomal gene sequences provide information at different scales, from slow evolving sites in stems to fast evolving sites in loops. Different periods of utilisation of 12S and 16S for phylogenetic reconstructions have been suggested, ranging from between 300 to 150 Ma. BP (Mindell and Honeycutt, 1990) to less than 65 Ma. (Hillis and Dixon, 1991). In fish, the 12S and 16S markers are usually successfully used for the reconstruction of phylogenies at the familial rank (*e.g*. Ortí *et al*.,

1996; Montoya-Burgos *et al.*, 1998; Murphy *et al*., 1999; Wilson *et al*., 2001; Rüber *et al*., 2006; Shimabukuro-Dias *et al.*, 2004; Campo *et al*., 2007; Hrbek *et al*., 2007; Almada *et al*., 2009; Cowman *et al*., 2009; Fernández and Schaefer, 2009; James Cooper *et al*., 2009; Javonillo *et al*., 2010; Straube *et al*., 2010).

Reticulons (RTNs) are membrane-bound proteins mainly anchored on the membrane of the smooth endoplasmic reticulum (van de Velde *et al*., 1994). The *rtn*s genes code for a large RTN protein family present in eukaryote's genome. RTNs have arisen during early eukaryotic evolution potentially concerned to the establishment of the endomembrane system (Oertle *et al*., 2003). In chordates, four paralogs of the *rtn* family are identified: namely *rtn1*, *rtn2*, *rtn3*, and *rtn4/nogo* (Oertle *et al*., 2003) that arose by duplication events before the divergence of sarcopterygians and actinopterygians (Diekmann *et al*., 2005). In fish, all paralogs have six C-termini exons encoding the Reticulon Homology Domain (RHD) (Oertle *et al*., 2003). Among *rtn*s' products, RTN4 (Nogo), and particularly RTN4-A has been intensively investigated in mammals. RTN4-A/Nogo-A is thought to be an inhibitor of neurite outgrowth, restricting the regenerative capabilities of mammalian central nervous system (CNS) after injury (Oertle *et al*., 2003). Contrary to mammals, lesioned axons regenerate in fish CNS due to different evolutionary origins of mammalians and fish *rtn4* N-termini (Diekmann *et al*., 2005). *Rtn*s contain multiple large introns. The variability of the first intron has been investigated in Loricariidae by Fisch-Muller *et al.* (in press) (Annex 3) as comparative nuclear marker in a barecoding study. In mammals, the first large intron contains promoters for alternative transcriptional initiation (Yan *et al*., 2006). In zebrafish (*Brachydanio rerio*) and fugu (*Takifugu rubripes*), r*tn4* is formed by at least nine and eight exons respectively (Diekmann *et al*., 2005). In zebrafish, three different mRNAs are generated from *rtn4* by alternative promoter usage, each consisting in a specific exon and the RHD (Diekmann *et al*., 2005). The highly conserved RHD is present in all RTN members and consists in 186 amino acids in zebrafish. The RHD is characterized by a hydrophilic loop of 66 amino acids flanked by two putative transmembrane segments and a hydrophilic tail (Yan *et al*., 2006). Part of the *f-rtn4* gene (excluding too conserved regions such as the RHD) has been successfully used for the reconstruction of the phylogenetic tree of the loricariid subfamily Hypoptopomatinae (Chiachio *et al*., 2008), and in a populational study of *Guyanancistrus brevispinis* within the Guianas using the first intron (Cardoso and Montoya-Burgos, 2009).

The COI gene encodes part of a large enzymatic complex of the mitochondrial respiratory chain. The sequence, due to the degenerate nature of the genetic code, possesses

high mutational rates in third and first positions of codons, despite relative conservation in amino acids (Ward and Holmes, 2007). These high mutational rates allow therefore the rapid accumulation of mutations between sequences that forms the basis of the barcode system. The differences accumulated are expected to be low within species due to the constant transmission of mitochondria, and high between species due to the absence of mitochondrial exchanges. The COI barcode system has already been efficiently used in quantifying and qualifying fish diversity (Ward *et al*., 2005; Hubert *et al.,* 2008; Ward *et al.,* 2009; Valdez-Moreno *et al*., 2009; Lara *et al*., 2010), and successfully highlighted cryptic species (*e.g*. Ward *et al*., 2008a; Ward *et al*., 2008b; Lara *et al.*, 2010; Fisch-Muller *et al.* (in press) (Annex 3)).

2.2.1.2- Alignments and reconstruction methods

The alignment represents one of the most crucial steps in the analysis of DNA sequences. This step determines to homology between positions of the different sequences that will be subsequently analysed by tree reconstruction methods. If the alignment is easily tractable manually in small datasets of coding regions between closely related taxa, this task becomes rapidly intractable with the progressive increase of taxa, and complexity of the data (*e.g*. due to insertion deletion events). Different automated methods have been developed to reconstruct multiple alignments such as MUSCLE (Edgar 2004a; 2004b), MAFFT (Katoh *et al*., 2002), or Clustal W (Thomson *et al.*, 1994). Probably one of the most used remains Clustal W or its version using a Graphical User Interface Clustal X. Automated alignment methods are powerful but often result in suboptimal solutions, especially in non coding regions which often display size polymorphism. Final alignment is consequently often optimized by eye by users (*i.e.* manually), and ambiguously aligned positions are thus simply discarded before tree reconstruction. However, simply removing ambiguous positions can result in the loss of a large amount of informative sites (Lutzoni *et al*., 2000), since only part of a column can be ambiguously aligned, and the process of determining which regions of the alignment are ambiguous is *ad hoc* and can be highly subjective (Redelings and Suchard, 2005). To minimize this potential bias, different solutions were proposed. Hall (2005) proposed to maximize an objective function such as the average quality score (or Q score; Thomson *et al*., 1997) using different values of gap penalties in Clustal X in order to obtain a

final alignment of maximal mean Q score. Löytynoja and Milinkovitch (2001) developed SOAP to explore the alignment's space using progressive increase of gap penalties in Clustal W to generate *n* multiple alignments. These *n* alignments are then simultaneously compared to a reference alignment (*e.g*. using default gap penalties parameters) to detect instable positions. However, the choice of values for gap penalties remains arbitrary for the determination of the alignment's space to explore. Moreover, different biases were also signaled such as those introduced by the guide tree computed by Clustal using a preliminary pairwise alignment to perform the final multiple alignment. Lake (1991) demonstrated that the order of the alignment (so the guide tree) dominated the reconstructed phylogenetic tree topology. Nevertheless, no consensus was reached on this question. Hall (2007) stated that their was no effect of the guide tree on multiple alignment, what was confirmed by Nelesen *et al*. (2008) who demonstrated that changes in the guide tree do not impact the accuracy of the estimated alignments. Alternatively Kumar and Filipski (2007) found a strong effect of the guide tree on downstream phylogenetic inferences, but concluded that "*the implicit consolation has been that at least incorrect phylogenetic clusters will not garner high statistical support*". So, what could be the consequences of ambiguously aligned positions on downstream phylogenetic reconstructions? Hall (2005), based on a widely accepted idea, stipulated that it was a truism that the quality of a tree could not be better than the quality of the alignment used to estimate that tree. Additionally, Rosenberg (2005) demonstrated that the accuracy of the alignment was largely dependent on the distances among sequences; the more closely related the more accurately aligned (and conversely). A first evaluation of this *a priori* was assessed by Ogden and Rosenberg (2006) who demonstrated that balanced reconstructed topologies were much less affected by alignment error than pectinate topologies, and that the degree to which the balanced trees were robust to alignment inaccuracy was unexpected. Essentially, alignments that were 50% inaccurate for balanced, ultrametric, equal branch length tree shapes, showed no average disadvantage as compared to the true alignments. In addition, in the same study, these authors demonstrated that probalistic tree reconstruction methods (Maximum Likelihood and Bayesian), outperformed distances (*e.g.* Neighbor Joining) and Maximum Parsimony based methods in terms of tree reconstruction accuracy. The Maximum Likelihood method consists in selecting the hypothesis maximizing the probability of observing the data. Introduced in phylogeny by Edwards and Cavalli-Sforza (1964) and Felsenstein (1981), the ML method tries to maximize the likelihood of the data, given a stochastic evolutionary model and a tree topology. Doing so, several parameters of the tree are optimized such as topology and branch lengths. The ML criterion is also at base of the

Bayesian inference. The assumption differs from the preceding in that the Bayesian method seeks the hypothesis of maximal probability knowing the data. In this case, Bayesian inference (*sensu* Rannala and Yang, 1996) searches for the tree of maximum posterior probability.

2.2.2- Morphological approach

Characterizing the biodiversity implies a clear delineation of what can be considered as distinct species. In systematics, the typological approach relies essentially on specimens, and perhaps the most straightforward mean to delineate species consists in an evaluation of the morphological characters. One can notice that using morphological characters to delineate species is far from the usual Mayr's (1963) definition of the biological species: a complex of interbreeding individual organisms co-existing at one point in time which is genetically isolated from other such complexes (for a reviews on species concepts see Kottelat, 1997; Bock, 2004). However, morphology remains one of the only mean to characterize and describe new taxa, and systematists often deal with a morphological species concept. The International Code of Zoological Nomenclature (1999) provides the legal frame for the establishment of new taxa and correct use of the newly formed names, and by the way ensures their stability. It establishes a set of rules and recommendations that must be followed. For example, to be valid when established, a new taxon (species-group level) must: follow the principle of the binomial nomenclature, be considered as the valid name with clear intention to publish this name as new, and provide a description of the distinctive characters (*i.e.* the diagnostic characters). In addition the name-bearing types (a holotype or syntypes) of a new species must be fixed and have to be held in trust for sciences as they are the international standards of reference. The holotype can be designated with one or more complementary specimens: the paratypes, both (holotype plus paratypes) constituting the type series. The paratypes are often added to integrate as much as possible the observed variability of the taxon in its formal description. The holotype not only fixes the taxonomy, but also the type locality of the species. The main approach consists thus to define species on the basis of observed similarities or differences. This step can be highly subjective, and different methods were developed to provide objective criteria in the delineation of species boundaries using morphological characters (*e.g.* numerical taxonomy). This approach assumes also *a priori* a

correlation between morphological evolution and genetic evolution leading to reproductive isolation, both being related at larger scale to genomic evolution. Moreover, even though apparently related, one must make a difference between searching for distinctive morphological characters to differentiate species and the cladistic method.

The cladistic method aims to classify the different species according to shared derived characters called synapomorphies. All species belonging to a monophyletic assemblage, or clade, possess thus these synapomorphic features in regard to their sister group that possess other distinct shared characters. Synapomorphies are not useful in species delineation since these features are shared by all close relatives. Unique characters for a given taxon, that are the main objective of the taxonomist, are called autapomorphies. By essence, these characters are uninformative in cladistics, but highly valuable as diagnostic features (*i.e.* features that distinguish the taxon from all others). This kind of character is often qualitative and usually coded in a binary fashion as presence-absence data or multistate data that are easily tractable by usual multivariate methods such as Correspondence Analysis (CA) and Multiple Correspondences Analysis (MCA).

Alternatively other methods relying on a quantitative approach have been developed for morphological studies by providing measurements of the form of specimens. This kind of method belongs to the field of morphometrics which is the study of form as the result of variations in shape and size (Richtsmeier *et al*., 2002). Morphometrics investigates shape and size variations and covariations, and their covariations with other variables (Claude, 2008). Two approaches using different data types were developped, the traditional morphometrics relying on the analysis of linear measurements, counts, angles, and ratios, and the geometric morphetrics relying on the analysis of landmark coordinates. Landmarks correspond to points located in homologuous positions between the compared objects (*e.g* base of fins' insertion, extremity of a given structure such as a bone…). Both approches possess their own advantages and disadvantages, and if geometric morphometrics was developped with a strong theoretical background (*e.g.* Bookstein, 1991; Zelditch *et al*., 2004; Claude, 2008), and seems effectively more powerful in the quantification of variations in shape and size, outputs of the traditional approach are often easier to interpret as the use of morphological variables is often more intuitive than landmarks. Moreover, both approaches often produce comparable results (*e.g*. Parson *et al*., 2003; Maderbacher *et al*., 2008; Sidlauskas *et al*., 2011). In addition,

traditional morphometric data are easily tractable using Principal Components Analysis (PCA) or Discriminant Analysis (DA).

2.2.3- field approach

Complementary to collection and lab works, I had the opportunity to participate in, and also to conduct different field trips in South America. These missions took place in French Guiana, Suriname, Guyana, and Peru, and followed several other missions previously organized in Brazil, Peru, Paraguay, or Panama. These field works allow the regular increase of MHNG collections in specimens and tissue samples, and provide often opportunities for the discovery of species unknown to science. Moreover, as stated above concerning the typological approach, type localities are fixed when such new species are described. Field collects represent thus a relevant and necessary mean to reach this goal (among other problematic) and allow a better description and characterization of these localities. Additionally to the collect of specimens and tissue samples for morphological and molecular analyses, field observations are consequently equally conducted. After catching, specimens are photographed and referenced using field numbers, fin clips for DNA studies are taken and identified using the same numbers, and specimens are fixed for long term preservation. Doing so, collecting points or localities are georeferenced using the Global Positioning System for latitude, longitude and altitude. Water parameters such as conductivity, pH, and temperature are also recorded, with the more recent addition of turbidity and amount of dissolved oxygen. Qualitative descriptive information on biotopes such as type of substrate (*e.g*. rocks, stones, gravels, sand, mud, or organic matter), water velocity, or type of river (*e.g*. forest creek, large river, water fall, or estuary) is also noted. This kind of data represents valuable information for characterizing fish environment and are classically used in fish ecological studies such as fish-habitat relationships (for French Guiana see *e.g*. Mérigoux *et al*., 1998; Mérigoux *et al*., 2001; for a review see de Mérona *et al*, in press).

2.2.4- Multi-tables approach

Standard approaches, such as those presented above, provide their own data type, reply to a single question at a time, and are often organized around the same central biological problematic. Unifying these different data sources, that can be of different statistical nature, into the same exploratory or descriptive frame remains a challenging task. These different data, organized into separate tables, often rely on the same statistical units (*e.g*. the specimens, the stations…) establishing therefore a link between tables, and can be described by multiple descriptors (*e.g*. genetic data, morphometric data, environmental parameters…). A possibility to unify these observations consists thus or to perform an analysis of the coupled tables and to search for a mean to gain independence from the nature of the data (quantitative and qualitative) to make them compatible, or to perform preliminary analyses on the separate tables according to the nature of the data, and to search for similar patterns in the different analyses, to include them into the same global analysis.

The first approach was reached by Hill and Smith (1976) who provided a mean to perform a PCA on a table mixing quantitative and qualitative data. In the Hill and Smith Analysis (HSA), quantitative data are first subjected to a PCA whereas qualitative data are subjected to a MCA. Then both types of data are made compatible by reweighting the columns to provide the same importance to each variable in the analysis, even though qualitative variables possess several modalities. While PCA looks for axes that maximize square of correlations of the quantitative variables, and MCA looks for axes that maximize the sum of ratios of correlations between modalities of the qualitative variables, the HSA establishes a compromise between these two analyses by looking for axes that maximize the mean of the square of correlations (quantitative variables) and the ratios of correlations (qualitative variables). This idea of compromise between different types of data represents the key stone of the multi-table approach, and in this sense, the HSA represents a first step in the simultaneous analysis of multiple data sources. This analysis was successfully used by Covain and Fisch-Muller (2007) (see Annex 2) to sort quantitative and qualitative morphological characteristics classically used as diagnostic features for the establishment of an identification key for the different genera of the Loricariinae.

The second point of view is reached by the so-called multi-table analyses. These analyses look for common structures present in the data sets, and include them in the same descriptive frame. Initially devoted to the study of ecological patterns such as species-habitat

relationships, the co-structure analyses try to bring out common information present in different data sets, for example, to establish the relationships between species distributions and environmental parameters recorded for the same localities. This aspect has been unified by Dolédec and Chessel (1994), when they developed the co-inertia analysis (CIA). The CIA aims to extract the joint structure between two tables relying on the same statistical units. The mathematical model of CIA is given in Dolédec and Chessel (1994) and in Dray *et al.* (2003b). The two studied tables (*e.g.* species occurrences and environmental parameters for several localities) are first submitted to preliminary analyses (*e.g.* PCA, MCA, CA, HSA) and united by the CIA to describe the common structure present in both tables. Results of the CIA consist in two new sets of scores of maximum covariance. The CIA indeed maximizes a compromise between the structure of the first table (*e.g.* a table containing species occurrences at different localities), the structure of the second table (*e.g.* a table containing environmental parameters for the same localities), and their link. Subsequently, Dolédec *et al.* (1996) extended the concept of co-inertia to three tables, and developed the RLQ analysis. The RLQ analysis aims to investigate the relationships between a table R (*e.g.* a table of species traits providing external information about rows) and a table Q (*e.g.* a table of sites' environmental variables providing external information about columns), united by a link table L (*e.g.* a species by site cross table), and establishes a compromise by extracting the joint structure between them. The RLQ differs from the CIA in that the relationships between the two tables R and Q is provided by the third table L, whereas in CIA this relationship is directly provided by the rows (*i.e.* the same statistical units) of the two studied tables. The mathematical model of RLQ is described in Dolédec *et al.* (1996) with adaptations in Dray *et al.* (2002), and Dray and Legendre (2008). The RLQ analysis consists in an eigenvalue decomposition of the cross-table L that provides ordination axes (*e.g.* species distribution) onto which scores obtained from preliminary analyses of both tables R and Q (*e.g.* traits and environmental data) are projected. Results of RLQ consist in two new sets of scores for the two tables R and Q of maximal covariance when the link table is submitted to a CA (Dolédec *et al.*, 1996). Finally, the co-inertia criterion was extended to K tables by Chessel and Hanafi (1996) who developed the multiple co-inertia analysis (MCOA). MCOA identifies the common structure present in multiple datasets ($n = k > 2$) relying on the same statistical units by providing a consensual typology (the compromise) maximizing the link with all tables simultaneously. This link is expressed by the sum of squared covariances between the linear combinations of the variables of each table and the compromise.

3- Objectives of this thesis

This manuscript is centred on the biological question of the evolution of the highly diversified Loricariinae. For this, I tried to benefit from the singularity of the three institutes (MHNG, LBBE, and GenEv) to investigate evolutionary patterns in this subfamily. Particularly, I tried to benefit from the recent developmental advances in multi-table analyses (and software through the free software R) and detection of new fast evolving markers, for the study of exhaustive and well documented datasets obtained from specimens, tissue samples for DNA analyses, and field observations. For this, since phylogenetic trees represent qualitative objects that can be easily converted into quantitative objects using patristic distances to create distances matrices (and as stated above, branch lengths are optimized for a given data set and evolutionary model using the ML criterion), phylogenetic information can be integrated in a first table that can be submitted to a Principal Coordinates Analysis (Gower, 1966). This first analysis represents thus the first step for the exploration of multiple tables relying on the same individuals. The use of multi-table analyses provided the necessary unifying descriptive frame to reach the main objective of the present thesis. The adaptation of multi-table analyses to a phylogenetic and evolutionary context allowed indeed the exploration of different types of data reinforcing significantly our knowledge of the group in integrative taxonomical and evolutionary purposes. When one of the data sets represents the phylogeny, all related structures in other tables can indeed be linked together to the phylogeny and interpreted in an evolutionary perspective. This approach allowed to explore relationships between morphological, ecological, distributional, and ethological data and to reveal evolutionary trends shaped through time in Loricariinae. Moreover, establishing a link between a phylogeny and different type of data implies that these data are under phylogenetic dependence. This central concept in comparative biology stipulates that because of the heritability of biological traits from common ancestors, the observations conducted between species are non independent (see Harvey and Pagel, 1991). Different methods have been developed to detect phylogenetic dependence in comparative data, one of the latest being the orthogram (Ollier *et al.*, 2006). The orthogram decomposes the trait variance along a phylogenetic tree represented as an orthonormal basis. However, in its original form, the orthogram can only deal with quantitative data. The extension of the orthogram to the quantitative and multivariate cases was thus a prerequisite for a comprehensive study of the

evolution of biological traits in the Loricariinae. This unifying structure, making each test directly comparable, allowed consequently the development of a new multivariate method for the exploration of patterns of co-evolution among multiple traits along a phylogeny. This new approach adapts the multi-scale pattern analysis (MSPA) technique developed for the analysis of spatial data (Jombart *et al*., 2009) into a phylogenetic context. This new analysis differs from the classical multi-table approach by the way to describe the phylogeny that is directly used as exploratory variable. The progressive establishment of robust and exhaustive phylogenies allowed the revision of the systematics of the Loricariinae, and the description of the new taxa highlighted by the combined analyses. The manuscript is organised in chapters centred on the main problematic and corresponding to the objectives stated below.

First, an evaluation of the multi-table approach was performed using the CIA to explore the co-structure between a phylogeny reconstructed using mitochondrial markers, and the morphological data set previously published by Covain and Fisch-Muller (2007) (Annex 2). In addition, the systematics of the Loricariinae was revised, and the phylogenetic dependence of the morphological characters classically used to describe the different genera was assessed.

Second, based on previously known collection specimens (a single in MHNG) and additional freshly collected material from Peru, a new genus and new species was described to clarify the systematics of the group. Additionally, an evaluation of the alignment, especially in intronic regions of the new *f-rtn4* marker was performed prior to reconstruct the first phylogeny of the Loricariinae mixing mitochondrial and nuclear information (for the characterization of the first intron of *f-rtn4* see Fisch-Muller *et al*., in press; Annex 3).

Third, the MCOA was evaluated in a global assessment of the diversity of a tribe of the Loricariinae, the Harttiini, within the Guianas. Following a first study restricted to a single genus of this tribe in a single country (Covain *et al*., 2006; Annex 1), genetic, morphometric, and ecological-distributional information of all Guianese populations and species of this tribe were united in the same descriptive frame to reveal underlying evolutionary forces shaping

their diversification throughout the Guianas. In addition several new highlighted taxa were described.

Fourth, the RLQ analysis was evaluated to detect co-dispersion events in two co-distributed groups of the Loricariidae: the Harttiini tribe, and the *Hypostomus* genus. The detection of common structures in both phylogenies being potentially related to co-dispersion events, the dating provided in one phylogeny for the dispersion of *Hypostomus* species were applied to the phylogeny of Harttiini to propose a phylogeographic hypothesis for the historical diversification of this tribe at the sub-continental scale.

Fifth, an exhaustive phylogeny was reconstructed (350 OTUs), the orthograms were generalized and the MSPA was evaluated on an extended data set mixing quantitative (discrete and continuous), qualitative (binary, multistate, and ordinal), intraphenotypic (morphology, ethology) or extraphenotypic (environmental parameters) to detect co-evolution among multiple traits along the phylogeny, and thus revealing variables involved in the main evolutionary innovations of the Loricariinae. In addition evolutionary patterns for these innovations were revealed and a dating for the appearance of these structures was proposed.

These five studies are developed in respective order in the subsequent chapters. Three of them are already published or in press, and two are presented as articles to be submitted. Three additional works directly related to the present thesis are appended in annexes to provide substantial complementary information.

Chapter 1

Assessing phylogenetic dependence of morphological traits using co-inertia prior to investigate character evolution in Loricariinae catfishes.

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An evaluation of the multi-table approach using the CIA is here performed to explore the co-structure between a phylogeny reconstructed using mitochondrial markers, and the morphological data set previously published by Covain and Fisch-Muller (2007) (Annex 2). In addition, the systematics of the Loricariinae is revised, and the phylogenetic dependence of the morphological characters classically used to describe the different genera is assessed.

Published in : *Molecular Phylogenetics and Evolution*, **46** (2008): 986-1002.

Abstract

With the increase of laboratory facilities, molecular phylogenies are playing a predominant role in evolutionary analyses. However, understanding the evolution of morphological traits remains essential for a comprehensive view of the evolution of a group. Here we present a new approach based on co-inertia analysis for identifying characters which variations are dependent to the phylogeny, a prerequisite for analyzing the evolution of characters. Our approach has the advantage of treating the full data set at once, including qualitative and quantitative variables. It provides a graphical output giving the contribution of each variable to the co-structure, allowing a direct discrimination among phylogenetically dependent and independent variables. We have implemented this approach in deciphering the evolution of morphological traits in a highly specialized group of Neotropical catfishes: the Loricariinae. We have first inferred a molecular phylogeny of this group based on the 12S and 16S mitochondrial genes. The resulting phylogeny indicated that the subtribe Harttiini was restricted to the single genus *Harttia*, and within the subtribe Loricariini, two sister subtribes were distinguished, Sturisomina (new subtribe), and Loricariina. Among Loricariina, the morphological groups *Loricariichthys* and *Loricaria* + *Pseudohemiodon* were confirmed. The co-inertia analysis highlighted a strong relationship between the morphological and the genetic data sets, and identified three quantitative and eight qualitative variables linked to the phylogeny. The evolution of quantitative variables was assessed using the orthogram method and showed a major punctual event in the evolution of the number of caudal-fin rays, and a more gradual pattern of evolution of the number of teeth along the phylogeny. The evolution of qualitative variables was inferred using ancestral states reconstructions and highlighted parallel patterns of evolution in characters linked to the mouth, suggesting co-evolution of the traits for adapting to divergent substrates.

Keywords: Siluriformes, Loricariidae, molecular phylogeny, ribosomal genes, morphology, co-inertia analysis, orthogram, character mapping.

1. Introduction

The increasing amount of robust molecular phylogenies, often based on multiple genes, is gradually setting aside the concern of reconciling the phylogenies based on molecules and morphology. As a consequence, studies based on morphological traits incur the risk of a significant decline. The evolution of morphology, visible traits or phenotypes, remain however essential for the understanding of the evolutionary history of a group. A meaningful approach for a comprehensive understanding of the evolution of a given taxon is to first generate a well supported molecular phylogenetic tree and thereafter interpret the evolution of morphological traits in the light of this phylogeny. This principle is followed in the character mapping methods available, either via parsimony or using stochastic models.

The evolution of traits (morphology, ecology, behavior …) may be plastic and stochastic or, to the contrary, traits may evolve according to a trend tightly linked to the phylogeny of the group. Only those characters displaying variations correlated to a given phylogeny may have their evolution interpreted in the light of that phylogeny. Therefore, testing for phylogenetic dependence is a first and unavoidable step to study the evolutionary relationship between a life trait and the phylogeny (Ollier et al., 2006). Several methods have been developed to detect phylogenetic dependence in comparative data (e.g. Felsenstein, 1985a; Cheverud et al., 1985; Gittleman and Kot, 1990; Harvey and Pagel, 1991; Lynch, 1991; Diniz-Filho et al., 1998; Pagel, 1999a; Abouheif, 1999, Blomberg et al. 2003; Ollier et al. 2006; for reviews see Rholf, 2001; Blomberg et al. 2003). Probably the most popular tests were developed by Abouheif (1999) who modified two previously existing tests, the Test For Serial Independence (TFSI) (von Neumann et al., 1941), and the RUNS test (Sokal and Rholf, 1995), which can detect phylogenetic autocorrelation for quantitative and qualitative variables respectively. These tests have the advantage of needing only the topological structure of the tree, which allows the use of a wide range of tree sources (cladograms, phylograms, consensus trees, supertrees …). Each character under study must be however individually tested according to its quantitative or qualitative nature. Therefore, this procedure becomes fastidious when the tree topology is complex, and when the number of traits under study is important.

After testing for the phylogenetic dependence of the character, their evolution can be reconstructed along the given phylogenetic tree. Several methods have been proposed for reconstructing ancestral states or for mapping characters on the tree in order to test hypotheses about the evolution of the selected characters (Schluter et al., 1997; Pagel, 1999b; Huelsenbeck et al., 2003; Pagel et al., 2004). They provide a graphical view of the best

possible reconstruction of the evolution of the trait assuming an implicit process of evolution (Maximum Parsimony mapping) or an explicit model of evolution (Maximum Likelihood, Stochastic, and Bayesian ancestral state reconstructions). Alternatively, the orthogram method developed by Ollier et al. (2006) represents a relevant approach that detects and characterizes phylogenetic dependence, and at the same time highlights different pattern of evolution along a phylogenetic tree. However, this method can treat only quantitative data.

Here we present a new approach to detect phylogenetic dependence of characters of different type in a fast, simultaneous and reliable way. Our approach is based on the co-inertia analysis (Dolédec and Chessel, 1994) to assess the common information present within a genetic and a morphological data set. It allows identifying the morphological variables that possess a strong covariation with the phylogeny in a pool of many different morphological variables, either quantitative or qualitative. The strength of our approach is to provide a direct graphical interpretation of the explored data sets. The phylogenetically informative morphological variables can be easily detected as well as the variables unlinked to the phylogeny. This last class of variables is discarded from further analyses as they represent evolutionary "noise". The co-structure can be represented in a phylogram summarizing the total amount of convergent information present in both molecular and morphological data sets.

We have implemented our new approach for identifying morphological characters varying dependently from the phylogeny, and have reconstructed their evolution in a group of highly derived catfishes, the Loricariinae. Our work has therefore started by the reconstruction of a robust molecular phylogeny of this group based on partial 12S and 16S mitochondrial genes. The Loricariinae represents a diversified subfamily among the large Neotropical catfish family Loricariidae, or armored catfish. Loricariids are characterized by a modification of the mouth structure into a sucker disk, by a body covered with bony plates, and by a unique pair of maxillary barbels. Loricariids have undergone an evolutionary radiation at a subcontinental scale, from Costa Rica to Argentina, which has been compared to that of the Cichlidae of the Great Lakes of the Rift Valley in Africa (Schaefer and Stewart, 1993). Among Loricariids, members of the Loricariinae subfamily are characterized by a long and depressed caudal peduncle and by the absence of an adipose fin. They live stuck to the substrate and show marked variations in body shape due to the various habitats colonized, from lotic to lentic systems, on inorganic or organic substrates. Some groups have numerous teeth, pedunculated, and organized in comb, while other groups have few teeth or even no teeth on premaxillae. These latter are often strongly differentiated, and can be bicuspid

straight and thick, spoon-shaped, reduced in size or very long. An important diversity in lips structure, which can be strongly papillose, filamentous or smooth, also characterizes this subfamily (Covain and Fisch-Muller, 2007).

Modern classification of Loricariinae started with Isbrücker (1979) who proposed a subdivision into four tribes and eight subtribes on the basis of morphology. These included the Loricariini (comprising six subtribes), the Harttiini (including two subtribes), the Farlowellini, and the Acestridiini. Schaefer (1987) established the monophyly of the Loricariinae on the basis of morphological data, and placed the Acestridiini into another subfamily, the Hypoptopomatinae (Schaefer, 1991). Rapp Py-Daniel (1997) confirmed the monophyly of the subfamily and of the Loricariini *sensu* Isbrücker (1979), and redefined the Harttiini comprising former Farlowellini. Further on, Montoya-Burgos et al. (1998) proposed the first molecular phylogeny of the family Loricariidae based on mitochondrial markers. They confirmed the position of the Farlowellini nested within Harttiini and provided the first evidence for a splitting of the subfamily into two lineages, *Harttia*, on one side and all other Loricariinae on the other side. They also found that *Farlowella* and *Sturisoma* form the sister group to the Loricariini. In a recent work, Covain and Fisch-Muller (2007) recognized 203 valid species distributed in 30 genera. Based on external morphological analyses, they partly confirmed the splitting of the subfamily into two tribes, the Harttiini and the Loricariini, and proposed four morphological groups within the Loricariini: (1) the *Pseudohemiodon* group, (2) the *Loricaria* group, (3) the *Rineloricaria* group, and (4) the *Loricariichthys* group. The morphological data set of Covain and Fisch-Muller (2007) was used here to test our new approach for detecting morphological characters linked to the phylogeny. Then, the evolution of the retained characters has been inferred.

2. Material and methods

2.1 Taxonomic sampling.

The molecular phylogeny was established for 14 genera totalizing 20 species of Loricariinae. Taxonomic sampling was chosen in a way to include at least one representative of the different morphological groups defined in Covain and Fisch-Muller (2007). The outgroup was chosen in another subfamily of Loricariidae. The list of material used for this study is given in Table 1. The analyzed samples came from the tissue collection of MHNG, Geneva, and the sequences were deposited in GenBank. The morphological characters analyzed in this study are presented in Covain and Fisch-Muller (2007) and summarized at the end of Table 3.

Table 1.Taxa list, specimen and sequence data for the 20 species of Loricariinae, and outgroup analyzed in this study. The abbreviations of institutions follow Leviton et al. (1985)

Species	Catalog Number	Field Number	Locality	mt 12S+16S bases + GenBank No.
Crossoloricaria venezuelae	INHS 35467	VZ 049	Venezuela, Rio Santa Rosa	1687 EU310444
Dasyloricaria tuyrensis	MHNG 2674.052	PA00–012	Panama, Rio Ipeti	1687 EU310445
Farlowella platoryncha	MHNG 2588.093	PE96–071	Peru, Rio Ucayali	1700 EU310446
Farlowella oxyrryncha	MHNG 2588.064	PE96–022	Peru, Rio Tambopata	1700 EU310443
Harttia guianensis	MHNG 2543.016	GF00–351	French Guiana, Marouini River	1704 EU310447
Hemiodontichthys acipenserinus	MHNG 2651.012	GY04–15	Guyana, Rupununi River	1688 EU310448
Lamontichthys stibaros	MHNG 2677.039	MUS 208	Peru, aquarium trade, Rio Itaya according to the exporter	1701 EU310449
Limatulichthys griseus	MHNG 2651.013	GY04–18	Guyana, Rupununi River	1689 EU310450
Loricaria clavipinna	MHNG 2640.044	PE98–002	Peru, Rio Putumayo	1693 EU310451
Loricaria parnahybae	MHNG 2602.067	BR98–274	Brazil, Rio Parnahyba	1689 EU310452
Loricariichthys maculatus	MHNG 2621.042	SU01–56	Surinam, Sarramacca River	1694 EU310453
Loricariichthys microdon	MHNG 2650.054	GY04–12	Guyana, Rupununi River	1694 EU310454
Metaloricaria paucidens	MHNG 2677.086	GF00–083	French Guiana, Marouini River	1703 EU310455
Planiloricaria cryptodon	MHNG 2677.038	MUS 211	Peru, aquarium trade, Rio Itaya according to the exporter	1690 EU310456
Rineloricaria platyura	MHNG 2651.009	GY04–83	Guyana, Rupununi River	1692 EU310458
Rineloricaria lanceolata	MHNG 2588.059	PE96–011	Peru, Rio Tambopata	1689 EU310457
Rineloricaria sp. Tocantins	UFRJ batch 6–EF4	BR 1114	Brazil, Rio Maranhão	1691 EU310459
Sturisoma nigrirostrum	MHNG 2588.055	PE96–001	Peru, Rio de las Piedras	1706 EU310460
Sturisoma monopelte	MHNG 2651.033	GY04–187	Guyana, Sawarab River	1707 EU310461
Sturisomatichthys citurensis	MHNG 2676.004	PA97–032	Panama, Rio Tuyra	1703 EU310462
Ancistrus cirrhosus[1]	MHNG 2645.037	MUS 202	Argentina, Rio Uruguay	1698 EU310442

[1] outgroup

2.2 DNA extraction, amplification and sequencing.

Tissue samples were preserved in 80% ethanol and stored at -20°C. Total genomic DNA was extracted with the DNeasy Tissue Kit (Qiagen) following the instructions of the manufacturer. The PCR amplification of partial 12S and 16S were carried out using the Taq PCR Core Kit (Qiagen). The primers used were: An12S–2D 5'–GCC AGC TTA CCC TGT GAA GG–3' and H3059 5'–CCG GTC TGA ACT CAG ATC ACG T–3'. The amplifications were performed in a total volume of 50 µl, containing 5 µl of 10x reaction buffer, 1 µl of dNTP mix at 10mM each, 1 µl of each primer at 10 µM, 0.2 µl of *Taq* DNA Polymerase equivalent to 1 unit of Polymerase per tube, and 1 to 4 µl of DNA. Cycles of amplification were programmed with the following the profile: (1) 3 min. at 94°C (initial denaturing), (2) 35 sec. at 94°C, (3) 30 sec. at 52–54°C, (4) 2 min. at 72°C, and (5) 5 min. at 72°C (final elongation). Steps 2 to 4 were repeated 27 to 39 times according to the quality and concentration of DNA. PCR products were purified with the High Pure PCR Product Purification Kit (Roche). Sequencing reactions were performed with the Big Dye Terminator Cycle Sequencing Ready Reaction 3.1 Kit (Applied Biosystems) following instructions of the manufacturer, and were loaded on an automatic sequencer 3100-Avant Genetic Analyzer (Applied Biosystems, Perkin-Elmer). To obtain the complete sequence of the amplified region, an internal primer was designed: Lor12S–3D 5'–CCT CGT ACC TTT TGC ATC ATG–3'.

2.3 Sequence alignment and phylogenetic reconstruction.

The DNA sequences were edited and assembled using BioEdit 7.0.1 (Hall, 1999). Alignment was realized using ClustalW (Thompson et al., 1994) and optimized by eye. Regions with ambiguous alignments were excluded from the analyses. Phylogenetic reconstructions were performed with PAUP* 4.0b10 (Swofford, 2003) following three methods: Neighbor-joining (NJ) (Saitou and Nei, 1987), Maximum parsimony (MP), and Maximum likelihood (ML) (Felsenstein, 1981). The model of substitution that best fitted the data was determined by Modeltest 3.06 (Posada and Crandall, 1998). The best fit model was used for the ML tree reconstructions and to correct the distance matrix for NJ analysis. Robustness of the results was estimated by resampling the data set with the nonparametric bootstrap (Efron, 1979) following Felsenstein's (1985b) methodology with 1000 replicates for NJ and MP methods, and with 200 replicates for ML method. Alternative topologies were investigated using the Shimodaira and Hasegawa (SH) test (1999) that allows comparison

between the best ML tree and an alternative topology (Goldman et al., 2000). SH tests were performed using PAUP* 4.0b10 with 2000 RELL replicates.

2.4 Co-structure analysis between morphology and genetics (CIA).

To highlight a possible relationship between the information of morphological data provided in Covain and Fisch-Muller (2007), and the one provided by our molecular data, both data sets were analyzed by Co-inertia Analysis (CIA) (Doledec and Chessel, 1994). Taxonomical sampling was modified in order to keep the same 14 genera present in both data sets. For this, when more than one representative of a given genus was present in the molecular data set, all except one were pruned, that is to say: *Loricaria parnahybae*, *Loricariichthys microdon*, *Rineloricaria* sp. Tocantins, *Rineloricaria platyura*, *Farlowella oxyrryncha*, and *Sturisoma nigrirostrum*. Because morphological characters analyzed here are homogenous within genera, only generic names are given in the CIA and subsequent analyses. Molecular data were converted into a distance matrix corrected according to the model of substitution re-estimated by Modeltest 3.06 (Posada and Crandall, 1998). This matrix was then rendered Euclidian using Lingoes' (1971) method. Principal Coordinate Analysis (PCoA) (Gower, 1966) was performed on this corrected distance matrix to reveal the structuring of the genetic data set. This analysis provide a tree free representation of the phylogenetic data set onto axes, where the pairwise distances between genera are exactly the genetic pairwise distances of the matrix. Morphological data were analyzed by Hill and Smith Analysis (HSA) (1976) to reveal their structuring. The HSA consists in a Principal Component Analysis (PCA) of a table mixing quantitative and qualitative variables. These two simple analyses (PCoA and HSA) were then coupled by a CIA to study a possible co-structure of each type of information. This analysis describes the common structure of both tables measured on the same statistical units (herein the genera). The mathematical model of CIA is given in Dolédec and Chessel (1994) and in Dray et al. (2003). Results of the CIA consist in two sets of scores (morphological in table $\mathbf{A} = [\mathbf{a}_1,\ldots,\mathbf{a}_p]$, and genetic in table $\mathbf{B} = [\mathbf{b}_1,\ldots,\mathbf{b}_p]$) of maximum covariance (i. e., maximization of product: $\mathrm{cov}(\mathbf{a},\mathbf{b}) = \mathrm{var}(\mathbf{a})^{1/2} \cdot \mathrm{var}(\mathbf{b})^{1/2} \cdot \mathrm{cor}(\mathbf{a},\mathbf{b})$). Thus, the CIA maximizes a compromise between the structure of morphological information ($\mathrm{var}(\mathbf{a})$), the structure of phylogenetic information ($\mathrm{var}(\mathbf{b})$), and their link ($\mathrm{cor}(\mathbf{a},\mathbf{b})$). To assess the significance of the CIA results, a Monte-Carlo permutation test was computed on the RV coefficient (Robert and Escoufier, 1976). This procedure tests the link between two tables by permuting simultaneously the rows of

both tables. Then, the common structure of the tables has been extracted by computing Euclidian distances between genera using the CIA scores of both tables. This new distance matrix was submitted to a hierarchical analysis using Fitch and Margoliash (FM) (1967) algorithm, resulting in a phylogram showing the relationships that are strictly congruent between both data sets. The FM phylogram was calculated using the global optimization criterion with negative branch lengths allowed, and 999 random permutations for the input order of taxa. The most external group within Loricariinae, according to the results of the phylogeny, was used to root the tree. Multivariate analyses were conducted using ADE–4 software (Thioulouse et al., 1997), and the phylogram was calculated with the Fitch module in PHYLIP 3.66 package (Felsenstein, 2004).

2.5 Identification of phylogenetically dependent variables.

Phylogenetically dependent variables are given by the CIA as those that show the strongest covariation with the phylogeny (i.e., variables with the most important absolute contributions, and the longest vectors when projected onto axes). In order to have an independent confirmation of the results obtained by our approach (CIA results), quantitative variables were submitted to the Test For Serial Independence (TFSI) (von Neumann et al., 1941), and qualitative variables to the RUNS test (Sokal and Rholf, 1995) following Abouheif's (1999) procedures, as implemented in Phylogenetic Independence version 2.0 (Reeve and Abouheif, 2003). These tests against phylogenetic autocorrelation allow to detect self-similarities among adjacent (ordered) observations. The computation of the statistics requires a topology and the value of a trait for the tips. An average statistics (C–mean for TFSI test, and Runs–mean for RUNS test) is calculated for a random representative sampling of all possible branch swapping. This average statistics (observed) was then compared to a null hypothesis sampling distribution of randomized average statistics obtained by calculating an average statistics on a representative sampling of all possible branch swapping on topologies obtained after randomly shuffling the tips of the original topology. The tree topology used here corresponds to the ML tree calculated from the molecular data set used for the CIA analysis. Average statistics were estimated after 10,000 random permutations of tips around nodes and compared to the randomized average statistics obtained after 10,000 random shuffling of tips.

2.6 Analysis of character evolution.

We first analyzed quantitative phylogenetically dependent variables by using a canonical procedure that allowed decomposition of their variance along the phylogenetic tree

(Ollier et al, 2006). Prior to the variance analysis, an orthonormal vectorial basis was constructed to represent the topology of the phylogenetic tree. This tree (root, nodes, and tips) was described by a set of ordered dummy variables corresponding to a tip or a node (and its descendant tips). These dummy variables were then orthonormalized to obtain the orthonormal basis. Vectors of this basis were linear combinations of the dummy variables ranked according to the initial ranking of the dummy variables. This allowed the interpretation of the successive vectors in terms of decreasing phylogenetic dissimilarities. Then, a linear regression was performed with the centered and standardized trait variable as response variable, and the orthonormal basis as explanatory variables. Regression coefficients allowed reconstructing the trait variable, and squared coefficients provided variance decomposition of the trait onto the orthonormal basis. The plotting of the squared coefficients and of the cumulative squared coefficients provides two graphical tools called orthogram and cumulative orthogram (Ollier et al., 2006). Four permutation procedures associated to orthograms are used to test the null hypothesis of phylogenetic independence. These procedures are based on different statistics and consider different alternative hypotheses. The R2Max statistics was used to test against the alternative hypothesis that one vector explained a significant part of the trait variance (punctual effect). SkR2k was used to test against the alternative hypothesis that vectors near the tips (or the root) explained a significant part of the trait variance. SkR2k is high when the trait variance was rather explained by last vectors (towards tips) and low when explained by first vectors (towards root). Dmax is a Kolmogorov-Smirnov-like statistic and was used to test if the vector of squared coefficients may be an ordered random sample of the uniform distribution on (0, 1). Dmax was used to test against the alternative hypothesis that some successive vectors explained a significant part of the trait variance. Finally, SCE is a measure of the average local variation of the orthogram and tests against the alternative hypothesis that there are significant differences in variance explained by vectors and their neighbors (precedent or subsequent). Distribution of the statistics under the null hypothesis and confidence limits of (cumulative) orthograms were built using 9999 random permutations of the trait values. Orthograms and associated tests (Ollier et al., 2006) were conducted using ade4 package (Chessel et al., 2004) in R 2.4.0 (Ihaka and Gentleman ,1996).

We then analyzed qualitative phylogenetically dependent variables using Maximum Likelihood ancestral state reconstruction as implemented in the Stochchar 1.1 package (Maddison and Maddison, 2006a), in Mesquite 1.12 (Maddison and Maddison, 2006b). This method estimates for each node the ancestral states that maximize the probability of observing

the different character states in the terminal taxa, given a stochastic model of evolution. We used the Lewis's (2001) Mk model which assumes equal rates of change from one state to another, for forward as well as backward rates. This global rate was directly estimated from the data, and scaled using our ML tree branch lengths.

3. Results

3.1 Phylogenetic analysis of the subfamily Loricariinae.

We sequenced the partial 12S and 16S mitochondrial genes of 20 Loricariinae species representing 14 genera. The sequence alignment included 1739 positions from which 238 corresponded to the 12S rRNA gene, 73 corresponded to the tRNA Val gene, and 1428 belonged to the 16S rRNA gene. The model GTR + G + I (Tavaré, 1986) fitted our data the best as indicated by Modeltest.

MP, ML and NJ analyses lead to comparable tree topologies. The MP tree (not shown) included 1572 steps (CI = 0.513; RI = 0.528). In the ML tree (-lnL = 9414.26784), shown in Fig. 1, the Loricariinae was split into two lineages: the Harttiini (Fig.1, clade 1), including the single genus *Harttia*, and the Loricariini (Fig.1, clade 2). The Loricariini was divided into two clades, the Sturisomina (new subtribe) (clade A), and the Loricariina (clade B). The genus *Lamontichthys* was the first diverging genus within the clade Sturisomina, a position strongly supported by bootstrap values (100/100/99). The remaining Sturisomina representatives were then split into two lineages, one comprising a species of *Farlowella* and the two representatives of *Sturisoma*, and a second comprising another species of *Farlowella* and *Sturisomatichthys*. However, the first group was not found in NJ and MP tree topologies, and the node giving *Farlowella platoryncha* as sister genus of *Sturisomatichthys* was weakly supported by the same two methods. The polyphyly of *Farlowella* was assessed by a one-tailed SH test with, as alternative topology, the enforced monophyly of *Farlowella* as sister group of *Sturisoma* and *Sturisomatichthys* (without hypotheses concerning their interrelationships). The result indicated that the monophyly of *Farlowella* was significantly rejected ($p = 0.0495$). Our phylogenetic reconstructions all showed the monophyly of the subtribe Loricariina yet only the ML analysis gave good bootstrap support. Within the Loricariina, *Metaloricaria* branched at the base of the clade. The sister group of *Metaloricaria* was strongly supported (100/99/100) with *Dasyloricaria* as sister genus of all remaining representatives of the subtribe. The sister group of *Dasyloricaria* was then split into two clades: the first corresponding to *Rineloricaria* representatives, and the second comprising the remaining genera studied herein. This last group contained two clades with on one hand

representatives of the *Loricariichthys* group, and on the other hand representatives of the *Loricaria* group plus *Crossoloricaria* and *Planiloricaria*, these last two genera belonging to the so called *Pseudohemiodon* group (*sensu* Covain and Fisch-Muller 2007). Within the *Loricariichthys* group, the nominal genus occupied a sister position to *Hemiodontichthys* and *Limatulichthys*. The NJ tree showed however an unresolved polytomy among these three genera. Within the *Loricaria-Pseudohemiodon* clade, all methods placed *Loricaria* representatives as the sister lineage to the *Pseudohemiodon* group.

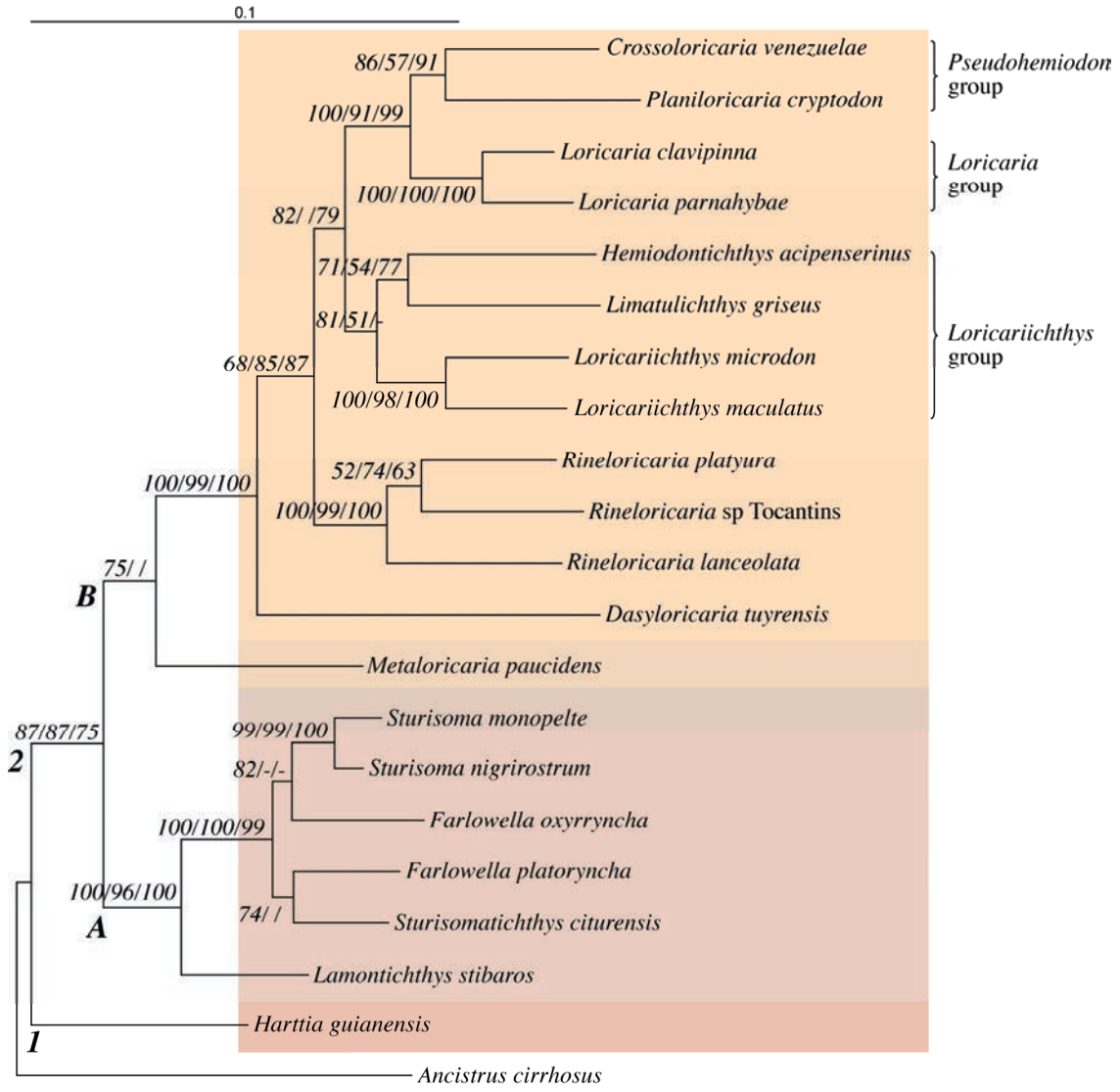


Fig. 1. Maximum likelihood tree of the Loricariinae including 14 genera and 20 species inferred from the analysis of partial 12S and 16S gene sequences (-lnL = 9414.26784). The best fit substitution model was GTR + G + I with the following parameter values: base frequencies: fA = 0.3686, fC = 0.2361, fG = 0.1856, fT = 0.2096; substitutions rates [A ↔ G] = 11.5316, [C ↔ T] = 37.5424, [A ↔ C] = 4.5376, [A ↔ T] = 4.8089, [C ↔ G] = 0.0089, [G ↔ T] = 1; proportion of invariable sites I = 0.4527; gamma shape parameter: α = 0.6563. Numbers above branches indicate bootstrap supports above 50 for ML, MP, and NJ trees respectively. Sign (-) indicates that the node was not found in some topologies. 1: Harttiini, 2: Loricariini, A: Sturisomina, B: Loricariina. Scale indicates the number of substitution per site as expected by the model.

3.2 Co-structure analysis of molecular and morphological data.

In order to highlight the co-structure of the morphological data as compared to the molecular data, the CIA was performed on the restricted data sets comprising the same taxonomic sampling. The new genetic distance matrix was calculated using a re-estimated model of substitutions which characteristics were: GTR + G + I: base frequencies: fA = 0.3563, fC = 0.2376, fG = 0.1970, fT = 0.2091; substitutions rates [A ↔ G] = 11.7966, [C ↔ T] = 42.5481, [A ↔ C] = 5.7480, [A ↔ T] = 4.6241, [C ↔ G] = 0.0714, [G ↔ T] = 1; proportion of invariable sites I = 0.4521; gamma shape parameter: α = 0.5508. A first assessment of the relationships between morphology and genetics was performed using the RV coefficient, and showed a strong and significant correlation between both data sets ($p<0.0001$; RV = 0.832). The projection of inertia axes of the PCoA of the genetic data and of the HSA of the morphological data onto co-inertia axes (Fig. 2c) placed plan 1–3 of the genetic data analysis in relation to plan 1–2 of the morphological data analysis. Thus, CIA found that both axes 1 were associated, and that axis 3 of the genetic data was associated to axis 2 of the morphological data. The first plan of CIA accounted for 85.84 % of the total co-structure (78.47 % for axis 1 and 7.37 % for axis 2) (Fig. 2d). CIA characteristics are given in Table 2. Covariance associated to the first axis was almost four times greater than the one associated to other axes. Co-inertia plan 1–2 was of the same quality than plans 1–3 and 1–2 of the initial analyses. The inertia projected onto co-inertia axes was equivalent to the one projected onto inertia axes of the initial analyses: 99.05 % (0.004643/0.004599) of the genetic data structure and 97.96 % (0.487/0.4971) of the morphological data structure was recovered by axis 1 of the co-structure analysis. Correlations between both data sets were also very high (more than 0.97 on the first co-inertia axis and 0.92 on the second one). Axis 1 of co-inertia analysis defined the tribal rank of the subfamily and split Harttiini, Sturisomina, and *Metaloricaria* on one hand and Loricariina on the other hand. Axis 2 defined the generic rank and ordered the genera according to their morphological and genetic proximity. The projection of morphological and genetic data coordinates onto co-inertia axes is given in Fig. 2. Superimposition of both sets of coordinates, after normalization for scaling, (Fig. 2a) allowed to display the most important differences between genetic (origin of arrows) and morphological data (extremity of arrows). These differences mainly concerned the generic rank (axis 2) and particularly genera *Planilocaria*, *Dasyloricaria*, and *Metaloricaria* among Loricariini, and *Harttia* concerning Harttiini. The co-structure highlighted concerned thereby the tribal rank and the grouping of genera in some groups (morphological and genetic) which were Sturisomina and the *Loricaria-Pseudohemiodon* group. The position of

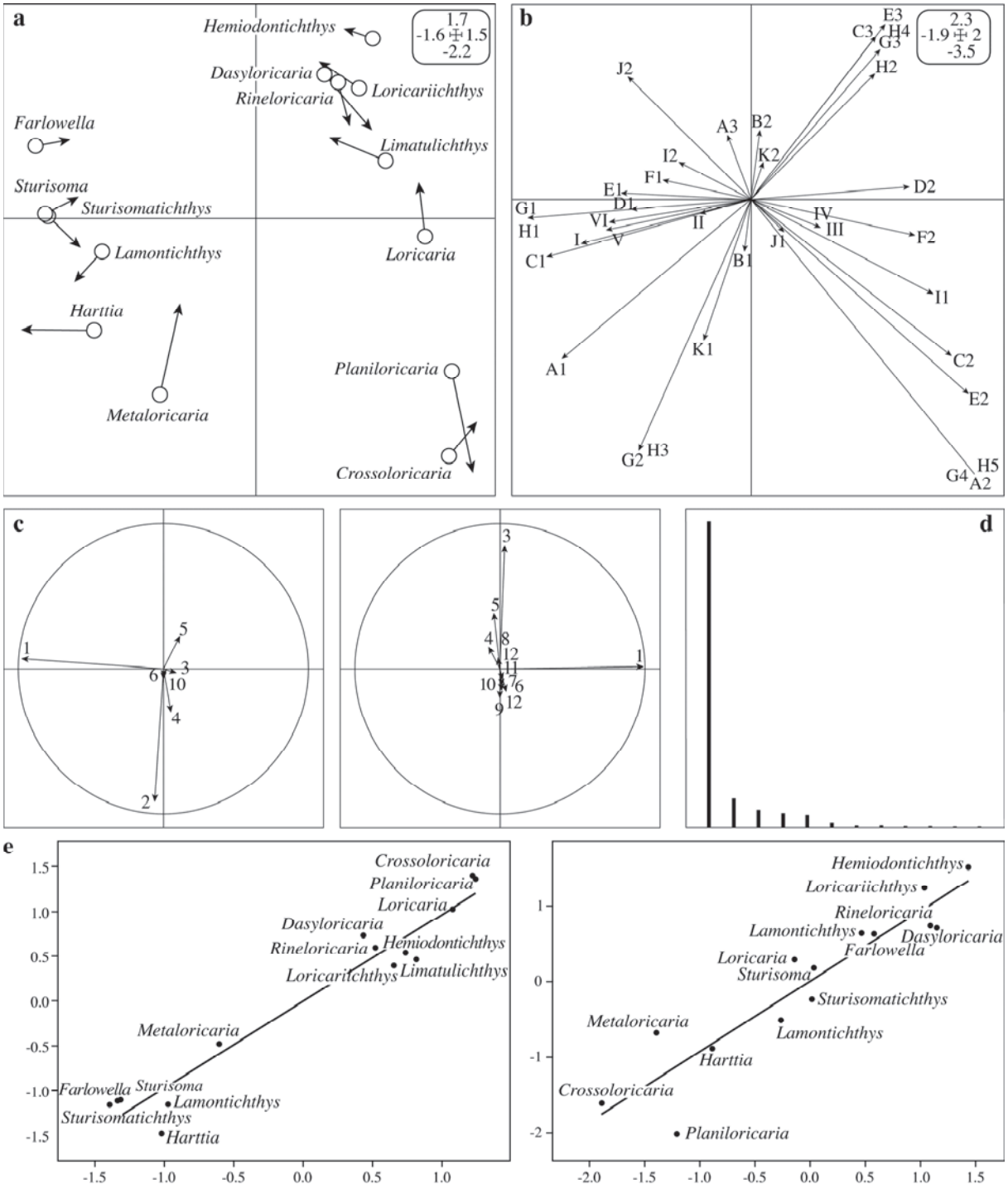


Fig. 2. Co-inertia analysis. Projection of data coordinates of preliminary analyses (PCoA of genetic data and HSA of morphological data) onto axes 1-2 of the co-inertia analysis. a: normalized individuals' scores in the co-inertia plan: genetic data (origin of arrows) and morphological data (extremity of arrows). b: coordinates of morphological variables in the co-inertia plan (numbered as in Table 3). c: projection of inertia axes of simple analyses onto co-inertia axes: inertia axes of PCoA of genetic data (left); inertia axes of HSA of morphological data (right). d: eigenvalues of co-inertia analysis. e: bivariate plots of correlations of normalized individuals' scores (genetic data in abscise and morphological data in ordinate) for the first (left) and second (right) co-inertia axes.

Table 2. Main characteristics of co-inertia analysis.

Co-inertia axes	Covariance	Variance 1	Variance 2	Correlation	Inertia 1	Inertia 2
1	0.04601	0.004599	0.487	0.9722	0.004643	0.4971
2	0.01419	0.0009029	0.2549	0.9295	0.001139	0.2752

Covariance: covariance between both systems of coordinates of co-inertia analysis (maximized by the analysis).
Variance 1: inertia of the genetic data projected onto co-inertia axes.
Variance 2: inertia of the morphological data projected onto co-inertia axes.
Correlation: correlation between both systems of coordinates of co-inertia analysis.
Inertia 1: maximum inertia projected onto axes of the simple analysis of genetic data (eigenvalues of PCoA).
Inertia 2: maximum inertia projected onto axes of the simple analysis of morphological data (eigenvalues of HSA).

Hemiodontichthys was also consistent between both representations, whereas *Metaloricaria* was placed together with Harttiini and Sturisomina. In the same manner, Sturisomina was grouped with Harttiini. The morphological variables involved the most in the co-structure were identified by the projection of the variables onto the first co-inertia plan (Fig. 2b) and by the inertia analysis. Absolute contributions of the variables to the axes are given in Table 3. Concerning axis 1 (tribal rank), these variables corresponded, in decreasing order, to: mouth and tooth shapes (variables G and H which contributed to 12.38 % of the explained inertia by this axis), the absence or presence of deep or weak postorbital notches (variable C, 12.04 % of the explained inertia), the number of caudal-fin rays (variable I, 10.72 % of the explained inertia), the lip structure (variable E, 8.9 % of the explained inertia), the number of premaxillary and dentary teeth (variables V and VI, respectively 7.84 % and 7.49 % of the explained inertia), the presence or absence of predorsal keels (variable D, 7.16 % of the explained inertia), the presence or absence of fringed barbels (variable F, 5.63 % of the explained inertia), and the characteristics of the maxillary barbels (variable I, 4.95 % of the explained inertia). Concerning axis 2 (generic rank), the strongest contributions were registered for: the tooth and mouth shape (variables H and G which contributed respectively to 21.16 % and 20.94 % of the explained inertia by this axis), the absence or presence of deep or weak postorbital notches (variable C, 13.47 % of the explained inertia), the absence or presence of a complete or incomplete abdominal cover (variable A, 13.39 % of the explained inertia), and the lip's structure (variable E, 12.13 % of the explained inertia). Bivariate plots of the individuals' normalized scores concerning co-inertia axes 1 and 2 (Fig. 2e) showed a better ordination of the genera along first axis, knowing the phylogenetic tree topology. Along axis 2, representatives of the *Pseudohemiodon* group were indeed grouped with Harttiini and *Metaloricaria*, whereas *Loricaria* was placed among Sturisomina.

Table 3. Main characteristics of variables tested for phylogenetic dependence. Variables are titled as in Covain and Fish-Muller (2007), and are ordered according to their absolute contributions to the first co-inertia axis.

	I	V	VI	III	IV	II	G	H	C	E	D	F	I	A	J	K	B
Absolute contibution to co-inertia axis 1 (in %)	10.72	7.84	7.49	1.90	1.90	0.93	12.38	12.38	12.04	8.90	7.16	5.63	4.95	3.83	1.54	0.21	0.01
Absolute contibution to co-inertia axis 2 (in %)	1.60	0.74	0.40	0.68	0.68	0.14	20.94	21.16	13.47	12.13	0.09	0.59	2.92	13.39	3.37	4.50	3.02
TFSI test (C-mean)	0.7103	0.5606	0.6086	-0.0411	-0.043	0.0554	-	-	-	-	-	-	-	-	-	-	-
P value (X ≤ X obs.)	0.9998	0.9995	0.9991	0.6205	0.5708	0.8028	-	-	-	-	-	-	-	-	-	-	-
P value (X ≥ X obs.)	**0.0003**	**0.0006**	**0.0010**	0.3796	0.4293	0.1973	-	-	-	-	-	-	-	-	-	-	-
RUNS test (Runs-mean)	-	-	-	-	-	-	5.3745	6.627	6.3082	3.9297	5.4107	4.3323	4.3323	3.9671	5.7954	5.2008	9.5661
P value (X ≤ X obs.)	-	-	-	-	-	-	**0.0002**	**0.0002**	**0.0017**	**0.0001**	**0.0318**	**0.0243**	**0.0243**	**0.0063**	0.4931	0.2447	0.9529
P value (X ≥ X obs.)	-	-	-	-	-	-	0.9999	0.9999	0.9984	1	0.9683	0.9758	0.9758	0.9938	0.5070	0.7554	0.0472
R2Max test	0.6851	0.5166	0.5082	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P value (X ≤ X obs.)	0.9995	0.8209	0.8525	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P value (X ≥ X obs.)	**0.0016**	0.2029	0.2016	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SkR2k test	2.5857	2.2934	2.6229	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P value (X ≤ X obs.)	**0.0007**	**0.0001**	**0.0005**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P value (X ≥ X obs.)	0.9996	1	0.9997	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dmax test	0.7002	0.7582	0.7225	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P value (X ≤ X obs.)	0.9995	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P value (X ≥ X obs.)	**0.001**	**0.0001**	**0.0002**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCE test	2.2602	2.4662	2.1879	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P value (X ≤ X obs.)	0.9996	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P value (X ≥ X obs.)	**0.0007**	**0.0001**	**0.0001**	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Quantitative variables I to VI. I: number of caudal-fin rays (including spines); II: number of pectoral-fin rays (including spine); III: number of pelvic-fin rays (including spine); IV: number of dorsal-fin rays (including spine); V: number of premaxillary teeth; VI: number of dentary teeth.

Qualitative variables A to K. A: abdominal cover with three modalities: 1 = absent, 2 = present incomplete, 3 = present complete; B: secondary organization in the abdominal cover with two modalities: 1 = absent, 2 = present; C: postorbital notches with three modalities: 1 = absent, 2 = present weak, 3 = present deep; D: predorsal keels with two modalities: 1 = absent, 2 = present; E: lips structure with three modalities: 1 = papillose, 2 = filamentous, 3 = rather smooth; F: fringed barbels with two modalities: 1 = absent, 2 = present; G: mouth shape with four modalities: 1 = elliptical, 2 = horse shoe like, 3 = bilobate, 4 = bilobate with trapezoidal opening; H: tooth shape with five modalities: 1 = pedunculated, 2 = straight bicuspid, 3 = pedunculated size reduced, 4 = straight bicuspid size reduced, 5 = spoon shaped size reduced; I: maxillary barbels with two modalities: 1 = conspicuous, 2 = inconspicuous; J: rostrum with two modalities: 1 = absent, 2 = present; K: snout shape with two modalities: 1 = rounded, 2 = pointed.

Absolute contribution to co-inertia axis: contribution of each variable to the total inertia explained by the axis.

TFSI and RUNS tests: tests against phylogenetic autocorrelation respectively for quantitative and qualitative variables as defined by Abouheif (1999).

R2Max, SkR2k, Dmax, and SCE tests: tests against phylogenetic dependence as defined by Ollier et al. (2006).

Bold types indicate significant tests for $\alpha = 5\%$.

A part of the incongruent information (background noise) was thus integrated on axis 2 and following ones, and these axes were consequently discarded for the calculation of the phylogram depicting the amount of phylogenetic information strictly congruent between the morphological and the genetic data sets. This strict congruence phylogram was thus reconstructed by taking, for each genus, the scores of the morphological and genetic data only on the first CIA axis to compute dissimilarities between individuals. *Harttia* was used as the rooting group according to previous results. The tree that best fit the distance matrix (Fig. 3) showed a topology comparable to the one of the ML tree. The first difference was that Sturisomina was partly retrieved by grouping *Sturisoma*, *Sturisomatichthys*, and *Farlowella* but not *Lamontichthys*. The relationships within Sturisomina stayed unresolved because of contradictions between genetics and morphology. The second difference lied within the Loricariina where *Rineloricaria*, *Dasyloricaria*, the *Loricariichthys* group and the *Loricaria* + *Pseudohemiodon* groups were all retrieved but with unresolved interrelationships. The last difference was the polytomy within the *Loricariichthys* group due to conflicting information between morphological and genetic data.

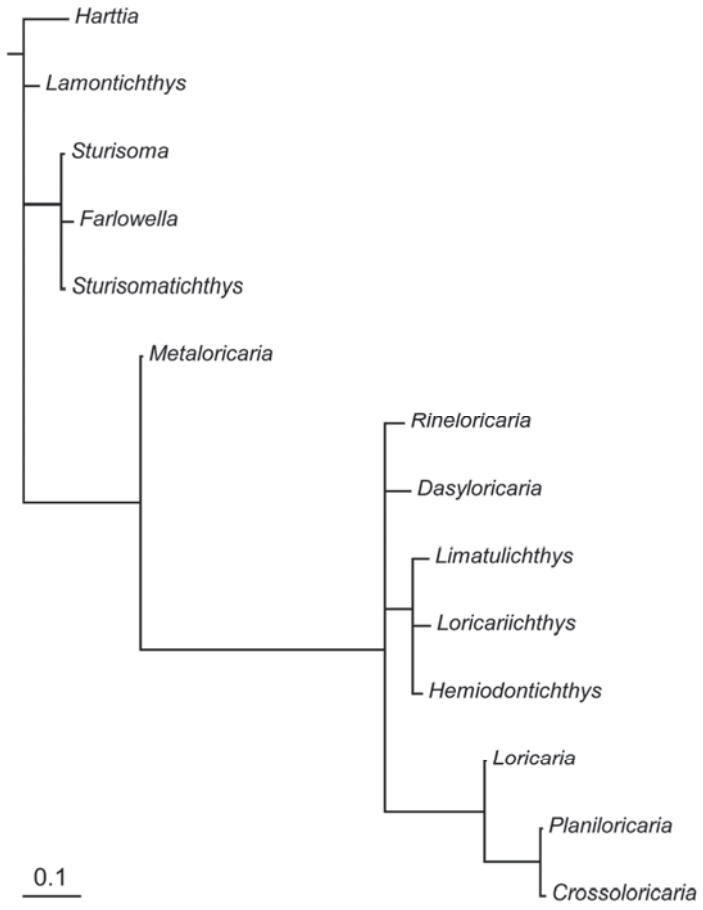
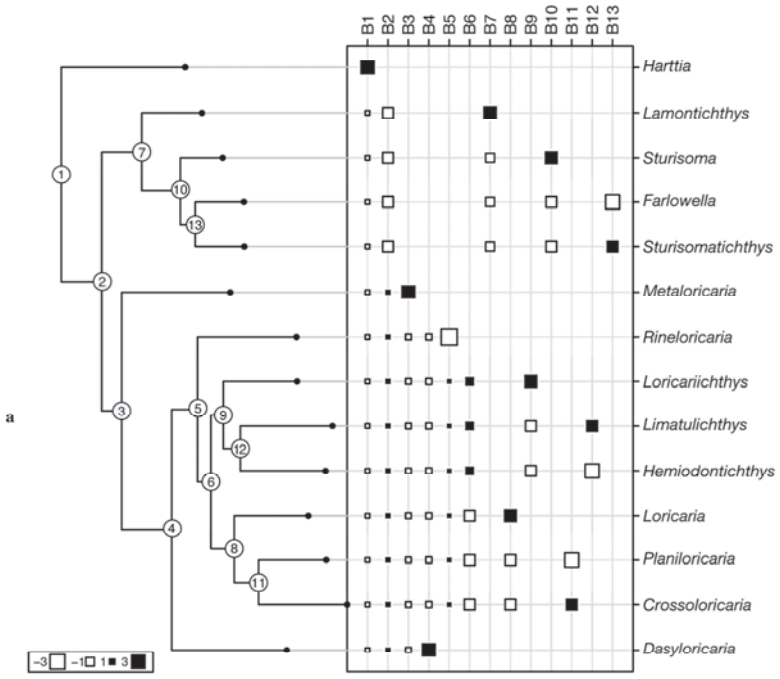


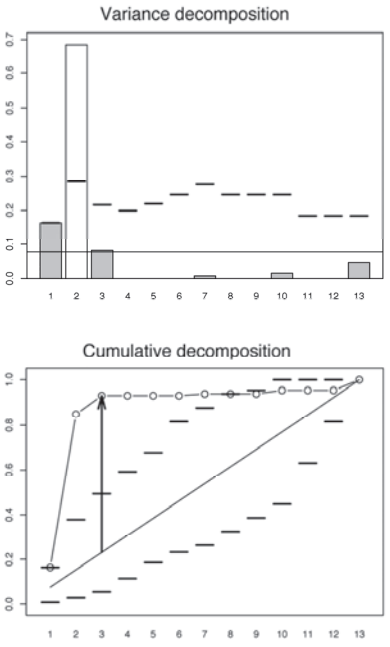
Fig. 3. Strict congruence phylogram computed from individuals' scores on the first co-inertia axis of the morphological and genetic data using Fitch and Margoliash algorithm. Sum of squares = 0.36173, average percent standard deviation (APSD) = 4.48288. Scale indicates the quantity of information computed from the morphological and genetic data sets.

3.3 Identification of morphological phylogenetically dependent variables.

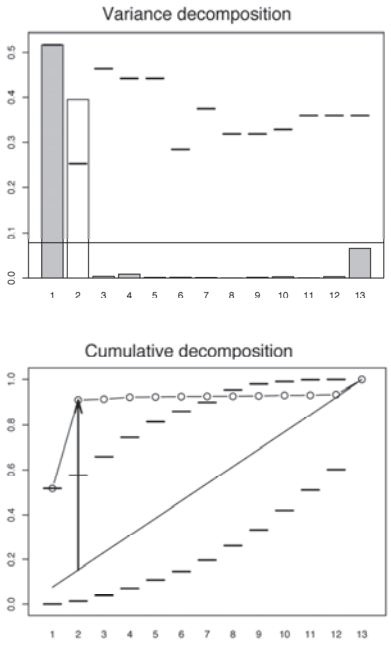
The quality of the obtained strict congruence phylogram allowed the recognition of several morphological groups that were congruent with the molecular phylogeny, and highlighted the level of resolution reached by the morphological variables to describe these groups from a phylogenetic point of view. The variables involved in the characterization of these groups were then tested for phylogenetic dependence following our new approach .The CIA results are summarized in the first two lines of Table 3. The contributions of quantitative variables to the first axis ranged from 10.72 to 0.93%, while quantitative variables ranged from 12.38 to 0.01 %. On axis 2, the absolute contributions of quantitative variables were small (1.6 to 0.14%), while qualitative variables showed generally high contributions (21.16 to 0.09%). These results were compared to the outputs of the TFSI tests (Table 3) which identified three quantitative variables to be strongly positively autocorrelated with the phylogeny: (1) the number of caudal-fin rays (I), (2) the numbers of premaxillary (V) and (3) dentary teeth (VI). These three variables also showed the strongest contributions to co-inertia axis 1, ranging from 10.72 % (I) to 7.49 % (VI). On axis 2, all quantitative variables were weakly informative. The CIA results were then compared to the outputs of the RUNS tests conducted on qualitative variables (Table 3) which showed a significant autocorrelation to the phylogeny for the following characters: abdominal cover present or absent (A), postorbital notches shape (C), predorsal keels present or absent (D), lip structure (E), fringed barbels present or absent (F), mouth shape (G), the tooth shape (H), and maxillary barbel length (I). The null hypothesis of absence of phylogenetic autocorrelation was consequently rejected for all these variables. To the contrary, absence of phylogenetic autocorrelation was not significantly rejected for the following variables: secondary organization in the abdominal plating (B), rostrum present or absent (J), and snout shape (K). All phylogenetically autocorrelated variables possessed the strongest contributions to axis 1, ranging from 12.38 % (G, H) to 3.83 % (A). On axis 2, phylogenetically autocorrelated variables such as predorsal keels present or absent (D), and fringed barbels present or absent (F) appeared weakly informative (0.09 % and 0.59 % respectively), whereas uninformative variables such as rostrum present or absent (J) and the snout shape (K) played a more important role on the axis, contributing respectively to 3.37 % and 4.5 %. This means that one part of the background noise was integrated on axis 2, and provided an *a posteriori* justification for the rejection of axis 2 and next ones in the calculation of the strict congruence phylogram. In summary, the variables that contributed more than 3.83 % to the co-inertia axis 1 were significantly correlated to the phylogeny according to TFSI and RUNS results.



b – NUMBER OF CAUDAL-FIN RAYS (I)



c – NUMBER OF PREMAXILLARY TEETH (V)



d – NUMBER OF DENTARY TEETH (VI)

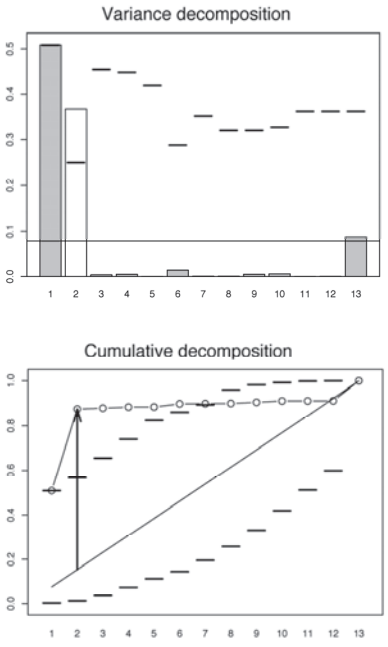


Fig. 4. Variance decomposition of three quantitative morphological traits across the orthonormal basis defined by the phylogenetic tree topology. a: Phylogenetic tree (left) and description of the topology of the tree by the orthonormal vectors B1 to B13 which represent nodes and descendent tips (right). Node numbering in the phylogenetic tree (1 to 13) indicates the number of the vector (B1 to B13) that accounts for the variance associated to the node. The indicative scale show squares with sizes proportional to the values of the orthonormal vectors (white and black for negative and positive values, respectively). b: Variance decomposition of the number of caudal-fin rays (I) using the orthogram plot (upper panel) and the cumulative orthogram plot (lower panel). c: Variance decomposition of the number of premaxillary teeth (V) using the orthogram plot (upper panel) and the cumulative orthogram plot (lower panel). d: Variance decomposition of the number of dentary teeth (VI) using the orthogram plot (upper panel) and the cumulative orthogram plot (lower panel). In the orthogram plots, the abscise gives the number of the vectors associated to nodes while the ordinate shows the contribution of the vector to the variance of the trait given by the squared regression coefficient (white and grey for positive and negative coefficients, respectively); dashes correspond to the upper confidence limit at 5 % deduced from 9999 Monte Carlo permutations; solid line represents the mean value. In the cumulative orthogram plots the ordinate shows the cumulated contribution of successive vectors to the variance; circles represent the observed value of cumulated squared regression coefficients; solid diagonal line represents expected value under absence of phylogenetic dependence; dashes correspond to the bilateral 95% confidence interval. Vertical arrow indicates the position of maximum deviation from the expected value (diagonal line).

3.4 Evolutionary analysis of phylogenetically dependent morphological variables

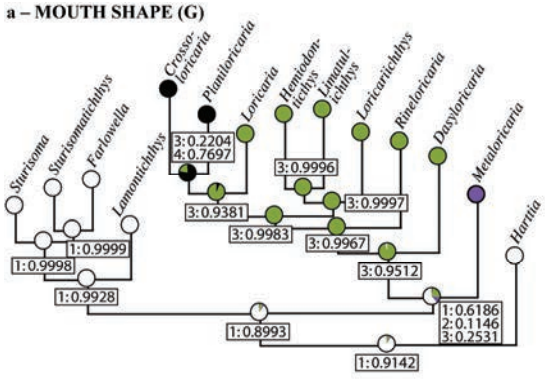
Quantitative variables I, V, and VI (Table 3) were analyzed using the orthogram approach (Fig. 4). The tree topology together with the vectorial basis (Fig. 4a) allowed the identification of the ranking of the nodes, and consequently to see which vector accounted for which node. The orthogram of the first quantitative variable analyzed, the number of caudal-fin rays (I) (Fig. 4b top), indicated that vector 2 explained the greatest part of the variance. This vector showed a strong departure from the expected value under the hypothesis of absence of phylogenetic dependence (given by the solid line in Fig. 4b top), and peaked outside of the confidence limit (given by the dashes). The cumulative orthogram (Fig. 4b down) confirmed predominance of vector 2 in the variance distribution. A significant departure from H0 was registered for this vector, and this pattern was preserved for several successive vectors. The maximum deviation from the expected value was given for the sum of the three first vectors (vertical arrow in Fig. 4b down) meaning that maximum variation was registered on these three vectors. All four statistical tests were also significant, particularly R2Max (Table 3; $p(X \geq X_{obs}) = 0.0016$), indicating that a single punctual modification of the trait (number of caudal fin rays) occurred at a particular node and that it stayed unchanged afterwards. Moreover, the variance distribution was rather skewed towards the root (Table 3; SkR2k: $p(X \leq X_{obs}) = 0.0007$), indicating that the deepest nodes of the phylogeny explained the variance distribution. These results suggested that this trait has been shaped deep in the phylogeny. In summary, a single major punctual event occurred at node 2, between

Sturisomina and Loricariina lineages, with a reduction of the number of caudal-fin rays in Loricariina.

In the second and third quantitative traits analyzed, the numbers of premaxillary (V) and dentary (VI) teeth, variance decomposition showed similar patterns. The orthogram plot (Figs. 4C and 4D up) pointed vectors 1 and 2 as explaining the major part of the variance distribution. Cumulative orthograms (Figs. 4C and 4D down) confirmed this fact with a maximum departure from the expected value under absence of phylogenetic dependence registered for the sum of two first vectors (arrow on vector 2). Out of the four statistics tested (Table 3), only R2Max was not significant meaning that a rather gradual effect was responsible of the variance distribution. Moreover, this distribution was skewed towards the root (Table 3, SkR2k: $p(X \leq X_{obs}) = 0.0001$ and $p(X \leq X_{obs}) = 0.0002$ for numbers of premaxillary and dentary teeth, respectively). Consequently, these two traits have been also shaped rather deep in the phylogeny. Two major successive events can be reconstructed in the overall gradual trend: a first decrease in the number of premaxillary and dentary teeth between Harttiini and Loricariini lineages (Fig 4a, node 1), and a second decrease between Sturisomina and Loricariina lineages (Fig 4a, node 2).

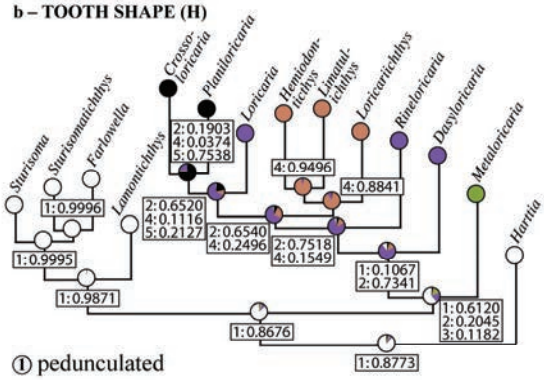
Qualitative variables A, C, D, E, F, G, H, and I were analyzed using Maximum Likelihood ancestral state reconstruction (Fig. 5). The mouth shape (Fig. 5a) evolved from circular in Harttiini and Sturisomina, to bilobate in all Loricariina except *Metaloricaria* which displays a horse shoe like mouth. Therefore, the ancestral state reconstruction showed an unclear state at the root of Loricariina, with a slight preference for the elliptical state ($p_{G1} = 0.6186$). A second step in the specialization of the mouth in Loricariina occurred in the *Pseudohemiodon* group which displays a bilobate mouth but with a trapezoidal opening. The tooth shape (Fig. 5b) showed a similar pattern of evolution than the mouth shape. Tooth evolved from pedunculated in Harttiini and Sturisomina to more specialized in Loricariina. A first step occurred at the basal diversification of the Loricariina where the teeth evolved from an ancestor possessing more probably pedunculated teeth ($p_{H1} = 0.6120$), to teeth pedunculated yet reduced in size in *Metaloricaria*, and straight and bicuspid in the sister lineage. In this last lineage, two other modifications occurred later on: a reduction in size in the *Loricariichthys* group and a change towards spoon shaped teeth reduced in size in the *Pseudohemiodon* group. The postorbital notches (Fig. 5c) appeared in the ancestor of the Loricariina. This feature regressed two times toward weak postorbital notches: a first time in *Limatulichthys*, and a second time in the *Pseudohemiodon* group. The lip structure (Fig. 5d) evolved from papillose in Harttiini, Sturisomina, and basal Loricariina, to rather smooth in the

a – MOUTH SHAPE (G)



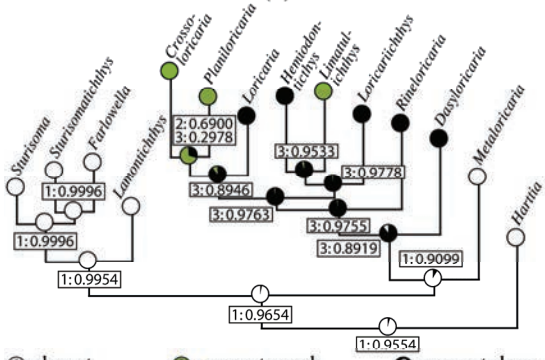
① elliptical ② horse shoe like ③ bilobate
④ bilobate with trapezoïdal opening

b – TOOTH SHAPE (H)



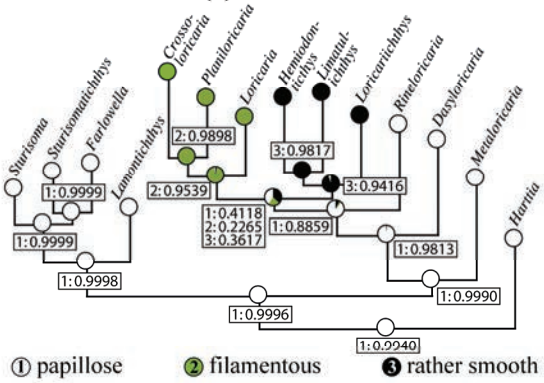
① pedunculated
③ pedunculated size reduced ② straight bicuspid
④ straight bicuspid size reduced ⑤ spoon shape size reduced

c – POSTORBITAL NOTCHES (C)



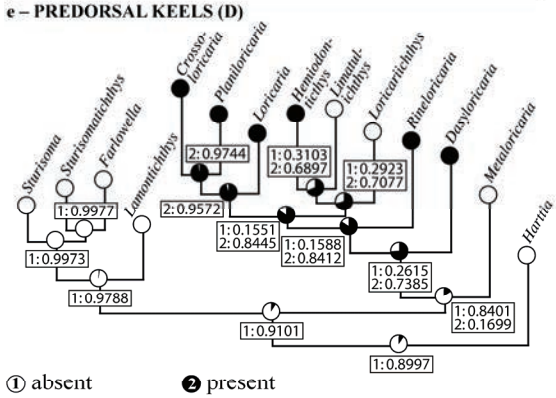
① absent ② present weak ③ present deep

d – LIPS STRUCTURE (E)



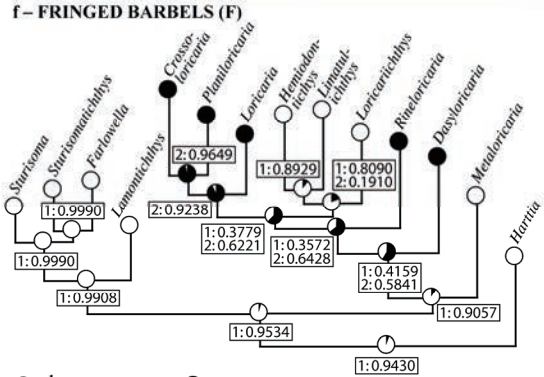
① papillose ② filamentous ③ rather smooth

e – PREDORSAL KEELS (D)



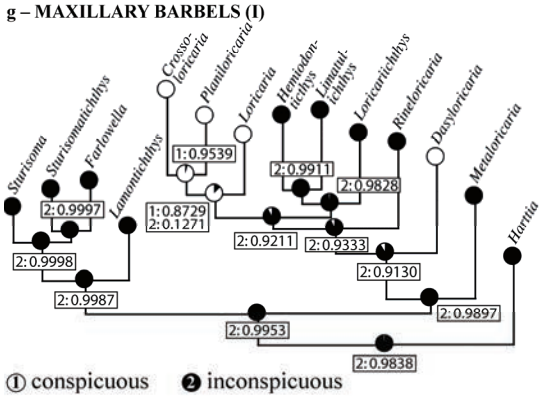
① absent ② present

f – FRINGED BARBELS (F)



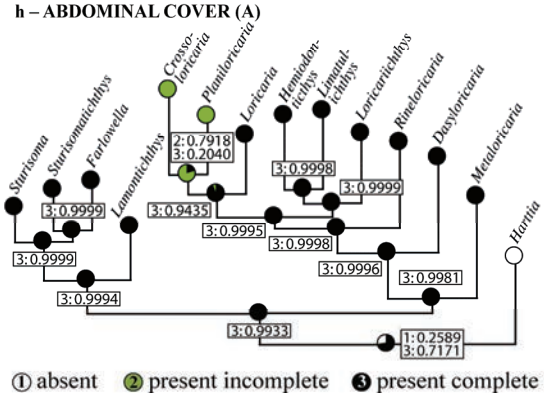
① absent ② present

g – MAXILLARY BARBELS (I)



① conspicuous ② inconspicuous

h – ABDOMINAL COVER (A)



① absent ② present incomplete ③ present complete

Fig. 5. Maximum likelihood ancestral state reconstructions of eight qualitative life-history traits along the phylogenetic tree using a single forward-backward rate (mK) model. Traits are ordered according to their absolute contribution to co-inertia axis 1. a: ancestral state reconstruction of the mouth shape with four modalities (G): estimated rate of change 1.981274554, -log L = 13.32290011; b: ancestral state reconstruction of the tooth shape with five modalities (H): estimated rate of change = 2.431420956, -log L = 18.364202556; c: ancestral state reconstruction of the postorbital notches with three modalities (C): estimated rate of change = 3.33717473, -log L = 12.268601550; d: ancestral state reconstruction of the lips structure with three modalities (E): estimated rate of change = 1.84519947, -log L = 10.47907210; e: ancestral state reconstruction of the predorsal keels with two modalities (D): estimated rate of change = 7.174725755, -log L = 8.39048636; f: ancestral state reconstruction of the fringed barbels with two modalities (F): estimated rate of change = 5.604381355, -log L = 8.07064024; g: ancestral state reconstruction of the maxillary barbels with two modalities (I): estimated rate of change = 4.041408096, -log L = 7.524279656; h: ancestral state reconstruction of the abdominal cover with three modalities (A): estimated rate of change = 1.88539421, -log L = 9.22821077. Boxes indicate the marginal probabilities of the most probable states. Likelihoods are reported as proportional likelihoods.

Loricariichthys group, and filamentous in the *Loricaria* and *Pseudohemiodon* groups. The sudden diversification of the lip structure made it difficult to reconstruct the ancestral state at the origin of this diversification (Fig. 5d, $p_{E1} = 0.4118$, $p_{E2} = 0.2265$, $p_{E3} = 0.3617$). Predorsal keels (Fig. 5e) appeared most probably in the ancestor of the Loricariina lineage not comprising *Metaloricaria* ($p_{D1} = 0.8401$). Thereafter, this feature regressed in several representatives of the *Loricariichthys* group such as *Loricariichthys* and *Limatulichthys*. Fringed barbels (Fig. 5f) are present only in some members of the Loricariina, yet the first appearance of this feature was difficult to assess and consequently none of the deeper ancestral nodes within this tribe displayed a clear state assignment. It seemed however clear that this feature regressed in representatives of the *Loricariichthys* group while it has never been present in *Metaloricaria.* The maxillary barbels (Fig. 5g) evolved from inconspicuous to conspicuous in two Loricariina lineages: the *Loricaria* and *Pseudohemiodon* groups. The abdominal cover (Fig. 5h) is absent in the species representing Harttiini and present in extant Loricariini, making it difficult to assess the state of the ancestor, yet the Maximum Likelihood ancestral state reconstruction method slightly favors the presence of an abdominal cover ($p_{A3} = 0.7171$). Latter on, this character evolved from a complete abdominal cover to an incomplete cover in the *Pseudohemiodon* group.

4. Discussion

In this work, we were interested in reconstructing the evolutionary history of the Loricariinae, a highly specialized group of neotropical catfishes, and in deciphering the evolution of their morphological traits. For this purpose, we used a new approach to detect phylogenetic dependence of character variations to the phylogeny, which is a prerequisite for a sensible evolutionary analysis of characters. Our approach using the CIA has the advantage

over existing methods to treat the full morphological data set at once, including qualitative and quantitative variables. The CIA offers a graphical output which allows a detailed analysis of the contribution of individual variables to the overall trend, within the frame of the phylogenetic tree. Contradictions and congruencies between both data sets are highlighted on the factorial map of individuals (Fig. 2a) by the relative position of both systems of coordinates (genetic and morphological) onto co-inertia axes. Incongruence between both data sets is given by the size of arrows representing the differences between genetics and morphology. Longer arrows, or origin of arrows in positive values and extremity in negative values imply strong contradictions between both data sets. In our case, no strong contradiction was highlighted by the CIA. The factorial map of variables (Fig. 2b), reveals the contribution of each variable to the co-structure, and identifies the groups defined by these variables. The graph of eigenvalues (Fig. 2d) identifies the axis explaining the major part of the congruent information between both data sets. Thus, the CIA provides an ordination of the variables according to their contribution to the co-inertia axes and by this mean allows the identification of phylogenetically dependent variables as well as the identification of the axes containing phylogenetic "noise" which are then discarded from the calculation of the strict congruence phylogram. The CIA approach has also the advantage of having no theoretical limitations and can be generalized to K tables displaying the same taxonomical sampling. These data can be of many different types (genetic, morphological, ethological, geographical, ecological…). The robustness of the CIA approach was assessed by comparing the level of correlation to the phylogeny as obtained by this method and the p-values obtained by classical tests, namely the TFSI test for quantitative variables, and the RUNS test for qualitative variables (Abouheif, 1999). The results of the comparisons (Table 3) show a strict correspondence between our approach and Abouheif's tests for assessing phylogenetic dependence and in this way we have shown that variables contributing more than 3.83 % to the co-inertia axis 1 were significantly correlated to the phylogeny.

In order to study the morphological evolution of the Loricariinae catfishes, we first inferred the phylogeny of the subfamily using 12S and 16S mitochondrial genes. The results show that Harttiini *sensu* Rapp Py-Daniel (1997) is not a monophyletic assemblage due to the scattered positions of its representatives in the phylogenetic tree, with a basal position of *Harttia* (type genus) as the sister group to all other Loricariinae analyzed. This corroborates the findings of Montoya-Burgos *et al.* (1998) who recovered this topology with a more restricted Loricariinae sampling. According to our results, we propose that the Harttiini should be restricted to the single genus *Harttia*. In the phylogenetic tree, the Loricariini *sensu*

Isbrücker (1979) was not retrieved. We thus redefine the Loricariini as the clade comprising two sister subtribes, (1) the Loricariina including all former Loricariini *sensu* Isbrücker (1979), and (2) a new monophyletic subtribe named Sturisomina, from the name of the first described genus of this group. Sturisomina includes at its base *Lamontichthys* as sister genus of *Farlowella*, *Sturisoma*, and *Sturisomatichthys* whose relationships still deserve further investigations. The paraphyly of *Farlowella*, even though surprising, is supported by the significant rejection of the constrained monophyly of the genus as assessed using the SH-test. A larger taxonomic sampling remains however necessary to definitely answer this question.

The relative position of *Metaloricaria* at the base of the Loricariina clade, is not consistent with the classification of Isbrücker (1979) who assigned it to the Harttiini tribe, and Metaloricariina subtribe. The position of *Metaloricaria* in our trees is poorly supported by bootstrap values for MP and NJ analyses, and should therefore be considered cautiously. However, the topology agrees with the hypothesis of Rapp Py-Daniel (1997) who suggested a placement within Loricariini (*sensu* Isbrücker 1979). Herein, the Loricariina constitutes the sister group of Sturisomina. Within the Loricariina, *Dasyloricaria* occupies a basal position, just after *Metaloricaria*, while *Rineloricaria* has a derived position relative to *Dasyloricaria* and constitutes the sister group to all other Loricariina. This topological situation renders the Rineloricariina subtribe proposed by Isbrücker, 1979 paraphyletic. Indeed, this subtribe comprised *Dasyloricaria, Rineloricaria*, *Ixinandria*, and *Spatuloricaria*, a grouping which is incompatible with our results. In addition, this subtribe was already questioned by Rapp Py-Daniel (1997) who found a paraphyly between *Spatuloricaria* and *Rineloricaria*. Here, the *Loricariichthys* group constitutes the sister clade of *Loricaria* plus the *Pseudohemiodon* groups. On the basis of the present taxonomic sampling, *Loricaria* is the sister clade of the *Pseudohemiodon* group represented here by *Crossoloricaria* and *Planiloricaria*. This agrees with Rapp Py-Daniel's (1997) results who found *Loricaria* branching at the base of the Planiloricariina (comprising *Planiloricaria* and *Crossoloricaria* among others). Nevertheless, these relationships deserve and wider taxonomic sampling for being confidently supported.

An overview of the morphological groups recently proposed by Covain and Fisch-Muller (2007) and the molecular phylogenetic results obtained herein, suggested that common information was shared between both data types. A strong correlation was indeed observed (RV = 0.832). This analysis suggested that several morphological groups were not obtained by chance or by character convergence, but followed a phylogenetic classification. The amount of congruent information between both data sets is in fact significant as summarized is the strict congruence phylogram (Fig. 3). This phylogram based on the co-structure analysis

confirmed the natural status of several morphological groups like the Harttiini and, among Loricariini: Loricariina (including *Loricariichthys*, and *Loricaria-Pseudohemiodon* groups), and one part of Sturisomina. The *Rineloricaria* group did not constitute a natural group as defined by incompatible molecular and morphological hypotheses. The co-structure showed that the variables used by Covain and Fisch-Muller (2007) were relevant to characterize tribal and subtribal ranks, as well as several morphological groups, but were insufficient to define the generic rank. Therefore, the lack of resolution at the generic level in the phylogram came mainly from the restricted morphological data set rather than from incompatibilities (6 discrete quantitative and 11 qualitative variables). However, the quality of the strict congruence phylogram obtained validates the co-inertia approach in a phylogenetic context by identifying morphological variables correlated to the phylogeny in a pool of different types of variables.

Maximum likelihood ancestral state reconstructions of qualitative variables underlined similar patterns of evolution of traits linked to the mouth. Moreover, the mouth characteristics appeared as the most important features for discriminating the different groups of this subfamily, as traits linked to this organ show the strongest variations correlated to the phylogeny. Therefore, we believe that the mouth shape, the tooth shape, the lips structure and the barbels shape may have co-evolved due to identical selective pressure acting on this organ. The co-variation of these traits may reflect adaptations to the large number of ecological niches conquered by the Loricariinae. For instance, species occurring over sandy substrates, such as the representatives of the *Pseudohemiodon* and *Loricaria* groups, possess a bilobate mouth with filamentous lips, whereas more rheophilic species like representatives of *Harttia* or *Lamontichthys* (which live on stones) possess an elliptical mouth with papillose lips. Our conclusions also highlight the difficulties in defining evolutionary independent morphological characters for phylogenetic purposes.

Some qualitative variables retained as phylogenetically dependent were homoplastic as referring to the molecular phylogenetic tree such as the predorsal keels, the fringed and maxillary barbels. The two first characters show local losses while the third displays two independent gains, which is a case of evolutionary convergence. This indicates that the CIA approach is not too restrictive and allows the retention of characters with some degree of homoplasy which can be of different nature (losses or independent gains). However, although retaining them as interesting characters, the CIA ordered them as the less informative among the retained ones (see absolute contributions on axis 1 in table 3).

The analysis of the quantitative variables with the orthogram method (Ollier et al., 2006) not only showed that these variables were shaped by the evolutionary history of this group but also described how these variables evolved along the phylogeny. The analysis of the number of caudal-fin rays indicated a significant drop at the base of the Loricariina lineage, with a reduction of rays from 14 (13 in *Farlowella*) in Sturisomina, to 12 in Loricariina (13 in *Metaloricaria*). We have noticed that in Loricariina, the loss of caudal-fin rays was accompanied by the appearance of a thicker caudal-spine bearing a whip used as a defensive weapon. These concomitant morphological changes may therefore be linked and the formation of the thicker caudal-spine with its whip may be the outcome fin rays fusions. Contrasting with the instance of caudal-fin rays number variation linked to the phylogeny presented above, the punctual reduction of caudal-fin rays in *Farlowella* and *Metaloricaria* were not dependent to the phylogeny but rather randomly distributed events and were thus discarded from an evolutionary interpretation. The analysis of the caudal-fin rays exemplifies the possibility that a given morphological trait may display changes that are linked to the phylogeny and others that arise in a stochastic manner. Yet, we have the tools to discern between these two situations. The study of the number of premaxillary and dentary teeth revealed a more gradual evolution of these features, as indicated by the non significativity of the R2Max test. The decrease in the number of teeth extended gradually along the phylogeny, from Harttiini (bearing 80 premaxillay and 70 dentary teeth) to Loricariini (bearing less than 60 premaxillay and 50 dentary teeth), and then between Sturisomina (bearing 20 to 60 premaxillay and 15 to 50 dentary teeth) and Loricariina (bearing 0 to 15 premaxillay and 3 to 15 dentary teeth).

As shown in our study, the orthogram method of Ollier et al. (2006) proved to be a powerful tool to detect phylogenetic dependence and to analyze the patterns of evolution of quantitative life-history traits. However, this method suffers from the fact that it can not treat qualitative variables; a weakness that can be partly overcame by using the CIA approach. The convincing results given by the orthograms encourage nevertheless the development of the method for analyzing qualitative data or even a complete table mixing different types of data. The theoretical background for generalizing the orthogram method is in progress and its implementation will be performed soon.

Acknowledgments

We are grateful to Volker Mahnert, MHNG, Geneva, for being the supervisor of first author during the early stage of this work, to Claude Weber, and Andreas Schmitz, MHNG, Geneva, and Lawrence M. Page, University of Florida, Gainesville for their helpful advices; Jan Pawlowski, University of Geneva, for the laboratory facilities, and José Fahrni, University of Geneva, for the sequencing process. We would also like to thank Philippe Debey, Poisson Ange Company, Lure, for providing specimens and samples from the trade; the G. and A. Claraz Foundation for their financial support for the missions in Surinam in 2001 and 2005; the Guyana Environmental Protection Agency for collecting permit; and the NRDDB and Iwokrama organization for their field logistic. This project was partly supported by the Fond National Suisse de la Recherche Scientifique (JIMB 3100A0–104005), and by a field grant of the Académie Suisse des Sciences Naturelles (ASSN). The figures were finalized by Florence Marteau, MHNG.

References

Abouheif, E., 1999. A method to test the assumption of phylogenetic independence in comparative data. Evol. Ecol. Res. 1, 895–909.

Blomberg, S.P., Garland, T.Jr., Ives, A.R., 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57, 717–745.

Chessel, D., Dufour, A.B., Thioulouse, J., 2004. The ade4 package–I– One-table methods. R News 4, 5–10.

Cheverud, J.M., Dow, M.M., Leutenegger, W., 1985. The quantitative assessment of phylogenetic constraints in comparative analyses: sexual dimorphism in body weight among primates. Evolution 39, 1335–1351.

Covain, R., Fisch-Muller, S., 2007. The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: Loricariidae): a practical key and synopsis. Zootaxa 1462, 1–40.

Diniz-Filho, J.A.F., de Sant'Ana, C.E.R., Bini, L.M., 1998. An eigenvector method for estimating phylogenetic inertia. Evolution 52, 1247–1262.

Dolédec, S., Chessel, D., 1994. Co-inertia analysis: an alternative method for studying species-environment relationships. Freshwater Biol. 31, 277–294.

Dray, S., Chessel, D., Thioulouse, J., 2003. Co-inertia analysis and the linking of ecological data tables. Ecology 84, 3078–3089.

Efron, B., 1979. Bootstrap methods: Another look at the jackknife. Ann. Statist. 7, 1–26.

Felsenstein, J., 1981. Evolutionary trees from DNA-sequences- a maximum-likelihood approach. J. Mol. Evol. 17, 368–376.

Felsenstein, J., 1985a. Phylogenies and the comparative method. Am. Nat. 125, 1–15.

Felsenstein, J., 1985b. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Felsenstein, J., 2004. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Fitch, W.M., Margoliash, E., 1967. Construction of phylogenetic trees. Science 155**,** 279–284.

Gittleman, J.L., Kot, M., 1990. Adaptation: statistics and null model for estimating phylogenetic effects. Syst. Zool. 39, 227–241.

Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49, 652–670.

Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53, 325–338.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41, 95–98.

Harvey, P.H.; Pagel, M.D. 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford, 248 pp.

Hill, M.O., Smith, A.J.E., 1976. Principal component analysis of taxonomic data with multi-state discrete characters. Taxon 25, 249–255.

Huelsenbeck, J.P., Nielsen, R., Bollback, J., 2003. Stochastic mapping of morphological characters. Syst. Biol. 52, 131–158.

Ihaka, R., Gentleman, R., 1996. R: A language for data analysis and graphics. J. Comput. Graph. Stat. 5, 299–314.

Isbrücker, I.J.H., 1979. Description préliminaire de nouveaux taxa de la famille des Loricariidae, poissons-chats cuirassés néotropicaux, avec un catalogue critique de la sous-famille nominale (Pisces, Siluriformes). Rev. Fr. Aquariol. 5, 86–116.

Leviton, A.E., Gibbs, R.H.Jr., Heal, E., Dawson, C.E., 1985. Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985, 802–832.

Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50, 913–925.

Lingoes, J.C., 1971. Some boundary conditions for a monotone analysis of symmetric matrices. Psychometrika 36, 195–203.

Lynch, M., 1991. Methods for the analysis of comparative data in evolutionary biology. Evolution 45, 1065–1080.

Maddison, W.P., Maddison, D.R., 2006a. StochChar: A package of Mesquite modules for stochastic models of character evolution. Version1.1 [http://mesquiteproject.org].

Maddison, W.P., Maddison, D.R., 2006b. Mesquite: a modular system for evolutionary analysis. Version 1.12 [http://mesquiteproject.org].

Montoya-Burgos, J.I., Muller, S., Weber, C., Pawlowski, J., 1998. Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences. In: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena Z.M.S., Lucena, C.A.S. (Eds.), Phylogeny and Classification of Neotropical Fishes. Edipucrs Porto Alegre, Porto Alegre, Brazil, pp. 363–374.

Ollier, S., Couteron, P., Chessel, D., 2006. Orthonormal transform to decompose the variance of a life-history trait across a phylogenetic tree. Biometrics 62, 471–477.

Pagel, M., 1999a. Inferring the historical patterns of biological evolution. Nature 401, 877–884.

Pagel, M., 1999b. The maximum likelihood approach to reconstructing Ancestral character states of discrete characters on phylogenies. Syst. Biol. 48, 612–622.

Pagel, M., Meade, A., Barker, D., 2004. Bayesian estimation of ancestral character states on phylogenies. Syst. Biol. 53, 673–684.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rapp Py-Daniel, L.H., 1997. Phylogeny of the Neotropical armored catfishes of the subfamilly Loricariinae (Siluriformes: Loricariidae). Unpublished Ph. D. Thesis, University of Arizona, Tucson, 280 pp.

Reeve, J., Abouheif, E., 2003. Phylogenetic Independence Version 2.0. Distributed freely by the authors on request. Department of Biology, McGill University.

Robert, P., Escoufier, Y., 1976. A unifying tool for linear multivariate statistical methods: the RV coefficient. Appl. Stat.-J. Roy. St C. 25, 257–265.

Rholf, F.J., 2001. Comparative methods for the analysis of continuous variables: geometric interpretations. Evolution 55, 2143–2160.

Saitou, N., Nei, M., 1987. The neighbor-joining method; a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Schaefer, S.A., 1987. Osteology of *Hypostomus plecostomus* (Linnaeus) with a phylogenetic analysis of the loricariid subfamilies (Pisces: Siluroidei). Contrib. Sci., Los Angeles Co. Mus. Nat. Hist. 394, 1–31.

Schaefer, S.A., 1991. Phylogenetic analysis of the loricariid subfamily Hypoptopomatinae (Pisces: Siluroidei: Loricariidae), with comments on generic diagnoses and geographic distribution. Zool. J. Linn. Soc.-Lond. 102, 1–41.

Schaefer, S.A., Stewart, D.J., 1993. Systematics of the *Panaque dentex* species group (Siluriformes: Loricariidae), wood-eating armored catfishes from tropical South America. Ichthyol. Explor. Freshwater. 4, 309–342.

Schluter, D., Price, T., Mooers, A.O., Ludwig, D., 1997. Likelihood of ancestor states in adaptive radiation. Evolution 51, 1699–1711.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Sokal, R.R., Rohlf, F.J., 1995. Biometry, 3rd ed. W.H. Freeman & Co. (Eds), New York, 887 pp.

Swofford, D.L., 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.

Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. In: Miura, R. M. (ed.), Some mathematical questions in biology-DNA sequence analysis. American Mathematics Society, Providence, RI, pp. 57–86.

Thioulouse, J., Chessel, D., Dolédec, S., Olivier, J.M., 1997. ADE–4: a multivariate analysis and graphical display software. Stat. Comput. 7, 75–83.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

von Neumann, J., Kent, R.H., Bellinson, H.R., Hart, B.I., 1941. The mean square successive difference. Ann. Math. Stat. 12, 153–162.

Corrigendum to « Assessing phylogenetic dependence of morphological traits using co-inertia prior to investigate character evolution in Loricariinae catfishes." Mol. Phylogenet. Evol. 46 (2008) 986-1002.

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A taxonomical problem was pointed out concerning the new proposed name Sturisomina. This name was unnecessary since a name for a tribe named Farlowellini was already available. A correction is here proposed.

Published in : *Molecular Phylogenetics and Evolution*, **55** (2010): 751.

In Covain *et al*. (2008), we proposed the new family-group name Sturisomina for the clade including the genera *Sturisoma*, *Farlowella*, *Sturisomatichthys* and *Lamontichthys*. This new name was not needed because the family-group name Farlowellini Fowler, 1958 is already available for any lineage including its type genus *Farlowella* (International Code of Zoological Nomenclature (ICZN), 1999).

The fact that Farlowellini was originally proposed as the name of a tribe within Loricariinae does not preclude its usage as the name of a subtribe (here Farlowellina). Article 36.1 of ICZN states the Principle of Coordination that applies to family-group names: a name established for a taxon at any rank in the family group is deemed to have been simultaneously established for nominal taxa at all other ranks in the family group; at all ranks the type genus remains the same (here *Farlowella*), and the name is formed from the stem of the name of the type genus (here *Farlowell-*) with the appropriate change of suffix (*-ini* for tribes, *-ina* for subtribes). The name has the same authorship and date at every rank.

According to the Principle of Priority, the valid name of a taxon is the oldest available name applied to it. Priority between names of the family group is not affected by the change of rank within the family, nor by any mandatory change in suffix of a family-group name consequent upon change in rank (Article 23.3.1). Accordingly, the correct name of a subtribe including *Farlowella* has to be Farlowellina Fowler, 1958.

In addition, the name Sturisomina proposed by the authors is not available because it does not satisfy the conditions of Article 13.1.1 of the ICZN, which requires for a new family-group name to become available to be accompanied by a description of definition that states in words characters that are purported to differentiate the taxon, and the fixation of a type genus (Article 64).

Acknowledgments

We are grateful to Maurice Kottelat for pointing out the nomenclatural problem.

Reference

International Commission on Zoological Nomenclature, 1999. International Code of Zoological Nomenclature, fourth ed. International Trust for Zoological Nomenclature, London.

Chapter 2

Intergeneric phylogenetic relationships in Loricariinae catfishes (Siluriformes: Locariidae), with description of *Fonchiiloricaria nanodon*: a new genus and species from Peru.

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Based on previously known collection specimens (a single in MHNG) and additional freshly collected material from Peru, a new genus and new species is here described to clarify the systematics of the group. Additionally, an evaluation of the alignment, especially in intronic regions of the new f-rtn4 marker is performed prior to reconstruct the first phylogeny of the Loricariinae mixing mitochondrial and nuclear information (for the characterization of the first intron of f-rtn4 see Fisch-Muller et al., in press; Annex 3). MSR wrote the morphological description and discussion, HO wrote the ecological part, and RC performed the molecular analyses and wrote the rest of the manuscript.

Accepted for publication in: *Journal of Fish Biology*.

Abstract

Recent investigations in the upper Río Huallaga in Peru revealed the presence of an intriguing species of the Loricariinae. To characterize and place this species within the evolutionary tree of the subfamily, a molecular phylogeny of this group is inferred based on the 12S and 16S mitochondrial genes, and the nuclear gene F-reticulon4. The resulting phylogeny indicates that this distinctive species of the Loricariinae is a member of the subtribe Loricariina. Given its phylogenetic placement, and its unusual morphology, this species is described herein as a new genus and new species of Loricariinae: *Fonchiiloricaria nanodon*. This new genus and new species is diagnosed by: usually possessing one to three premaxillary teeth that are greatly reduced in size; lips with globular papillae on the surface; the distal margin of lower lip bearing short, triangular filaments; the premaxilla greatly reduced; the abdomen completely covered by plates, with the plates between lateral abdominal plates small and rhombic; a caudal fin with 14 total rays; the orbital notch absent; five lateral series of plates; dorsal-fin spinelet absent; preanal plate present, large and solid, and of irregular, polygonal shape, and; the caudal peduncle becoming more compressed posteriorly for the last seven to 10 plates.

Key words: Neotropics - molecular phylogeny – morphology – systematics.

Introduction

Neotropical freshwaters comprise approximately one quarter of the total diversity of fishes with a prediction of around 8,000 extant species out of the estimated 31,500 to 32,500 (Lévêque *et al*., 2008). In South and Central America, the Loricariidae, or armored catfish, represents the most species-rich family of the Siluriformes in the world with 716 valid species and an estimated 300 undescribed species distributed in 96 genera (Ferraris, 2007). Loricariids are characterized by a depressed body covered by bony plates, a single pair of maxillary barbels, and above all, by the modification of the mouth into a sucker disk. Within the Loricariidae, members of the subfamily Loricariinae are united by a long and depressed caudal peduncle and by the absence of an adipose fin, but they exhibit substantial variation in body shape, lip morphology and dentition. There are currently 220 valid species of Loricariinae, distributed in 30 genera (for a review see Covain & Fisch-Muller, 2007; also Ghazzi, 2008; Ingenito *et al*., 2008; Fichberg & Chamon, 2008; Rapp Py-Daniel & Fichberg, 2008; Rodriguez & Miquelarena, 2008; Rodriguez & Reis 2008; Rodriguez *et al*., 2008; Thomas & Rapp Py-Daniel, 2008; de Carvalho Paixão & Toledo-Piza, 2009; Thomas. & Sabaj Pérez, 2010).

The evolutionary history of the Loricariinae has been only recently explored by Covain *et al*. (2008), who proposed the first molecular phylogeny of the subfamily and assessed the phylogenetic dependence of the morphological traits classically used as diagnostic features. Although their analysis included only 20 representatives of the Loricariinae, they redefined its systematics with the restriction of the tribe Harttiini to *Harttia*, and the placement of all remaining genera of the study within the tribe Loricariini. Within the latter, they redefined the subtribes Loricariina and Farlowellina (incorrectly named Sturisomina in Covain *et al*., 2008; corrected in Covain *et al*., 2010). Covain *et al*. (2008) furthermore demonstrated that the characteristics of the mouth and the caudal fin are autocorrelated with the phylogeny and that they are sufficient to define tribal and subtribal ranks, as well as several of the morphological groups proposed in Covain & Fisch-Muller (2007).

Recent investigations conducted in the Rio Huallaga drainage near Tingo Maria in Peru, revealed the presence of an unusual form of the Loricariinae characterized by distinct morphological characters. On first examination, the species resembles *Rineloricaria* or *Spatuloricaria*, but possesses unusual dentition. The Peruvian ichthyologist Fonchii Chang identified this form as a possible new species and new genus and deposited one specimen as a

future paratype in the Muséum d'histoire naturelle de la Ville de Genève (MHNG) in February 1999. Unfortunately, she died tragically in a boat accident on the Rio Pastaza in August 1999, prior to describing the species. Chang's remaining material for the new genus was also temporally misplaced shortly thereafter and the MHNG´s specimen was for a long time the only known representative of the taxon. Subsequently, some of Chang's collection has been found, and additional specimens have been found in museum collections and via ongoing fieldwork. The objectives of the present study are: 1) to place this new species of Loricariinae in the evolutionary tree of the subfamily, by reconstructing a molecular phylogeny based on mitochondrial and nuclear genes, and 2) to formally describe this new species.

Material and Methods

The molecular phylogeny was reconstructed based on the taxonomic sampling described by Covain *et al*. (2008) with the addition of two representative species of *Spatuloricaria*, and of the new taxon. One additional outgroup, *Pseudorinelepis genibarbis* (Valenciennes 1840), was added to root the tree following the results of Montoya-Burgos *et al.* (1998). The list of material used for this study is provided in Table I. The analyzed samples came from the tissue collection of MHNG, and the sequences were deposited in GenBank (accession numbers in Table I).

Tissue samples were preserved in 80% ethanol and stored at -20°C. Total genomic DNA was extracted with the DNeasy Tissue Kit (Qiagen) following the manufacturer's instructions. The PCR amplifications of partial 12S, 16S, and Fish Reticulon-4 (F-RTN4) for the phylogeny, as well as the 648-bp region of the cytochrome *c* oxidase I (COI) mitochondrial gene required for DNA barcodes, were carried out using the Taq PCR Core Kit (Qiagen). The methodology for PCR amplifications followed Covain *et al.* (2008) for 12S and 16S, Chiachio *et al*. (2008) for F-RTN4, and Vari & Ferraris (2009) for COI. PCR products were purified with the High Pure PCR Product Purification Kit (Roche). Sequencing reactions were performed with the Big Dye Terminator Cycle Sequencing Ready Reaction 3.1 Kit (Applied Biosystems) following instructions of the manufacturer, and were loaded on an automatic sequencer (3100-Avant Genetic Analyzer, Applied Biosystems, Perkin-Elmer).

Table I. Taxa list, specimen and sequence data for the 26 species of Loricariinae, and outgroup analyzed in this study. The acronyms of institutions follow Fricke & Eschmeyer (2010).

Species	Catalog Number	Field Number	Locality	mt 12S+16S bases + GenBank No.	Reference	F-RTN4 bases + GenBank No.	Reference
Crossoloricaria venezuelae	INHS 35467	VZ 049	Venezuela, Rio Santa Rosa	2416 EU310444	Covain *et al.* 2008	1994 HM623647	This study
Dasyloricaria tuyrensis	MHNG 2674.052	PA00–012	Panama, Rio Ipeti	2416 EU310445	Covain *et al.* 2008	2005 HM623639	This study
Farlowella platoryncha	MHNG 2588.093	PE96–071	Peru, Rio Ucayali	2429 EU310446	Covain *et al.* 2008	2301 HM623649	This study
Farlowella oxyrryncha	MHNG 2588.064	PE96–022	Peru, Rio Tambopata	2430 EU310443	Covain *et al.* 2008	2237 HM623650	This study
Harttia guianensis	MHNG 2643.016	GF00–351	French Guiana, Marouini River	2435 EU310447	Covain *et al.* 2008	2112 FJ013232	Chiachio *et al.* 2008
Hemiodontichthys acipenserinus	MHNG 2651.012	GY04–15	Guyana, Rupununi River	2419 EU310448	Covain *et al.* 2008	2246 HM623645	This study
Lamontichthys stibaros	MHNG 2677.039	MUS 208	Peru, aquarium trade, Rio Itaya[2]	2430 EU310449	Covain *et al.* 2008	2038 HM623648	This study
Limatulichthys griseus	MHNG 2651.013	GY04–18	Guyana, Rupununi River	2423 EU310450	Covain *et al.* 2008	1959 HM623644	This study
Loricaria clavipinna	MHNG 2640.044	PE98–002	Peru, Rio Putumayo	2424 EU310451	Covain *et al.* 2008	1964 HM623653	This study
Loricaria parnahybae	MHNG 2602.067	BR98–274	Brazil, Rio Parnahyba	2421 EU310452	Covain *et al.* 2008	1985 FJ013231	Chiachio *et al.* 2008
Loricariichthys maculatus	MHNG 2621.042	SU01–56	Surinam, Sarramacca River	2425 EU310453	Covain *et al.* 2008	2221 HM623642	This study
Loricariichthys microdon	MHNG 2650.054	GY04–12	Guyana, Rupununi River	2424 EU310454	Covain *et al.* 2008	1949 HM623643	This study
Metaloricaria paucidens	MHNG 2677.086	GF00–083	French Guiana, Marouini River	2435 EU310455	Covain *et al.* 2008	2073 HM623637	This study
Planiloricaria cryptodon	MHNG 2677.038	MUS 211	Peru, aquarium trade, Rio Itaya[2]	2415 EU310456	Covain *et al.* 2008	2006 HM623646	This study
Rineloricaria platyura	MHNG 2651.009	GY04–83	Guyana, Rupununi River	2420 EU310458	Covain *et al.* 2008	2219 HM623641	This study
Rineloricaria lanceolata	MHNG 2588.059	PE96–011	Peru, Rio Tambopata	2420 EU310457	Covain *et al.* 2008	2226 HM623640	This study
Rineloricaria osvaldoi	UFRJ batch 6–EF4	BR 1114	Brazil, Rio Maranhão	2424 EU310459	Covain *et al.* 2008	2023 HM623652	This study
Sturisoma nigrirostrum	MHNG 2588.055	PE96–001	Peru, Rio de las Piedras	2437 EU310460	Covain *et al.* 2008	2556 HM623636	This study

Sturisoma monopelte	MHNG 2651.033	GY04–187	Guyana, Sawarab River	2436 EU310461	Covain *et al.* 2008	1980 HM623651	This study
Sturisomatichthys citurensis	MHNG 2676.004	PA97–032	Panama, Rio Tuyra	2435 EU310462	Covain *et al.* 2008	2268 HM623635	This study
Spatuloricaria aff. *puganensis*	MHNG 2710.050	PE08-230	Peru, Rio Huallaga	2418 HM592624	This study	1981 HM623654	This study
Spatuloricaria sp. Nanay	MHNG 2677.071	PE05-014	Peru, aquarium trade, Rio Nanay[2]	2419 HM592625	This study	1979 HM623655	This study
Fonchiiloricaria nanodon	MHNG 2710.048	PE08-199	Peru, Rio Monzon	2429 HM592626	This study	2015 HM623656	This study
Fonchiiloricaria nanodon	MHNG 2710.060	PE08-336	Peru, Rio Aucayacu	2429 HM592627	This study	2015 HM623657	This study
Ancistrus cirrhosus[1]	MHNG 2645.037	MUS 202	Argentina, Rio Uruguay	2420 EU310442	Covain *et al.* 2008	1809 HM623638	This study
Pseudorinelepis genibarbis[1]	MHNG 2588.079	PE96-040	Peru, Rio Ucayali	2434 HM592623	This study	1926 HM623634	This study

[1] outgroup
[2] according to the exporter

The DNA sequences were edited and assembled using BioEdit 7.0.1 (Hall, 1999), and aligned manually. This alignment was then compared to *n* multiples alignments generated by ClustalX 1.83 (Thompson *et al*., 1997), using default parameters for pairwise alignment and computation of the guide tree. For multiple alignments, Gap Opening Penalties (GOP) ranged from seven (around half default parameter) to 30 (twice default parameter) with a progressive increase of one. Gap Extension Penalties (GEP) started to a value representing 30% of the GOP of reference with a progressive increase of 30% at each step until reaching 90% of the GOP. These alignments were then submitted to SOAP 1.2a4 (Löytynoja & Milinkovitch, 2001) to detect and remove unstable blocks. To evaluate the influence of unstable positions in the alignment on the tree reconstruction, different statistics were computed. These were the final length of the alignment in number of bases, the sum of branch lengths of the phylogenetic trees, the mean nodal support, and the standardized Colless' index (Colless, 1982). The final length of the alignment was used to evaluate the loss of information in the alignment. It was obtained from SOAP 1.2a4. The sum of branch lengths provided an estimation of the total amount of evolution recovered by the phylogenetic tree quantified as the number of substitutions per site, and was computed using the *ape* 2.5 package (Paradis *et al*., 2004; Paradis, 2006) in R 2.10.1 (R Development Core Team, 2009). Prior to its computation, Maximum likelihood (ML) (Felsenstein, 1981) tree reconstructions were performed using a general model as implemented in Treefinder (Jobb *et al*., 2004) version of October 2008. The appropriate substitution model was estimated with the corrected Akaike Information Criterion (Sugiura, 1978) as implemented in Treefinder. The degree to which the set of branch lengths approximates the actual number of substitutions is governed by the adequacy of the model. Robustness of the results was estimated using Local Rearrangements of the Expected-Likelihood Weights (LR-ELW) (Strimmer & Rambaut, 2002). The mean nodal support was used to evaluate the global robustness of the tree. LR-ELW were computed using 1,000 replicates with Treefinder, and their mean computed using *ape* in R. The Colless' index provided an estimation of the tree shape by a measure of the imbalance of the topology. After testing for the best model, this index was standardized using the Equal Rate Markov (ERM) or Proportional to Distinguishable Arrangements (PDA) distribution of tree shape (Mooers & Heard, 1997). A small index characterizes a more balanced topology of the tree. The likelihood test that evaluated the ERM model against the PDA model, and the computation of the Colless' index were performed using the apTreeshape 1.4-3 package (Bortolussi *et al*., 2006) in R. To allow direct comparisons in the behaviour of these four statistics that were expressed in different units, all were standardised before plotting.

To detect a potential conflict in the phylogenetic signal present in the different parts of the manual alignment, the combinability between mitochondrial and nuclear markers was assessed using the Incongruence Length Difference (ILD) test (Farris *et al*., 1994) as implemented in PAUP* 4.0b10 (Swofford, 2003). Since mitochondrial DNA is presumably transmitted through maternal lineage as a single not recombining genetic unit (Meyer, 1993), a first partition corresponding to mitochondrial genes was created. In addition, because the mutational pattern in non coding (introns) and coding (exons) regions of F-RTN4 are different, two additional partitions were created. The ILD test was conducted using a heuristic search with 1,000 replicates, TBR branch swapping, and random addition of taxa with 10 replicates. Appropriate substitution models corresponding to each potential partition were accordingly estimated, and a partitioned ML phylogenetic reconstruction was performed. Gaps in the alignment were considered as missing data. Robustness of the results was estimated by resampling the data set with the nonparametric bootstrap (Efron, 1979) following Felsenstein's (1985) methodology with 1,000 pseudoreplicates.

All available specimens (n = 26) of the new taxon were secondarily compared with representatives of all genera of the subfamily Loricariinae, except for the monotypic *Rhadinoloricaria macromystax* (Günther 1869), for which comparisons were made to descriptions in Isbrücker & Nijssen (1974), and Covain & Fisch-Muller (2007). *Rhadinoloricaria macromystax* is rare in collections, and the holotype is lost for the time being (J. Maclaine, The Natural History Museum, London, pers. com.), therefore no specimens of *R. macromystax* were available for this study.

Morphometric variables were measured with a digital caliper (0.1 mm precision). Measurements and counts follow Rodriguez *et al.* (2008), except for the premaxillary ramus length (due to difficulty of measurement), and the orbital diameter excluding the notch. These two measurements were excluded. Terminology of osteological characters follows Schaefer (1997). Osteological observations were made on two cleared and stained specimens (CS), prepared according to the method of Taylor & Van Dyke (1985), with modifications. The illustrations were made using a stereomicroscope Leica M50.

In the list of material examined, institutional acronyms and catalog numbers are presented first, followed by the number of specimens in that lot, size range, locality, date of collection and collector. Institutional acronyms follow Fricke & Eschmeyer (2010), with the addition of LBP (Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista "Júlio de Mesquita Filho") (for Comparative Material see Appendix S1).

Results

Phylogenetic reconstruction

Manual sequence alignment included 6,898 positions from which 970 corresponded to the 12S rRNA gene, 72 to the tRNA Val gene, 1,468 to the 16S rRNA gene, 891 to the exonic regions of the F-RTN4 gene, and 3,497 to the intronic regions of the F-RTN4 gene. Seventy-two multiple alignments were generated by ClustalX 1.83 with progressive increase of GOP and GEP, and were simultaneously compared to the manual alignment. Three thousand eight hundred and forty three positions were found to be unstable corresponding to the almost complete intronic part of F-RTN4 and parts of the loop regions of the ribosomal genes. Eleven consensus alignments were computed ranging from no removal of information (manual alignment) to complete removal of unstable blocks, with a 10% progressive increase of removal of positions supported by less than a given percentage of alignments. Phylogenetic trees were reconstructed for these 11 alignments using the general best fit model J2+I+G (Jobb *et al*., 2004), except for the manual alignment for which the best fit model was GTR+G (Tavaré, 1986). The final length of the alignment, sum of branch lengths, mean nodal support, and standardized Colless' index were then computed for these 11 alignments and corresponding phylogenetic trees. After standardization, these four statistics were plotted as a function of the progressive removal of unstable positions to follow their behaviour (Fig. 1). Progressive removal of unstable blocks, supported by less than 10% to 100% of the alignments, led to a significant loss of information. This loss represented 45% to 55% of the total alignment's length. From 6,898 positions, the length of the alignment immediately dropped to 3,807 positions after removal of unstable blocks that were found in more than 10% of the alignments, to finally reach 3,055 positions after removal of all unstable blocks. Tree reconstructions performed on these 11 alignments using a general model led to identical tree topologies except for the manual alignment. The likelihood tests conducted on those topologies resulted in the significant rejection of the ERM model against the PDA model ($2.23 < X < 2.42$; $0.016 < p.value < 0.026$). The PDA model was accordingly used to standardize the Colless' index that increased from 0.84 for the topology computed from the whole data set, to 0.89 for all other topologies. A smaller index indicated a more balanced topology. The two differences recorded in the topologies concerned the branches leading to

the possible new genus and *Metaloricaria*. The sum of branch lengths is directly proportional to the alignment's length, and both curves follow the same behaviour. The sum of branch

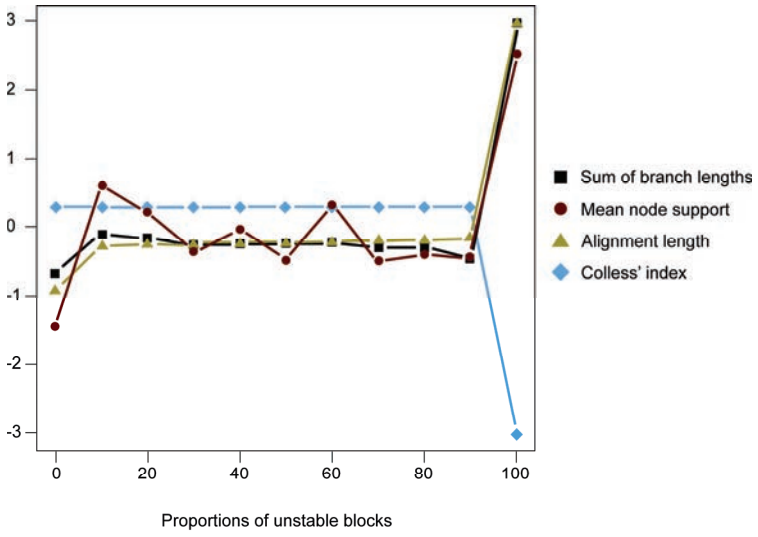

Fig. 1. Effect of removal of unstable blocks of the alignment on the descriptive statistics computed from phylogenetic reconstructions. Alignment length expressed in number of bases; sum of branch lengths expressed in number of substitutions per sites using a general best fit model; mean node support expressed in mean Local Rearrangements of the Expected-Likelihood Weights using 1,000 replicates; standardized Colless' index computed using the Proportional to Distinguishable Arrangements model of tree shape distribution. All statistics were standardized to allow direct comparisons, and plotted as a function of percentages of unstable blocks present in the alignment.

lengths immediately drops from 1.32 (manual alignment) to 0.98, then slightly decreases to finally reach a minimum of 0.92 after complete removal of ambiguous positions. The model of substitutions perfectly fits the data for the manual alignment and intermediary values of alignment's length. For smaller alignments, the model overestimates the number of substitutions with the curve of branch lengths located above the curve of alignment's length, whereas for longer alignments it underestimates them, with the curve located below. The mean nodal support is also significantly affected with a mean LR-ELW of 90.8 dropping to between 87.3 and 85.3 for the progressive removal of unstable positions, and reaches a minimum of 83.5 after complete removal of unstable blocks. No conflicting phylogenetic signal was detected in the data set, as the ILD test failed to reject the null hypothesis of congruence between data partitions (ILD: $p(X>X_{obs}) = 0.15$). Given that no conflict between data partitions is detected, and that the removal of unstable positions negatively affects the reconstructed trees, the manual alignment was used in subsequent analyses to consider all the available information. The sequences were consequently concatenated, and three partitions corresponding to mitochondrial genes, exonic parts of F-RTN4, and intronic parts of F-RTN4 were used to reconstruct the tree. The models GTR+G for mitochondrial genes, TN+G (Tamura & Nei, 1993) for exonic regions of F-RTN4, and TVM+G (Rodriguez *et al*,. 1990) for intronic regions of F-RTN4 fits the data the best as indicated by Treefinder.

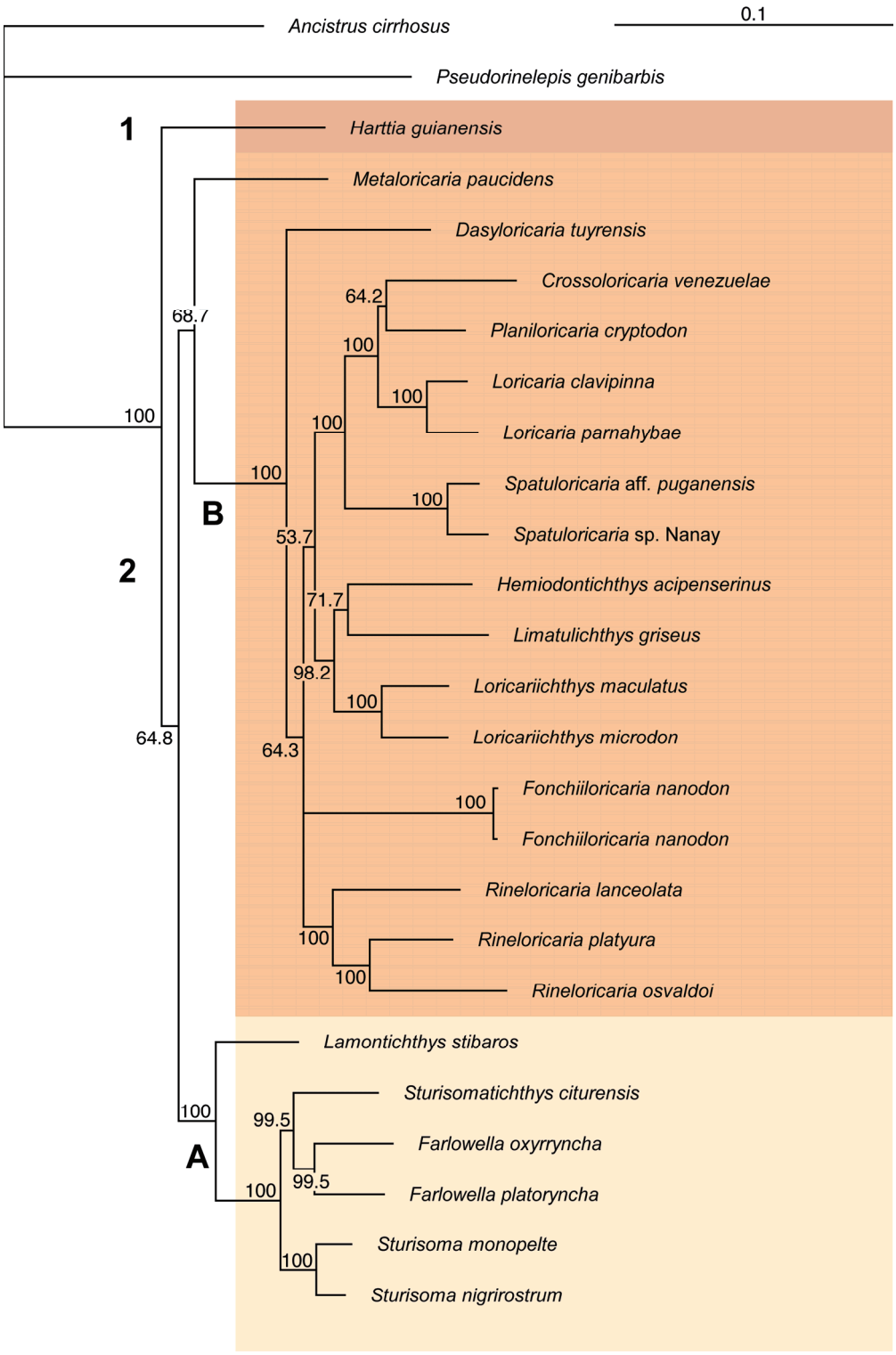

Fig. 2. Bootstrap majority rule consensus tree (consensus level = 50) over 1,000 pseudoreplicates of the best Maximum Likelihood tree of the Loricariinae inferred from the combined analysis of sequences of partial 12S and 16S mitochondrial genes, and partial F-RTN4 nuclear gene. Numbers above branches indicate bootstrap supports above 50. Clades: 1, Harttiini; 2, Loricariini; A, Farlowellina; B, Loricariina. Scale indicates the number of substitutions per site as expected by the model.

The phylogenetic reconstruction leads to a tree topology comparable to the one obtained by Covain *et al.* (2008). The best ML tree (-lnL = 29666.32) splits the Loricariinae into two lineages: the Harttiini (clade 1) including only *Harttia*, and the Loricariini (clade 2) including all the other Loricariinae (Fig. 2). The Loricariini is divided into two clades: the Farlowellina (clade A) comprising *Lamontichthys*, *Sturisoma*, *Farlowella* and, *Sturisomatichthys*; and clade B comprising Loricariina with the 17 remaining representatives of Loricariinae. Within the Loricariina, *Metaloricaria* is recovered at the base of the clade. The sister group of *Metaloricaria* is strongly supported (100% bootstrap) with *Dasyloricaria* recovered as the sister genus to the remainder of the subtribe. The sister group of *Dasyloricaria* is split into two clades: the first one corresponds to *Rineloricaria*, and the second one consists of the remaining genera studied herein. The sister clade of *Rineloricaria* contains the possible new genus in a sister position to two groups: one formed by the *Loricariichthys* group (*sensu* Covain & Fisch-Muller, 2007), and a second comprising *Spatuloricaria* in a sister position to *Loricaria* plus the *Pseudohemiodon* groups (*sensu* Covain & Fisch-Muller, 2007). The position of the possible new genus is weakly supported with a bootstrap majority rule consensus tree (consensus level = 50) leading to a polytomy (Fig. 2). In the ten most frequently obtained bootstrap topologies (Fig. 3), the possible new genus never connects within an extant genus but always in a sister position to genera or entire groups. In the first and sixth topologies (Fig. 3a-f) the possible new genus connects in a sister position to all Loricariina except for *Metaloricaria* and *Dasyloricaria*. In the second and tenth topologies (Fig. 3b-j), it is recovered in a sister position to *Dasyloricaria,* both in turn forming the sister group of the Loricariina except for *Metaloricaria*; while in the third topology (Fig. 3-c) it clusters in a sister position to a clade including *Spatuloricaria* as the sister genus of the *Loricaria* + *Pseudohemiodon* groups. In the fourth and seventh topologies (Fig. 3d-g) it clusters in a position corresponding to the one of the best ML trees where it forms the sister group of the *Loricariichthys* and *Spatuloricaria-Loricaria-Pseudohemidon* groups. In the fifth and eighth bootstrap topologies (Fig. 3e-h) the possible new genus is recovered in a sister relationship to the *Loricariichthys* group. In the ninth topology (Fig. 3i) the possible new genus forms the sister group of *Dasyloricaria*, both forming in turn the sister group of *Rineloricaria*.

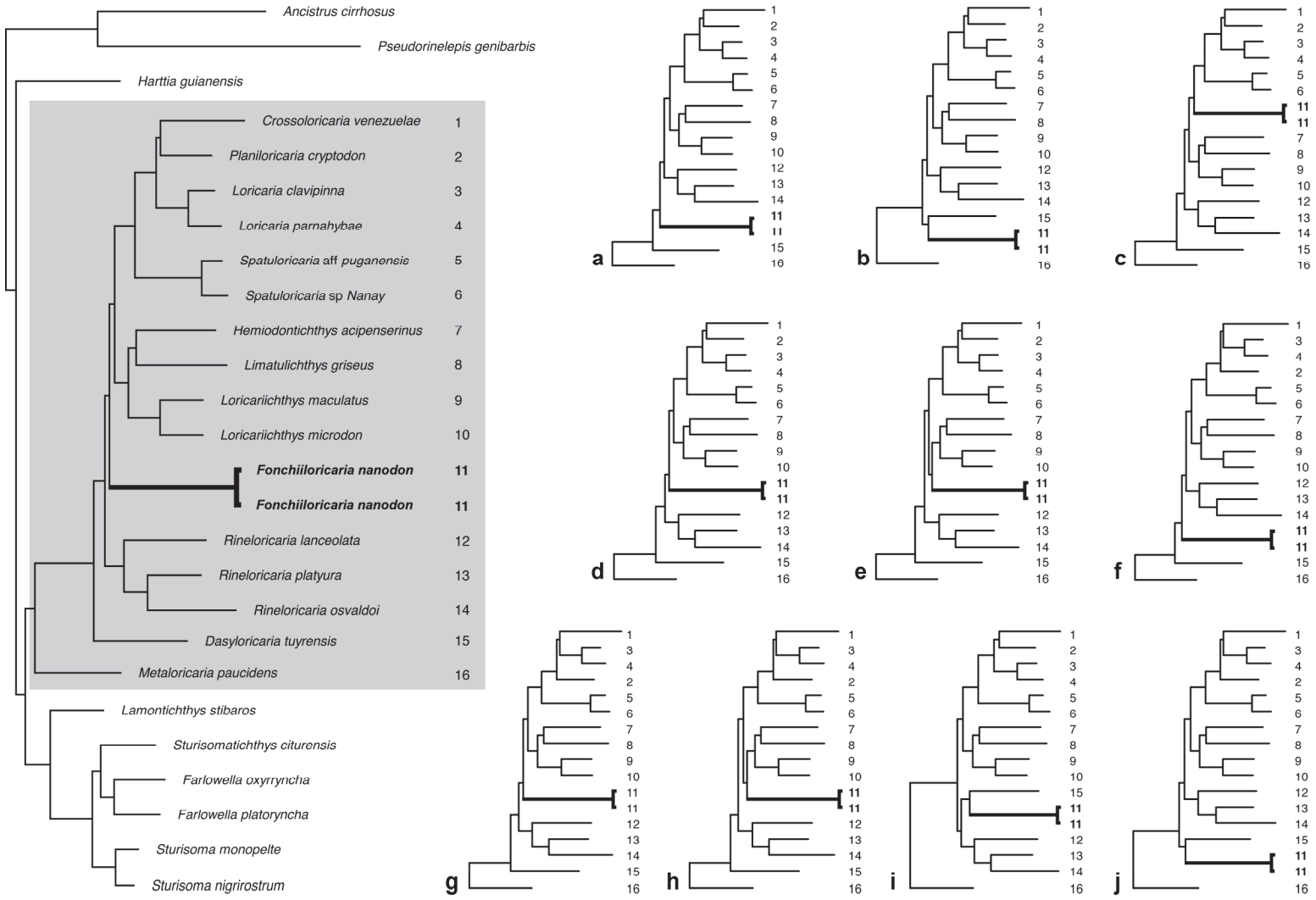

Fig. 3. Ten most frequent topologies obtained from a bootstrap analysis of the best ML tree. Only the subtribe Loricariina was represented to evaluate the possible positions of *Fonchiiloricaria nanodon* within its own group. Bold style refers to the branch leading to *F. nanodon*.

Morphological description

Based on the phylogenetic data the new species cannot be assigned to any known genus, therefore, a new genus is described herein. Thirteen of the 18 specimens from the type series, ranging from 91.4 to 174.6 mm L_S were measured. Specimens smaller than 90 mm L_S were excluded to minimize bias due to the allometry. Eight specimens (MUSM 32153) were excluded from the type series because they were previously dissected, making measurements difficult.

***Fonchiiloricaria*,** new genus

Type species *Fonchiiloricaria nanodon*, new species

Diagnosis: The new genus is distinguished from all other genera of Loricariinae by usually possessing one to three premaxillary teeth (although these are often missing), that are much reduced in size, particularly in comparison to the dentary teeth (Fig. 4). The following

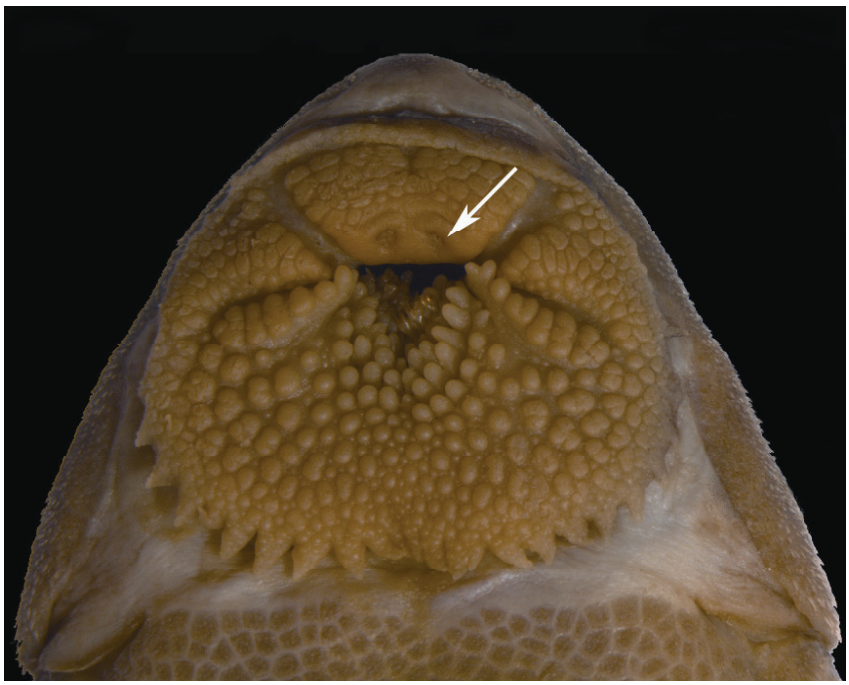

Fig. 4. Detail of the mouth of *Fonchiiloricaria nanodon*, MUSM 10583, paratype, 160.1 mm L_S (ventral view). White arrow shows the very reduced teeth.

combination of characters also differentiates this genus from all other members of the Loricariinae: lips with globular papillae on surface, except for some areas close to the opening of the mouth where the papillae are prolonged and digitiform; distal margin of lower lip with short, triangular filaments; premaxilla very reduced (Fig. 5A & B; the non-reduced condition

of the premaxilla is represented in Fig. 5C & D); abdomen totally covered by plates, medial plates small and rhombic between lateral abdominal plates; caudal fin with 14 total rays (12 branched); orbital notch absent; five lateral series of plates; dorsal-fin spinelet absent; preanal

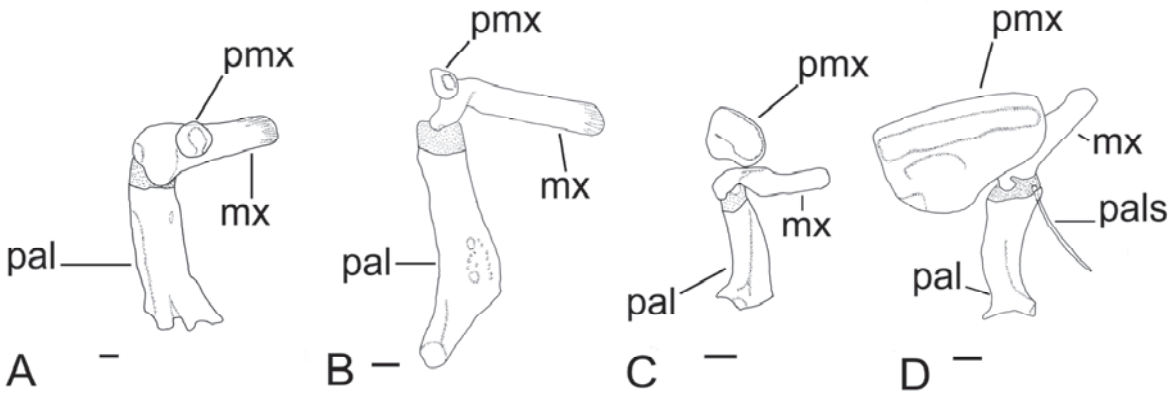

Fig. 5. Schematic drawing of the left premaxilla and associated bones in: A) *Fonchiiloricaria nanodon* (MUSM 32153, 163.6 mm L_S), B) *Pseudohemiodon laticeps* (Regan) (NUP 3462, 175.4 mm L_S), C) *Rineloricaria aequalicuspis* (MCP 26910, 85.3 mm L_S) and D) *Harttia kronei* Miranda Ribeiro (MZUSP 82617, 87.2 mm L_S). pmx: premaxilla, mx: maxilla, pal: palatine, pals: palatine splint. Scale: 1 mm.

plate present, large and solid, and of irregular, polygonal shape. Trunk and caudal peduncle becoming more compressed posteriorly for last seven to 10 plates (Fig. 6).

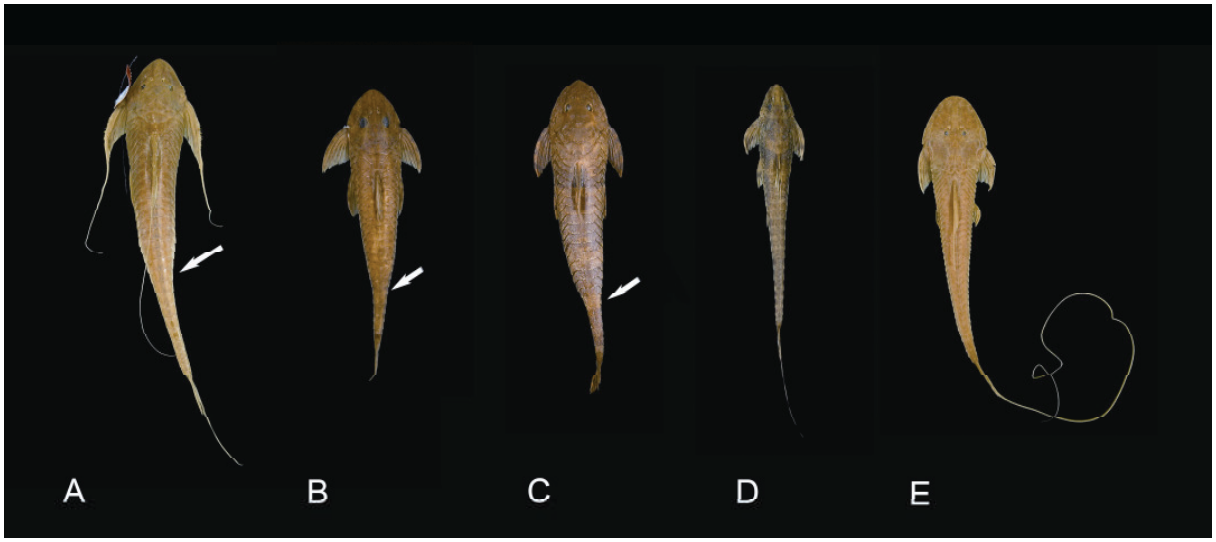

Fig. 6. Dorsal views showing the shape of the caudal peduncle in: A) *Lamontichthys filamentosus* (La Monte) (LBP 162, 181.7 mm L_S), B) *Harttia duriventris* Rapp Py-Daniel & Oliveira (MZUSP 34228, 104.9 mm L_S), C) *Fonchiiloricaria nanodon* (ANSP 138947, paratype, 160.2 mm L_S), D) *R. heteroptera* (LBP 6948, 129.5 mm L_S) and E) *P. cryptodon* (MZUSP 57653, 111.3 mm L_S). White arrow shows the abrupt beginning of the more compressed part of the caudal peduncle.

Fonchiiloricaria nanodon**,** new species

Holotype (Fig. 7)

MUSM 37953, 160.8 mmL_S, Río Huallaga between Tingo Maria and Aucayacu, Leoncio Prado, Huanuco, Peru, 13/09/2008, fishermen, MHNG/MUSM expedition.

Paratypes: all from Peru, Huanuco Department, Leoncio Prado Province: ANSP 138892, 1, 34.7 mm L_S, vicinity of Tingo Maria, back-water near Puerto Nuevo, flowing into Río Tullumayo, and mainstream Río Tullumayo, 25/09/1955, Catherwood Peruvian Expedition station 1; ANSP 138944, 1, 38.2 mm L_S, vicinity of Tingo Maria, Río Huallaga, 24/09/1955; ANSP 138947, 1, 160.2 mm L_S, vicinity of Tingo Maria, back-water near Puerto Nuevo, flowing into Río Tullumayo, and mainstream Río Tullumayo, 25/09/1955, Catherwood Peruvian Expedition station 1; ANSP 138951, 1, 101.5 mm L_S, vicinity of Tingo Maria, Río Rondós (tributary of Río Monzón), just above new bridge site, 29/09/1955, Catherwood Peruvian Expedition station 1; MUSM 10583, 4, 156.11-163.0 mm L_S, Tingo Maria, Río Huallaga, 01/10/1996, F. Chang; MUSM 38338, 1, 170 mm L_S, Río Monzón, 17/10/2007, H. Ortega; MHNG 2603.015, 1, 154.7 mm L_S, Tingo Maria, Río Huallaga, 12/07/1998, F. Chang and M. Velásquez; MHNG 2710.048, 1, 45.4 mm L_S, tributary of Río Huallaga in vicinity of Tingo Maria, Río Monzón at mouth of Quebrada Bella, 12/09/2008, S. Fisch-Muller *et al.*; MHNG 2710.051, 4, 158.1-174.6 mm L_S, same data as holotype; MHNG 2710.060, 1, 56.8 mm L_S, tributary of the Río Huallaga, Río Aucayacu River, 14/09/2008, S. Fisch-Muller *et al.*; MHNG 2710.067, 1, 91.4 mm L_S, Río Huallaga, upstream of Tingo Maria on road to Huanuco, Tingo Maria, 19/09/2008, S. Fisch-Muller *et al.*

Non type material

MUSM 32153, 6 + 2 CS, 157.1-181.3 mm L_S, Tingo Maria, Río Huallaga, Leoncio Prado, Huanuco, Peru, 11/07/1998, F. Chang.

Diagnosis: Same as for genus.

Description: Morphometric data summarized in Table II. General aspect of fish depressed, especially posterior to dorsal fin. Dorsal profile of body convex from snout to dorsal-fin spine, slightly convex from the end of dorsal fin to approximately the middle of caudal peduncle, continuing straight from this point to one or two plates anterior of caudal fin (Fig. 7).

Fig. 7. Holotype of *Fonchiiloricaria nanodon*, MUSM 37953, 160.8 mm L_S, in dorsal (top), lateral (middle), and ventral (bottom) views.

Outline of head triangular in dorsal view, with sides straight or slightly rounded. Upper margin of orbit smooth, not raised. Orbital notch absent. Interorbital region large and flat. Paired anterior postrostral and cheek plates angled ventrally and barely expanded along head margin. Odontodes short, densely covering head, trunk, and fin rays, making fish somewhat hyspid. Snout tip with globular protuberance of naked skin, without odontodes along its ventral region. Rounded naked area not reaching anteriormost pore of infraorbital ramus of sensory canal. Gill opening small.

Mouth rounded with short upper lip (folded inwards) and well-developed lower lip with medial notch (Fig. 4). Large globular papillae arranged in regular rows on surface of lips, except for some modified digitiform papillae, at angles of mouth opening and below dentaries. Distal margin of lower lip fringed with short, triangular filaments. Maxillary barbel very small, often inconspicuous; when evident shorter than one-half length of eye. Premaxillary ramus very reduced; edentulous (n=9) or with one to three (modally two, n=12) small teeth in functional series (many teeth with tips broken off, but included in counts). When present, teeth bicuspid with inner cusp slightly longer than outer cusp. Premaxillary teeth embedded in soft tissue and very small compared to dentary teeth. Dentary ramus with large, well-developed bicuspid teeth in functional series (two to five; modally four, n=21); inner cusp slightly longer than outer cusp.

Abdomen completely covered by small rhombic plates between lateral abdominal plates. Plates reaching gill opening (Fig. 7). Preanal plate large and solid with irregular polygonal shape (usually one plate present; two specimens with two and three preanal plates respectively). Five lateral series of plates. Six to 10 (modally eight, n=13) lateral abdominal plates. Twenty-seven to 30 (modally 28, n=13) plates in median lateral series, with moderately weak keels formed by hypertrophied odontodes. Keels coalesced along last nine to eleven plates (modally 10, n=13). Middorsal series with 12 plates visible in cleared and stained material. Predorsal plates arranged in regular pattern, forming transverse rows. Supraoccipital and predorsal plates without keels.

Posterior margin of dorsal fin straight, with first or second branched ray longest. Tip of depressed dorsal fin reaching third or fourth plate posterior of fin insertion. Dorsal-fin spinelet absent. Posterior margin of pectoral fin concave, unbranched ray longest, reaching beyond the pelvic-fin origin. Posterior margin of pelvic fin straight or slightly rounded; first unbranched ray longest, reaching to or slightly beyond anal-fin origin. Posterior margin of anal fin straight, with first unbranched ray longest. Tip of depressed anal fin, reaching fifth or sixth plate posterior to anal-fin insertion. Three or four ventral plates along anal-fin base. Posterior

Table II: Descriptive morphometrics of the holotype and paratypes of *Fonchiiloricaria nanodon* expressed as percentages of standard length or head length. Ranges provided only include paratypes.

	N	Min	Max	Mean	SD	Holotype
Standard length (mm)	12	91.4	174.6	150.7	-	160.8
Percentage of standard length						
Predorsal length	12	32.9	35.3	34.1	0.76	34.9
Postdorsal length	12	56.5	70.3	64.3	5.62	69.7
Postanal length	12	51.6	56.4	53.0	1.23	53.5
Dorsal-fin spine length	8	19.6	22.2	20.7	0.97	21.5
Anal-fin spine length	11	18.3	21.5	20.0	0.89	20.8
Pectoral-fin spine length	12	17.8	21.3	20.1	1.01	21.7
Pelvic-fin spine length	12	16.4	19.5	17.9	0.82	18.4
Uppermost caudal-fin ray	4	26.6	53.0	41.4	13.51	97.1
Lowermost caudal-fin ray	11	9.9	15.5	13.6	1.64	15.1
Thoracic length	12	13.8	18.2	15.9	1.10	15.5
Abdominal length	12	15.6	18.2	16.8	0.71	18.1
Cleithral width	12	19.1	21.6	20.1	0.78	19.9
Depth of caudal peduncle	12	1.5	1.7	1.6	0.06	1.6
Width of caudal peduncle	12	3.2	3.9	3.5	0.21	3.9
Pelvic-fin origin to caudal-fin	12	66.5	70.5	68.7	1.06	70.6
Snout tip to pelvic-fin origin	12	31.2	34.3	32.4	0.95	32.2
Body width at dorsal-fin origin	12	15.1	20.5	18.6	1.61	18.5
Body depth at dorsal-fin origin	12	13.3	17.4	15.6	1.45	15.1
Body width at anal-fin origin	12	12.8	17.1	15.6	1.22	16.4
Body depth at anal-fin origin	12	10.2	12.6	11.5	0.73	11.4
Head length	12	21.7	23.2	22.4	0.49	22.4
Percentage of head length						
Head depth	12	52.5	79.9	63.5	7.22	56.5
Snout length	12	61.5	64.7	63.1	0.81	62.7
Interorbital width	12	24.8	30.2	27.8	1.65	27.9
Internareal width	12	11.2	13.4	12.2	0.65	12.4
Eye diameter	12	11.5	15.1	13.0	1.18	16.5
Width of lower lip	12	21.4	29.6	26.7	2.27	26.6

margin of caudal fin concave, with 14 rays in total (12 branched rays). Upper unbranched ray extending as a long filament up to 97.1 % L_S. Anal, pelvic and pectoral fins with odontodes on unbranched rays. Usually five (sometimes four) supracaudal plates covering base of caudal-fin rays. Trunk and caudal peduncle becoming more compressed posteriorly, and straight in the lateral margin of last seven to 10 plates of caudal peduncle (Fig. 6).

Barcodes: GenBank accession numbers for the cytochrome *c* oxydase I nucleotide sequences are: paratype MHNG 2710.060 (PE08-336): GU722207; paratype MHNG 2710.048 (PE08-199): GU722208.

Sexual Dimorphism: Males with weakly hypertrophied odontodes on sides of head (also in ventral view) and on dorsal surface of pectoral-fin rays. Such hypertrophied odontodes lacking in females. Unbranched pectoral-fin ray thickened in males (vs. females without unbranched pectoral-fin ray hypertrophied) (Fig. 8)**.**

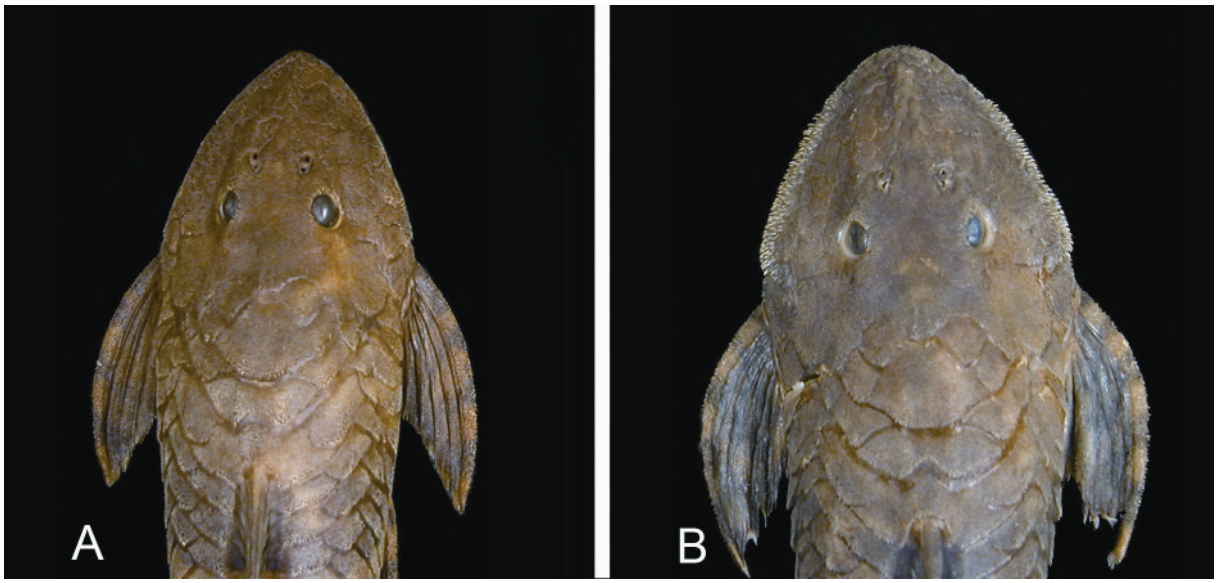

Fig. 8. Sexual dimorphism in *Fonchiiloricaria nanodon* (dorsal view). A) female (ANSP 138947, paratype, 160.2 mm L_S) and B) male (MUSM 32153, 181.4 mm L_S).

Colour in alcohol: Background colour of dorsal surface of head and body brown with four (rarely five) wide, transverse dark brown saddles. First saddle, at origin of dorsal fin, narrowest. Second saddle at end of dorsal-fin base, and last two or three on caudal peduncle. All fin rays yellowish tan with numerous dark brown spots arranged in bands. Caudal fin with conspicuous dark band on its base and numerous small spots close to distal margin, sometimes forming wide black stripe. Ventral surface yellowish, except darkly spotted in one mature male (MUSM 32153, 181.4 mm L_S). Sides of head, snout, and upper lip, frequently with small dark spots or dark vermiculations. Upper caudal filament hyaline.

Colour in life: Young specimens with head and body pale greenish grey anterior to dorsal-fin origin. Transverse saddles almost black. Body pale greyish tan posterior to first saddle. Adults with head brown until dorsal-fin origin and darker than rest of body. Transverse saddles deep brown with first one darker. Body yellowish tan, lighter posterior to first saddle.

Ontogenetic comments: In the examined specimens (ANSP 138892, 34.7 mm L_S; ANSP 138944, 38.2 mm L_S and ANSP 138951, 101.5 mm L_S), the premaxillary teeth are not visible. However, in the largest of these (101.5 mm L_S) their previous presence is suggested by empty holes in the soft overlying tissue. These same specimens have two to four well-developed teeth on each dentary. The lower lip has numerous well-developed digitiform papillae. The abdominal plates are represented by scarce isolated odontodes scattered over the entire abdomen. The lateral abdominal plates are also represented by few odontodes. In ANSP 138892 (34.7 mm L_S), the naked area of the tip of the snout is large. In one specimen (ANSP 138951, 101.5 mm L_S) the abdominal plates are separated by narrow regions of skin. The series of digitiform papillae in the lower lip are more developed than in the other young specimens studied. The naked area of the snout is more reduced in proportion to other young specimens examined, indicating that the size of this area is reduced proportionately during growth of the fish.

Etymology: The name of the genus honours the late Dr. Fonchii Chang, a Peruvian ichthyologist who collected and identified this species as new to science. The specific epithet is from the Greek *nano*, meaning reduced, and *odon*, meaning teeth.

Distribution: The species was collected in the middle Río Huallaga drainage, in the vicinity of Tingo Maria, Peru (around 9°19'22''S 76°01'50''W).

Ecology: This rheophilic species has been collected in the main stream of the Río Huallaga and its tributaries, in swift current, over rocky substrates of stones, shingles, gravels, and sand. Some type specimens (MUSM-MHNG specimens) were collected with representatives of rheophilic fauna such as *Chaetostoma*, *Hypostomus*, *Lamontichthys*, *Spatuloricaria*, *Ancistrus*, *Farlowella*, *Pimelodella*, *Centromochlus*, *Parodon, Hemibrycon, Knodus* and *Eigenmannia*. The type localities are located at an altitude of 600 to 700 meters above sea level, between the eastern slopes of the Andean Cordillera and the western slopes of the Cordillera Azul. In this region the Río Huallaga is shallow during the dry season (30 to 250 cm depth), but may rise four meters after heavy rains, or during the rainy season. The Huallaga is a white water river ranging from 50 to 90 meters width in the main channel during low water level. Dense vegetation grows along the banks. The pH, at various sites along Huallaga River where type specimens were collected, ranged from 7.3 to 7.6 and the conductivity from 250 to 432 $\mu S.cm^{-1}$. The Río Monzón, next to the bridge located seven km

upstream of Tingo Maria, where paratypes MUSM 38338 and MHNG 2710.048 were collected, is a clear water river with 50 cm of visibility, and with sandy areas and pebbles along the shore.

Discussion

The phylogenetic analysis recovers *Fonchiiloricaria nanodon* as a member of the Loricariina. Although support values are low for the clade comprising *Fonchiiloricaria* + other Loricariina, *Fonchiiloricaria* was always recovered as sister to a group of Loricariina genera but never as part of any named genus (Figs. 2 & 3). This justifies its placement in a new genus.

In a recent overview of the morphological diagnostic characters of the different genera of the Loricariinae, Covain *et al.* (2008) demonstrated that several of the characters are strongly autocorrelated with the phylogeny of this group. These include features linked to the morphology of the mouth including mouth shape, tooth shape, lip structure, and the number of premaxillary and dentary teeth. Other features under phylogenetic dependence include the presence or absence of a postorbital notch, the presence or absence of an abdominal plating and the number of caudal-fin rays. In this context, *Fonchiloricaria nanodon* is an unusual species. No other species of the Loricariinae exhibits the extreme reduction in size and number of premaxillary teeth (when not missing) relative to dentary teeth as occurs in *F. nanodon*. The reduction in size and number of teeth coupled with the extreme reduction of the premaxilla is observed among species of the *Pseudohemiodon* group (*Dentectus barbarmatus* Martín Salazar, Isbrücker & Nijssen 1982, *Pyxiloricaria menezesi* Isbrücker & Nijssen 1984, *Pseudohemiodon* spp., *Reganella depressa* (Kner 1853), and *Planiloricaria cryptodon* (Isbrücker 1971), as well as in the *Loricariichthys* group (*Hemiodontichthys acipenserinus* (Kner 1853), *Loricariichthys derbyi* Fowler 1915, and especially *Loricariichthys edentatus* Reis & Pereira 2000). Among members of the *Pseudohemiodon* group, the reduction in size and number of teeth is also associated with changes in shape, in particular with the appearance of spoon shaped teeth.

The general shape of the mouth of *Fonchiiloricaria* is similar to that of *Rineloricaria* and *Ixinandria steinbachi* (Regan 1906) and the presence of digitiform papillae of different degrees of development on the mouth, also occurs in *Rineloricaria daraha* Rapp Py-Daniel &

Fichberg 2008, *Rineloricaria heteroptera* Isbücker & Nijssen 1976, *Metaloricaria paucidens* Isbücker 1975, and *Spatuloricaria* sp.

The postorbital notch is another well known character in the Loricariinae that is generally present in most genera and species of the subtribe Loricariina. The postorbital notch is, however, absent in *Fonchiiloricaria*, *Metaloricaria*, *Loricaria lentiginosa* Isbrücker 1979, *Loricaria prolixa* Isbrücker & Nijssen 1978, and *Loricaria piracicabae* Ihering 1907 (Thomas & Sabaj Pérez, 2010, pers. obs.).

Fonchiiloricaria has an abdominal region entirely plated, as in almost all species of *Rineloricaria*, and has a well-defined preanal plate, whereas it is absent in *Rineloricaria setepovos* Ghazzi 2008*, I. steinbachi*, *P. menezesi*, some species of *Harttia* and *Spatuloricaria*. Many species of the Loricariinae have small platelets in the region anterior of the anus; however the homology of small platelets to the anal plate is untested (for example: *Loricaria apeltogaster* Boulenger*, Loricaria lentiginosa*, *Loricaria prolixa*, *Paraloricaria* spp.*, Brochiloricaria* spp.*, P cryptodon, R. macromystax*, *D. barbarmatus*, *Apistoloricaria* spp.).

In addition, *F. nanodon* shares with *Sturisoma*, *Sturisomatichthys, Lamontichthys, Pterosturisoma microps* (Eigenmann & Allen 1942) of the subtribe Farlowellina the presence of 12 branched caudal-fin rays. This contrasts with the 10 or 11 branched rays of the other genera of Loricariina (Covain & Fisch-Muller 2007). The presence of 12 branched caudal-fin rays is unique to *Fonchiiloricaria* in the Loricariina. Covain *et al*. (2008) noted that the loss of caudal-fin rays in Loricariina was concomitant with the presence of a thicker upper caudal-spine bearing a whip used as a defensive weapon, and hypothesized that these morphological changes could be the outcome of ray fusion. *Fonchiiloricaria* also bears a whip like structure on the upper caudal-fin spine but because it possesses 12 rays, this hypothesis cannot apply. The last seven to 10 plates of the caudal peduncle are abruptly compressed relative to the anterior portion of the body whereas they are gradually compressed in nearly all other genera of the subfamily. This characteristic is also observed in *Harttia* and *Lamontichthys,* groups of rheophilic fishes which also live over stones in swift currents of the main channel of rivers. This abrupt compression of the distal end of the caudal peduncle may represent an adaptation to a benthic life in swiftly flowing currents.

Acknowledgments

We would like to thank M. Sabaj Perez (ANSP), S. Fisch-Muller (MHNG), J.I. Montoya-Burgos (University of Geneva), V. Abilhoa (MHNCI), A. Miquelarena (MLP, ILPLA); R. Reis (MCP); F. Langeani (DZSJRP); F. Carvajal Vallejos (UMSS); M. Retzer (IHNS); J. Lima de Figueiredo, O. Oyakawa and K. Ferreira (MZUSP); C. Oliveira (LBP); E. Cordiviola de Yuan and M. Marchese (INALI); G. Chiaramonte (MACN-Ict); F. Cancino and C. Butí (CI-FML); F. Bockmann, A.C. Aquino and R. M. Corrêa e Castro (LIRP), W. Crampton (University of Central Florida) and Carla Pavanelli (NUP) for diverse types of help. During 2008 MHNG and MUSM´s field trip, RC and HO benefited from help and logistical assistance from M. Hidalgo and V. Meza Vargas (MUSM), T. Pequeno and collaborators from CIMA, P. De Rham, and L. Rodriguez (Conservación de Recursos Naturales). MSR was supported by a postdoctoral fellowship from FAPESP (Process nº 2007/04701-2) and from a grant from the All Catfish Species Inventory (NSF-DEB 0315963). Two anonymous reviewers provided useful comments that leaded to improvements in the manuscript.

References

Bortolussi, N., Durand, E., Blum, M. & François, O. (2006). apTreeshape: statistical analysis of phylogenetic tree shape. *Bioinformatics* **22**, 363–364.

Chiachio, M.C., Oliveira, C. & Montoya-Burgos, J. (2008). Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). *Molecular Phylogenetics and Evolution* **49**, 606-617.

Colless, D. H. (1982). Review of phylogenetics: The theory and practice of phylogenetic systematics. *Systematic Zoology* **31,** 100–104.

Covain, R. & Fisch-Muller, S. (2007). The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: Loricariidae): a practical key and synopsis. *Zootaxa* **1462**, 1- 40.

Covain, R., Dray, S., Fisch-Muller, S. & Montoya-Burgos, J.I. (2008). Assessing phylogenetic dependence of morphological traits using co-inertia prior to investigate character evolution in Loricariinae catfishes. *Molecular Phylogenetics and Evolution* **46**, 986-1002.

Covain, R., Dray, S., Fisch-Muller, S. & Montoya-Burgos, J. (2010). Corrigendum to "Assessing phylogenetic dependence of morphological traits using co-inertia prior to investigate character evolution in Loricariinae catfishes." *Molecular Phylogenetics and Evolution* **55**, 751.

de Carvalho Paixão, A. & Toledo–Piza, M. (2009). Systematics of *Lamontichthys* Miranda-Ribeiro (Siluriformes: Loricariidae), with the description of two new species. *Neotropical Ichthyology* **7,** 519-568.

Efron, B. (1979). Bootstrap methods: Another look at the jackknife. *Annals of Statistics* **7**, 1–26.

Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. (1994). Testing significance of incongruence. *Cladistics* **10**, 315–319.

Felsenstein, J. (1981). Evolutionary trees from DNA-sequences- a maximum-likelihood approach. *Journal of Molecular Evolution* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Ferraris, Jr., C.J. (2007). Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. *Zootaxa* **1418**, 1-628.

Fichberg, I. & Chamon, C.C. (2008). *Rineloricaria osvaldoi* (Siluriformes: Loricariidae): a new species of armored catfish from rio Vermelho, Araguaia basin, Brazil. *Neotropical Ichthyology* **6**, 347-354.

Ghazzi, M.S. (2008). Nove espécies novas do gênero *Rineloricaria* (Siluriformes,Loricariidae) do rio Uruguai, do sul do Brasil. *Iheringia, Sér Zool* **98**, 100-122.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids. Symposium Series* **41**, 95–98.

Ingenito, L.F.S., Ghazzi, M.S., Duboc, L.F. & Abilhoa, V. (2008). Two new species of *Rineloricaria* (Siluriformes: Loricariidae) from the rio Iguaçu basin, southern Brazil. *Neotropical Ichthyology* **6**, 355-366.

Isbrücker, I.J.H. & Nijssen, H. (1974). *Rhadinoloricaria* gen. nov. and *Planiloricaria*, two genera of South American mailed catfishes (Pisces, Siluriformes, Loricariidae). *Beaufortia* **22**, 67-81.

Jobb, G., von Haeseler, A. & Strimmer, K. (2004). TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology* **4**, 18.

Lévêque, C., Oberdorff, T., Paugy, D., Stiassny, M.L.J. & Tedesco, P.A. (2008). Global diversity of fish (Pisces) in freshwater. *Hydrobiologia* **595**, 547-567.

Löytynoja, A. & Milinkovitch, M.C. (2001). SOAP, cleaning multiple alignments from unstable blocks. *Bioinformatics* **17**, 573-574.

Meyer, A. (1993). Evolution of mitochondrial DNA in fishes. In: *Biochemistry and Molecular Biology of Fishes, Volume 2: Molecular Biology Frontiers.* (Hochachka, P.W. & Mommsen, P., eds), pp 1-38. Amsterdam, the Netherlands: Elsevier Science.

Montoya-Burgos, J.I., Muller, S., Weber, C. & Pawlowski, J. (1998). Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences. In: *Phylogeny and Classification of Neotropical Fishes* (Malabarba, L.R, Reis, R.E., Vari, R.P., Lucena, Z.M.S. & Lucena, C.A.S., eds), pp 363-374. Porto Alegre: EDIPUCRS.

Mooers, A.Ø. & Heard, S.B. (1997). Inferring evolutionary process from phylogenetic tree shape. *Quarterly Review of Biology* **72**, 31-54.

Paradis, E. (2006). Analysis of Phylogenetics and Evolution with R. Springer, New York, 211 pp.

Paradis, E., Claude, J. & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289-290.

Rapp Py-Daniel, L. & Fichberg, I. (2008). A new species of *Rineloricaria* (Siluriformes: Loricariidae: Loricariinae) from rio Daraá, rio Negro basin, Amazon, Brazil. *Neotropical Ichthyology* **6**, 339-346.

Rodriguez, M. & Miquelarena, A. (2008). *Rineloricaria isaaci* (Loricariidae: Loricariinae), a new species of loricariid catfish from the Uruguay River basin. *Journal of Fish Biology* **73,** 1635–1647 doi:10.1111/j.1095-8649.2008.02037.x

Rodriguez, M.S. & Reis, R.E. (2008). Taxonomic Review of *Rineloricaria* (Loricariidae: Loricariinae) from the Laguna dos Patos drainage, Southern Brazil, with the descriptions of two new species and the recognition of two species groups. *Copeia* **2008**, 333-349.

Rodriguez, M.S., Cramer, C.A., Bonatto, S.L. & Reis, R.E. (2008). Taxonomy of *Ixinandria* Isbrücker & Nijssen (Loricariidae: Loricariinae) based on morphological and molecular data. *Neotropical Ichthyology* **6**, 367-378.

Rodriguez, F., Oliver, J.L., Marin, A. & Medina, J.R. (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142**, 485-501.

Schaefer, S.A. (1997). The Neotropical cascudinhos: systematics and biogeography of the *Otocinclus* catfishes (Siluriformes: Loricariidae). *Proceedings of the Academy of Natural Sciences of Philadelphia* **148**, 1-120.

Strimmer, K. & Rambaut, A. (2002). Inferring confidence sets of possibly misspecified gene trees. *Proceedings of the Royal Society of London B: Biological Sciences* **269**, 137-142.

Sugiura, N. (1978). Further analysis of the data by Akaike's information criterion and the finite corrections. *Communications in Statististics, Theory and Methods* M **A7**, 13-26.

Swofford, D.L. (2003). PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.

Tamura, K & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512-526.

Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. In: *Some mathematical questions in biology-DNA sequence analysis* (Miura, R.M., ed), pp 57–86. Providence, RI: American Mathematics Society.

Taylor, W.R. & Van Dyke, G.C. (1985). Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* **9**, 107-119.

Thomas, M.R. & Rapp Py-Daniel, L.H. (2008). Three new species of the armored catfish genus *Loricaria* (Siluriformes:Loricariidae) from river channels of the Amazon basin. *Neotropical Ichthyology* **6**, 379-394.

Thomas, M.R. & Sabaj Pérez, M.H. (2010). A new species of whiptail catfish, genus *Loricaria* (Siluriformes: Loricariidae), from the Rio Curuá (Xingu Basin), Brazil. *Copeia* **2010**, 274-283

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876-4882.

Vari, R.P. & Ferraris, Jr., C.J.. (2009). New species of *Cetopsidium* (Siluriformes: Cetopsidae: Cetopsinae) from the upper rio Branco system in Guyana. *Neotropical Ichthyology* **7**, 289-293.

Electronic References

Fricke, R. & Eschmeyer, W.N. (2010). A guide to Fish Collections in the Catalog of Fishes database. On-line version of 15 January 2010. http://research.calacademy.org/redirect?url=http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp.

R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Appendix S1 – Comparative material:

Apistoloricaria ommation Nijssen & Isbrücker, 1988: ANSP 182331, 3 + 1 CS, Rio Amazonas vicinity of Iquitos, Maynas Prov., Loreto dept., Peru; *Aposturisoma myriodon* Isbrücker, Britski, Nijssen & Ortega, 1983: MHNG 2584.029, 1, tributary of Río Aguaytia, Huanuco, Río Huacamayo, Madre de Dios, Peru; *Brochiloricaria chauliodon* Isbrücker, 1979: ILPLA 1357, 1, Laguna El Rey, Río Paraná basin, Santa Fe, Argentina; *Brochiloricaria macrodon* (Kner, 1853): NUP 2248, 4 + 1 CS, Manso dam, affluent of the Río Paraguai, Chapada dos Guimarães, Mato Grosso, Brazil; *Crossoloricaria* aff. *Bahuaja* Chang & Castro, 1999: MHNG 2710.084, 1, tributary of left Río Cushabatay, Quebrada Raya, Ucayali, Peru; *Dasyloricaria filamentosa* (Steindachner, 1878): INHS 60296, 2 + 1 CS, Río Maticora, Caribbean drainage, Falcón, Venezuela; *Dentectus barbarmatus*: ANSP 131631, 4 CS + 5, Hacienda Mozambique, Río Metica, Meta, Colombia; *Farlowella amazonum* (Günther 1864): MZUSP 92763, 5 + 1 CS, Lago do Maiacá, at right side of Rio Amazonas, close to Santarém, Pará, Brazil; *Farlowella nattereri* Steindachner, 1910: MZUSP 57658, 3 + 1 CS, Rio Madeira, Amazonas, Brazil; *Farlowella vittata* Myers, 1942: INHS 28302, 3 + 1 CS, tributary of Río Suripa, , Río Orinoco drainage, Barinas, Venezuela; *Furcodontichthys novaesi* Rapp Py-Daniel, 1981: MZUSP 58191, 7 + 2 CS, Rio Tapajós , Pará, Brazil; *Harttia carvalhoi* Miranda Ribeiro, 1939: MZUSP 48622, 4 + 2 CS, stream affluent of Ribeirão Grande, Pindamonhangaba, São Paulo, Brazil; *Harttia duriventris*: MZUSP 34228, 2 + 2 CS, Igarapé Águas Claras, Rio Itacaiúnas, Serra dos Carajás, Pará, Brazil; *Harttia kronei*: MZUSP 82617, 3 + 1 CS, Iporanga, São Paulo, Rio Betari, Brazil; *Harttia loricariformis* Steindachner, 1877: MZUSP 79390, 3 + 1 CS, Ribeirão Grande, São Paulo, Paraíba do Sul, Brazil; *Harttia maculata* Boeseman, 1971: MHNG 2643.027, 1 CS, Grand rivière Inini, bief, French Guiana, France; *Harttia punctata* Rapp Py-Daniel & Oliveira, 2001: MZUSP 88561, 5 + 2 CS, Serra da Mesa hydroelectric dam, Rio Tocantins basin, Minaçu, Goiás, Brazil; *Harttiella crassicauda* (Boeseman, 1953): MHNG 2674. 053, 1, Ijkreek, Nassau Mountain, Suriname; *Hemiodontichthys acipenserinus*: MZUSP 56804, 5 + 2 CS, Rio Trombetas, Pará, Brazil; *Ixinandria steinbachi*: UMSS 215, 22 + 6 CS, Río Orosas, Aniceto Arce, Tarija Province, Bolivia; *Lamontichthys filamentosus*: LBP 162, 3 + 1 CS, Rio Branco, Rio Acre, Acre State, Brazil; *Lamontichthys llanero* Taphorn & Lilyestrom, 1984: MZUSP 85799, 4 + 1 CS, Río Orituco, Río Orinoco basin, Guarico, Venezuela; *Limatulichthys griseus*

(Eigenmann, 1909): MCP 37161, 1 + 1 CS, Praia Agua Blanca, Río Nanay, Loreto, Peru; *Loricaria apeltogaster*: MCP 12414, 1 + 1 CS, Rio Uruguai, Rio Grande do Sul, Brazil; *Loricaria holmbergi* Rodriguez & Miquelarena, 2005: ILPLA 347, 3 + 1 CS paratypes, Arroyo Agua Caliente, Río San Francisco basin, Santa Bárbara dept., Jujuy, Argentina; *Loricaria lentiginosa:* DZSJRP 1562, 1 + 1 CS, Mendonça–Lima, Rio Grande, São Paulo, Brazil; *Loricaria prolixa* : DZSJRP 6312, 2 + 1 CS, Nova Aliança, Rio Borá between Nova Aliança and Potirendaba, Rio Tietê basin, São Paulo, Brazil; *Loricaria simillima* Regan, 1904: ILPLA 1368, 1 + 1 CS, Puerto Valle, Río Paraná basin, Ituzaingó dept., Corrientes, Argentina; *Loricariichthys anus* (Valenciennes, 1835): MCP 11221, 2 + 1 CS, Lagoa de Cidreira, Tramandai, Rio Grande do Sul, Brazil; *Loricariichthys brunneus* (Hancock 1828): INHS 35491, 3 + 1 CS, Caño Capa, Río Masparro, Río Apuré drainage, El Tambor, Barinas, Venezuela; *Loricariichthys derbyi*: MCP 23378, 2 + 1 CS, stream Pinto on road Pará/ Maranhão (BR-136), Río Parnaíba, Maranhão, Brazil; *Loricariichthys edentatus*: MCP 34612, 1 + 1 CS, Rio Ibicui in Itaqui, Rio Uruguai basin, Rio Grande do Sul, Brazil; *Loricariichthys labialis* (Boulenger, 1895): ILPLA 1284, 2 +1 CS, Riacho Carrizal, Bella Vista, Río Paraná basin, Corrientes, Argentina; *Metaloricaria paucidens*: ANSP 189123, 2 + 1 CS, rivière Litanie at onfluence with Marowini River, Kondre, Sipalawini, Suriname; *Paraloricaria agastor* Isbrücker, 1979: MLP 9623, 3, Yaciretá hydroelectric dam, Río Paraná basin, Argentina; *Planiloricaria cryptodon*: MZUSP 57653, 2 + 1 CS, Rio Amazonas, downstream of Rio Madeira, Amazonas, Brazil; *Pseudohemiodon laticeps* (Regan, 1904): NUP 3462, 10 + 1 CS , Rio Cuiabá, Rio Paraguai basin, Santo Antônio de Leverger, Mato Grosso, Brazil; *Pseudoloricaria laeviuscula* (Valenciennes 1840): MZUSP 8542, 3 + 1 CS, Rio Tapajós, Santarém, Pará, Brazil; *Pterosturisoma microps* (Eigenmann & Allen, 1942): MHNG 2677.072, 1, Aquarium trade import, Iquitos, Peru; *Pyxiloricaria menezesi*: MZUSP 78897, 1, Rio Cachoerinha, , Cáceres, Mato Grosso, Brazil; *Reganella depressa*: MZUSP 57729, 7 + 1 CS, Rio Tapajós, Pará, Brazil; *Ricola macrops* (Regan, 1904): MLP 3874, 3 + 1 CS, San Pedro, Buenos Aires, Argentina; *Rineloricaria aequalicuspis*: MCP 26910, 2 CS, Arroio Carvalho (affluent of Rio Três Forquilhas), Tramandaí, Rio Grande do Sul, Brazil; MCP 23558, 6, stream affluent of Rio Sertão, Mampituba, Santa Catarina, Brazil; *Rineloricaria beni* (Pearson, 1924): MACN-Ict 6895, 4, Río Quizer, Santa Cruz, Bolivia; MACN-Ict 6884, 10 + 1 CS, San Javier, Santa María creek, Bolivia; *Rineloricaria catamarcensis* (Berg, 1895): MACN-Ict 3585, 1 syntype, Catamarca, Argentina; CI-FML 10260, 2 + 2 CS, Río Marapa, Juan Bautista Alberdi, Tucumán, Argentina; *Rineloricaria formosa* Isbrücker & Nijssen, 1979: MZUSP 92132, 3 + 1 CS, Rio Tiquié, Rio Negro basin, Amazonas, Brazil; *Rineloricaria heteroptera*: LBP 6948, 15 + 2 CS, Igarapé Nouba Uba, São Gabriel da Cachoiera, Rio Negro, Amazonas, Brazil; *Rineloricaria isaaci* Rodriguez & Miquelarena, 2008: ILPLA 1715, 1 CS paratype, and MLP 9668, 1 paratype, Arroyo El Pelado,Uruguay River basin, Entre Rios, Argentina; *Rineloricaria lanceolata* (Günther, 1868): MCP 28859, 3 + 2 CS, stream on BR 364, Rio Purus basin, Acre, Brazil; *Rineloricaria latirostris* (Boulenger, 1900): MZUSP 22864, 2 + 1 CS, stream of Píccoli, Río Paraná basin, Corumbataí, São Paulo, Brazil; *Rineloricaria maquinensis* Reis & Cardoso, 2001: MCP 23641, 3, Rio Morto, Santa Catarina, Brazil; MCP 10622, 1 CS, Rio Itoupava, close to Ermo, Araranguá, Santa Catarina, Brazil; *Rineloricaria osvaldoi* Fichberg & Chamon, 2008: LBP 4954, 12 + 3 CS, Rio Vermelho, Rio Araguaia basin, Goiás, Brazil; *Rineloricaria parva* (Boulenger, 1895): INALI 1008, 8, Campo Rostagno, La Capital dept., Santa Fe, Argentina; MCP 40426, 5 CS, Laguna El Rey, Río Salado basin, Santa Fe, Argentina; *Rineloricaria pentamaculata* Langeani & de Araujo, 1994: DZSJRP 10101, 2 + 1 CS, Ribeirão da Quinta, Río Paraná basin, Itatinga, São Paulo, Brazil; *Rineloricaria rupestris* (Schultz, 1944): INHS 34977, 3 + 1 CS, Río Chama, Lago Maracaibo

drainage, El Vigia, Mérida, Venezuela; *Rineloricaria strigilata* (Hensel, 1868): MCP 27304, 4 + 1 CS, Arroio Candiota, Jaguarão, Rio Grande do Sul, Brazil; *Rineloricaria uracantha* (Kner, 1863): ANSP 104110, 5 + 1 CS, creek at 12 mi. W of Santiago, Veraguas Prov., Panamá; *Spatuloricaria* sp: LBP 1616, 1 + 2 CS, Aragarças, Rio Araguaia, Goiás, Brazil; *Sturisoma festivum* Myers, 1942: IHNS 35603, 3 + 1 CS, Río Muyapa, Lago Maracaibo drainage, Muyapa, Mérida, Venezuela; *Sturisoma rostratum* (Spix & Agassiz, 1829): MZUSP 52311, 3 + 1 CS, Rio Araguaia, Mato Grosso, Brazil; *Sturisomatichthys leightoni* (Regan, 1912): ANSP 84177, 1 and ANSP 84178, 1, Honda, Río Magdalena basin, Colombia.

Chapter 3

The Harttiini (Siluriformes, Loricariidae) from the Guianas: a multi-table approach to assess their diversity, evolution, and distribution.

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The MCOA is evaluated in a global assessment of the diversity of a tribe of the Loricariinae, the Harttiini, within the Guianas. Following a first study restricted to a single genus of this tribe in a single country (Covain et al., 2006; Annex 1), genetic, morphometric, and ecological-distributional information of all Guianese populations and species of this tribe are united in the same descriptive frame to reveal underlying evolutionary forces shaping their diversification throughout the Guianas. In addition several new highlighted taxa are described.

Accepted for publication in: *Cybium*. Special issue on the Guianese fish fauna [project deposited by R. Covain and S. Fisch-Muller and accepted by the Société française d'ichtyologie (RC & SFM guest editors)].

ABSTRACT.- The Harttiini are a tribe of Loricariinae poorly characterized morphologically. Within the Guianas (French Guiana, Suriname, and Guyana), six valid species were recognized, including five *Harttia*, and the monotypic *Harttiella crassicauda*. Recent investigations conducted during the last decade by the authors and their co-workers, revealed several unidentified populations of Harttiini. Using a multivariate and multi-table approach unifying morphometry, genetics through DNA barcodes, and ecology-distribution of all populations and species, the global diversity and main evolutionary trends of this group were assessed. The separate analyses highlighted strong structures supporting the validity of three genera: *Harttiella*, *Harttia*, and *Cteniloricaria*, as well as nine new species (six *Harttiella*, two *Harttia*, and one *Cteniloricaria*), and one synonym. The combined analysis established a compromise between the preliminary ones, and revealed their common structure. This structure was found to be linked to the evolutionary history of Harttiini. Their evolution was driven toward adaptations to a definite type of biotope. These included modifications in size and shape, particularly of the caudal peduncle, depending on whether the species colonised rivers or mountainous forest creeks. A longitudinal evolutionary gradient was also highlighted in the geographical distribution of the species despite large overlaps. Notably, *Harttiella* possessed the greatest number of species with the smallest distribution, making each of them highly vulnerable to anthropic perturbations of their environment. Nine new species are described and a key to all species from the Guianas is proposed.

RÉSUMÉ. – Les Harttiini représentent une tribu de Loricariinae assez peu différenciée morphologiquement. Dans les Guyanes (Guyane française, Suriname et Guyana), six espèces valides étaient reconnues, incluant cinq *Harttia* et le monotypique *Harttiella crassicauda*. De récentes collectes réalisées lors de la dernière décennie par les auteurs et leurs collègues, ont révélé la présence de plusieurs populations non identifiées de Harttiini. En utilisant une approche multivariée et multi-tableaux unifiant morphométrie, génétique aux travers des codes barres ADN et écologie-distribution de toutes les populations et espèces, la diversité globale et les principales tendances évolutives de ce groupe ont été évaluées. Les analyses séparées ont révélé de fortes structures supportant la validité de trois genres : *Harttiella*, *Harttia* et *Cteniloricaria*, ainsi que neuf nouvelles espèces (six *Harttiella*, deux *Harttia* et une *Cteniloricaria*) et un synonyme. L'analyse combinée établit un compromis entre les analyses préliminaires et révèle leur structure commune. Cette structure s'est avérée liée à l'histoire évolutive des Harttiini. Leur évolution a conduit à des adaptations à un type défini de biotope. Celles-ci incluent des modifications de taille et de forme, en particulier du pédoncule caudal, selon que l'espèce a colonisé les rivières ou les criques forestières de montagne. Un gradient évolutif longitudinal de la distribution géographique des espèces a également été mis en évidence malgré de forts chevauchements. Le genre *Harttiella* possède ainsi le plus grand nombre d'espèces ainsi que la plus petite distribution, rendant chacune d'entre elles particulièrement vulnérable aux perturbations anthropiques de leur environnement. Neuf nouvelles espèces sont décrites et une clé de toutes les espèces des Guyanes est proposée.

Key words. – Morphometry – DNA barcodes – COI gene - Ecology – Multiple co-inertia analysis – New species descriptions.

INTRODUCTION

The Neotropical freshwaters are home to one quarter of the total world ichthyodiversity, with a prediction of around 8,000 extant species out of a mean estimation of 32,000 (Lévêque *et al*., 2008). In this context, the Guiana Shield region represents one of the most species rich regions of South America, with an estimated 2,200 freshwater fish species, representing one quarter of the Neotropical fish diversity, among which 700 are considered endemic (source WWF: http://wwf.panda.org/about_our_earth/ecoregions/guianan_freshwater.cfm). For the Guiana Shield Vari and Ferraris (2009) listed 1,168 valid species of fish distributed in 15 orders, and 49 families. Therein, excluding lowlands species, they reported 429 species in Guyana, 309 in Suriname with 34% species overlap between these two countries, and 298 in French Guiana with 46% of shared species between French Guiana and Suriname. Planquette *et al*. (1996) listed 429 species in French Guiana alone. Among this tremendous diversity, 80 valid species of Loricariidae were recorded from the three Guianas (Vari and Ferraris, 2009), including 38 species in Guyana, 45 species in Suriname, and 25 species in French Guiana.

The Loricariidae is a highly diversified catfish family comprising about 1,000 species, characterized by a depressed body covered by bony plates, a single pair of maxillary barbels, and by an important modification of the mouth structure into a sucker disk. Recent investigations conducted by the authors in Guyana, Suriname, and French Guiana revealed the presence of several new species and populations of Loricariidae belonging to the tribe Harttiini.

The Harttiini represents a group of rheophilic fishes mainly distributed in the eastern part of South America, in rivers flowing across Brazilian and Guiana Shields. Most inhabit the main stream of rivers over rocky and sandy bottoms, in swift currents where the water is clear and well oxygenated. The systematics of Harttiini has remained confused until now, due to their low morphological diversity. Isbrücker (1979) defined the Harttiini as having the dorsal fin originating approximately opposite to the pelvic-fin origin, the caudal fin with 12 (rarely 11) soft rays, no orbital notch, and little variability in tooth and lip structures. In the same work, Isbrücker and Nijssen described *Cteniloricaria*, distinguishing it from *Harttia* by a slender body shape, a more deeply forked caudal fin, and the abdomen wholly covered by medium sized plates. Isbrücker (1979) placed *Sturisoma*, *Harttia*, *Lamontichthys*, *Harttiella*, *Pterosturisoma*, *Cteniloricaria*, *Sturisomatichthys*, and *Metaloricaria* within Harttiini. Montoya-Burgos *et al.* (1998) proposed the first molecular phylogeny of the family

Loricariidae, and provided evidence that the Harttiini, as defined by Isbrücker (1979), was not a monophyletic assemblage. Isbrücker (in Isbrücker *et al*., 2001) described *Quiritixys* only based on the unusual sexual dimorphism of *Harttia leiopleura*. Rapp Py-Daniel and Oliveira (2001) described seven species of *Harttia*, and put *Cteniloricaria* in the synonymy of *Harttia* mainly based on the characteristics of *H. fowleri* but without consulting the type species: *Cteniloricaria platystoma*. Ferraris (2003, 2007) maintained the validity of *Cteniloricaria*, and put *Quiritixys* in the synonymy of *Harttia*. Provenzano *et al*. (2005), Covain *et al*. (2006), and Covain and Fisch-Muller (2007) maintained the synonymy of *Cteniloricaria* and *Harttia*. The latter also tentatively placed the monotypic genus *Harttiella* into Harttiini, suggesting *Harttiella* as a dwarf form closely related to *Harttia*. Covain *et al.* (2008) proposed the first molecular phylogeny of the subfamily, and redefined the systematics of the Loricariinae. They placed *Metaloricaria* within Loricariina, and *Lamontichthys*, *Farlowella*, *Sturisoma*, and *Sturisomatichthys* within Farlowellina, both subtribes belonging to the tribe Loricariini. In the same work, they restricted Harttiini to *Harttia*. The Harttiini comprises currently 23 valid species, including eight species distributed on the Guiana Shield (including part of Brazil and Venezuela). Within the Guianas (Guyana, Suriname, and French Guiana), six valid species of Harttiini are recorded, including five species of *Harttia*, and one *Harttiella*.

Harttiella crassicauda was initially collected by Geijskens and Creutzberg in the Nassau Mountains during the 1948-1949 Suriname expedition (Bakker and Lanjouw, 1949; Boeseman, 1953). This unusual species was described as a representative of *Harttia* by Boeseman (1953), but due to its particular morphology, Boeseman (1971) created the genus *Harttiella* to accommodate the species. *Harttiella* was characterized by a depressed body shape, broad head, body, and caudal peduncle, the absence of lateral and predorsal keels, the strongly spiny body plates, the naked belly, and a thick caudal peduncle (Boeseman, 1971). The species is only known from the Nassau Mountains in Suriname, an isolated plateau (570 meters above mean sea level) in Northeastern Suriname. Geijskens and Creutzberg described the habitat of *Harttiella crassicauda* as a small forest creek on top of Nassau Mountains, with a rocky bottom covered with sand and stones, and some falls. They located the creek as a tributary of the Marowijne River, but without providing more details. Mol and Ouboter (2004) mentioned that *H. crassicauda* was at risk of extinction or possibly already extinct because of mining activities in Nassau Mountains. However, in 2005 Mol and co-workers collected the species for the second time, 56 years after its original collection. At the same time, they noted that *H. crassicauda* was still an endangered species due to potential degradation of its habitat by both small and large scale mining, and its restricted distribution in a single creek (Mol *et*

al., 2007). By allowing a better grasp of its morphology and ecology, the rediscovery of *H. crassicauda* led to the discovery of additional populations potentially belonging to *Harttiella* in French Guiana.

In the present study, we provide a global assessment on the diversity of Harttiini within the Guianas that includes all species and populations collected during the last decade. Based on a multi-table approach integrating genetics through DNA barcodes, morphometry, and ecology-distribution of the different species and populations, the systematics of Harttiini is revised, their main evolutionary trends are revealed, and new taxa are described.

MATERIAL AND METHODS

Morphometry

This study was based on 622 measured specimens and included all populations and species of Harttiini collected from the Guianas. Part of this material was previously analysed by Covain *et al*. (2006), and is not listed again. The additional material, including type specimens of *H. fowleri*, *H. guianensis*, *H. crassicauda*, *H. platystoma*, and *H. maculata*, was deposited in the Muséum d'histoire naturelle, Geneva (MHNG), the Muséum National d'Histoire Naturelle, Paris (MNHN), the National Museum of Natural History – Naturalis, Leiden (RMNH), the British Museum of Natural History, London (BMNH), the Centre for the Study of Biological Diversity, University of Guyana, Georgetown (CSBD), and the Academy of Natural Sciences, Philadelphia (ANSP).

In the list of measured material, institutional acronyms and catalogue numbers are presented first, followed by the number of specimens in the lot, locality, collector and date of collection. Institutional acronyms follow Fricke and Eschmeyer (2010). To prevent artificial groupings, the different populations collected in different basins were considered independently. The taxonomy followed Covain *et al*. (2006), and the abbreviations used in the different analyses are provided between square brackets.

H. platystoma: **Guyana, Essequibo River drainage [Hplat].** - BMNH 1866.8.14.124 lectotype of *Loricaria platystoma* Günther, 1868, Surinam (?); MHNG 2651.080 (3), CSBD uncat. (2, ex MHNG 2651.080),

Burro-Burro River, 2.5 km upstream of the confluence with the Siparuni River, Montoya-Burgos *et al.*, 2.11.2004; MHNG 2650.093 (2), CSBD uncat. (3, ex MHNG 2650.093), Jamas Rapids at Kurupukari Cross, Montoya-Burgos *et al.*, 31.10.2004; MHNG 2651.035 (3), CSBD uncat. (2, ex MHNG 2651.035), upper Rupununi, near Dadanawa ranch, Montoya-Burgos *et al.*, 26.10.2004; MHNG 2650.090 (2), CSBD uncat. (1, ex MHNG 2650.090), Essequibo River, Kurupukari Cross, Montoya-Burgos *et al.*, 31.10.2004; MHNG 2650.082 (2), CSBD uncat. (3, ex MHNG 2650.082), Siparuni River, just downstream of Georges Creek, Montoya-Burgos *et al.*, 2.11.2004; ANSP 182390 (3), Essequibo River, Kurupukari Cross, Sabaj *et al*., 24.10.2002; ANSP 182341 (6), Kuyuwini River, 48.3 km E of Kuyuwini Landing, 182 km SE of Lethem, Sabaj *et al*., 6.11.2003.

H. maculata: **Suriname, Corantijn River drainage [HmacC].** – RMNH 26381 holotype of *Parasturisoma maculata* Boeseman, 1971, upper Corantijn River basin, Sipaliwini, near airstrip; MHNG 2704.021 (1), Corantijn River, Sir Walter Raleigh's Falls, Montoya-Burgos *et al*., 15.10.2007; MHNG 2704.016 (6), MHNG 2704.017 (7), MHNG 2704.019 (3), Sipaliwini River, Paikali Rapids, Montoya-Burgos *et al*., 14.10.2007; MHNG 2704.015 (2), Sipaliwini River, Yavi Sowa Rave Creek, Montoya-Burgos *et al*., 14.10.2007; MHNG 2704.022 (4), Curuni River, at Sir Walter Raleighwallen, Montoya-Burgos *et al*., 15-16.10.2007; MHNG 2704.027 (5), Sipaliwini River, in rapids, Montoya-Burgos *et al*., 22.10.2007; MHNG 2704.026 (7), Manicouni River, at confluence with Sipaliwini River, Montoya-Burgos *et al*., 20.10.2007; MHNG 2704.024 (10), Sipaliwini River, 15 min upstream of Kwamalasamutu village, Montoya-Burgos *et al*., 17.10.2007; MHNG 2704.020 (11), Corantijn River, in Sir Walter Raleighvallen, Montoya-Burgos *et al*., 15.10.2007. **Suriname, Suriname River drainage [HmacS].** - MHNG 2673.073 (1), Gran Rio River, Assigon, Montoya-Burgos *et al*., 31.10.2005; MHNG 2671.047 (1), Gran Rio River, Cajana around 200 m downstream of Kossindo village, Montoya-Burgos *et al*., 2.11.2005; MHNG 2673.026 (3), Gran Rio River, Cajana Creek, Montoya-Burgos *et al*., 2.11.2005; MHNG 2674.003 (5), Gran Rio River, Awaradam, Montoya-Burgos *et al*., 29.10.2005. **French Guiana-Suriname, Maroni/Marowijn River drainage [HmacM].** - MHNG 2643.001 (1/2), Tampoc River, Pièrkourou Falls, Fisch-Muller *et al*., 13.10.2000; MHNG 2683.037 (1), Crique Voltaire, Voltaire Falls, Fisch-Muller *et al*., 13.11.2006; MHNG 2683.027 (3), Crique Voltaire, Voltaire camp, Fisch-Muller *et al*., 12.11.2006; MHNG 2643.013 (1), Tampoc River, Pièrkourou Falls, Fisch-Muller *et al*., 13.10.2000; MHNG 2643.029 (1), Tampoc River, st. 6, Le Bail and Keith, 17.11.1998; MHNG 2643.027 (4/5), Grand Inini River, in reach, Le Bail *et al*., 28.9.1997; MHNG 2717.042 (26), Paloemeu River, tributary of Tapanahony River at Weyu camp, Montoya-Burgos *et al*., 28-30.10.2008. **French Guiana, Mana River drainage [HmacMn].** – MHNG 2700.054 (1), Crique Aya, 400m downstream of Aya camp, Montoya-Burgos and Melki, 28.11-4.12.2007.

H. surinamensis: **Suriname, Suriname River drainage [Hsur].** - MHNG 2674.007 (27), Gran Rio River, Awaradam, Montoya-Burgos *et al*., 29.10.2005; MHNG 2674.042 (9), Gran Rio River, Cajana around 150 m downstream of Kossindo village, Montoya-Burgos *et al*., 28.10.2005; MHNG 2673.014 (5), Gran Rio River, Cajana near Kossindo, Montoya-Burgos *et al*., 2.11.2005; MHNG 2673.033 (13/33), Gran Rio River, Cajana Creek, Montoya-Burgos *et al*., 2.11.2005.

H. fowleri: **French Guiana, Oyapock River drainage [Hfow].** – MNHN 1901-0372 holotype of *Oxyloricaria fowleri* Pellegrin, 1908, Camopi River; MHNG 2680.091 (18), Oyapock River, Alikoto Falls, Covain *et al*., 3.11.2006; MHNG 2643.023 (2), Oyapock River, upstream of Maripa Falls, Fisch-Muller *et al*., 20.10.1999; MHNG 2681.091 (1), Oyapock River, at mouth of Crique Mouloukoulou, Covain *et al*., 4.11.2006.

H. guianensis: **French Guiana, Approuague River drainage [HguiAp].** – MNHN 1998-0395 holotype of *Harttia guianensis* Rapp Py-Daniel & Oliveira, 2001, Approuague River, Saut Athanase (52°1'W, 4°11'N); MNHN 1998-0396 (2), paratypes, same data as holotype; MHNG 2621.097 (3/7), Approuague River, Mapaou Falls, Weber *et al*., 4.11.2001; MHNG 2662.093 (4), Arataï River, Pararé Falls, Fisch-Muller *et al*., 21.11.2003; MHNG 2662.099 (1), Arataï River, Crique Nourague, Fisch-Muller *et al*., 21.11.2003; MHNG 2662.100 (2), Arataï River, Crique Nourague, Fisch-Muller *et al*., 22.11.2003; MHNG 2662.091 (3), Arataï River, Grand Japigny Falls, Fisch-Muller *et al*., 22.11.2003. **French Guiana, Maroni River drainage [HguiMr].** - MHNG 2643.019 (1), Maroni River, Creek near power station of Antecume Pata, Fisch-Muller and Weber, 20.10.2000; MHNG 2643.010 (3), Litany River, W-SW Falls from Antecume Pata, Jégu *et al*., 24.10.2000. **French Guiana, Sinnamary River drainage [HguiSi].** - MHNG 2661.009 (5), Sinnamary River, Deux Roros Falls, Vigouroux, 17.11.2004; MHNG 2680.053 (3/34), Sinnamary River, Takari Tanté Falls, Vigouroux, 15.10.2003.

H. crassicauda: **Suriname, Marowijn River drainage [Hcras].** - RMNH 19418 (8/15) holotype and paratypes of *Harttia crassicauda* Boeseman, 1953, Nassau Mountains, in creek, Suriname; MHNG 2674.051 (11), MHNG 2674.053 (3), Nassau Mountains, Paramaka Creek N1, Mol, 2.11.2005; MHNG 2679.098 (4/5), Nassau Mountains, Paramaka Creek, Mol, 04.2006.

Unidentified Harttiini: *H*. aff. *maculata* **[Haffmac]. -** MHNG 2704.030 (12), Sipaliwini Savannah, Trio Amerindian territory, Suriname-Brazil border, Four Brothers Mountains, Paru de Oeste River, Trio tribe, 20-21.10.2007. *H*. aff. *trombetensis* **[Hafftrom]. -** MHNG 2704.029 (27), Sipaliwini Savannah, Trio Amerindian territory, Suriname-Brazil border, Four Brothers Mountains, Paru de Oeste River, Trio tribe, 20-21.10.2007. *H*. sp. Coppename **[HCopp]. –** MHNG 2690.012 (7), Suriname, Coppename River at Raleighvallen, Mol, 29.11.2006; MHNG 2690.013 (17), Suriname, Coppename River at Raleighvallen, Mol, 30.11.2006. *H*. sp. Arataï **[HArata]. –** MHNG 2723.094 (16), French Guiana, Approuague River, Balenfois Mountains, Crique Cascades, Gaucher, 02.2008. *H*. sp. Atachi Bakka **[Hatach]. –** MHNG 2723.093 (6), French Guiana, Maroni River, Atachi Bakka Mountains, Gaucher, 06.2009. *H*. sp. Kotika **[HCotic]. –** MHNG 2695.059 (80), French Guiana, Maroni River, Kotika Mountains, Tostain, 05.09.2007. *H*. sp. Lucifer **[Hlucif]. –** MHNG 2721.088 (10), French Guiana, Mana River, Lucifer Mountains, West of Crique Cascade, Montoya-Burgos and Fischer 10.02.2010; MHNG 2721.091 (7), French Guiana, Mana River, Lucifer Mountains, headwater of flowing toward Citron, Montoya-Burgos and Fischer 11.02.2010. *H*. sp. Mana **[HMana]. –** MHNG 2699.070 (45/53), French Guiana, Mana River, Trinité Mountains, Crique Baboune, Crique Aya around 100m in front of Aya Camp, Montoya-Burgos and Melki, 28.11.–04.12.2007; MHNG 2699.098 (4), French Guiana, Trinité Mountains, Mana River, Crique Aya at foot of inselberg, Montoya-Burgos and Melki, 28.11–04.12.2007. *H*. sp. Orapu **[HOrap]. –** MHNG 2682.055 (10), French Guiana, Tortue Mountains, Orapu River, Crique Grillon at ONF camp, Covain *et al.*, 8.11.2006; MHNG 2724.002 (1), French Guiana, Tortue Mountains, Orapu River, Crique Grillon at ONF camp, Vigouroux *et al*., 7.11.2003. *H*. sp. Saul **[Hsaul]. –** MHNG 2712.085 (6), French Guiana, Maroni River, Galbao Mountains, Crique Limonade, Tostain 18.03.2008. *H*. sp. Sinnamary **[HSinna]. –** MHNG 2723.095 (1, ex MHNG 2643.030), French Guiana, Sinnamary River, Crique Coeur Maroni, Le Bail *et al.*, 15.10.1982 or 02.02.1983. *H*. sp. Trinité **[Htrinit]. –** MHNG 2713.087 (5), French Guiana, Sinnamary River, Tabular Mountain of Trinité massif, Crique Grand Leblond, Tostain and Ravet 6.10.2009.

All specimens were measured with a digital calliper to the nearest 0.01 mm. The measurements and counts follow Covain *et al.* (2006) except for: (1) the premaxillary ramus length, due to difficulty and inaccuracy of the measurement; (2) the measurements related to the tail characteristics (total length, upper and lower caudal-spine lengths, and minimum caudal-fin length) due to numerous broken tails; and (3) the angular measurements of the snout α and β because these two measurements are highly correlated to the head depth and cleithral width respectively, and increased consequently redundancy in the dataset. While these seven measurements were excluded, we added the thoracic and abdominal lengths according to Isbrücker (1973), and the distances between the anus and the tip of the snout, and the anus and the insertion of the anal, pelvic and pectoral fins. The dataset therefore included 29 continuous morphometric variables, and 4 discrete meristic variables. The list of variables is provided in table I. Specimens smaller than 20 mm were excluded from the analyses to minimize the bias introduced by allometric growth. Because morphometric data are highly correlated between them, missing data (representing less than 0.45% of the whole data set) were estimated for specimens belonging to a given population using the least squares method with the standard length (SL) used as explanatory variable.

In order to highlight the morphological structure of the species and populations under study, the data were subjected to multivariate analyses. Prior to the analyses, all measurements were standardized by the SL and log transformed. This transformation, equivalent to the additive log ratio of Aitchinson (1986), controls for size effect, preserves and linearizes allometric growth, and prevents spurious correlations of simple ratios (Atchley *et al.*, 1976; Corrucini, 1977; Hills, 1978; Dodson, 1978; Albrecht, 1978; Atchley and Anderson, 1978). The final table included data on 618 specimens of Harttiini, from 23 different populations, and 32 columns. This table was then centered and reduced to allow comparison of variables expressed in different units (here no unit for log ratio transformed measurements, and number of objects for the meristic data), and submitted to a principal components analysis (PCA) to reveal its structuring. PCA was performed with the ade4 1.4-14 (Dray and Dufour, 2007) and ade4TkGUI 0.2-5 (Thioulouse and Dray, 2007) packages in R 2.10.1 (R Development Core Team 2009).

Table I. - Descriptive morphometrics and meristics of all Guianese Harttiini. Morphometric data expressed as percents of standard length (SL) or head length (HL). Abbreviations of the different morphometric variables used in the analyses are provided between square brackets. N: number of specimens measured. Computed statistics include holotype.

	Cteniloricaria platystoma			*Cteniloricaria napova*			*Harttiella crassicauda*		
N	147			12			26		
	range	mean ± sd	lectotype	range	mean ± sd	holotype	range	mean ± sd	holotype
Standard length (SL)	38.36 - 180.26	90.05 ± 41.36	171.40	71.04 - 128.73	111.83 ± 15.36	113.20	24.09 - 47.96	32.40 ± 5.37	47.96
Percents of SL									
Head length [Ltet]	18.48 - 23.93	21.11 ± 1.19	19.59	20.38 - 24.62	21.36 ± 1.09	21.23	23.69 - 28.12	25.83 ± 1.17	23.69
Predorsal length [LpreDo]	27.85 - 32.66	29.91 ± 0.90	29.39	29.97 - 31.41	30.91 ± 0.38	31.05	36.99 - 39.69	38.56 ± 0.74	36.99
Postdorsal length [LpostDo]	58.48 - 64.75	61.60 ± 1.14	61.34	59.61 - 62.08	60.73 ± 0.74	59.62	46.16 - 49.79	48.05 ± 0.92	49.79
Caudal peduncle length [LpostAn]	47.95 - 55.82	51.74 ± 1.17	50.15	49.34 - 53.00	51.03 ± 0.90	50.43	32.77 - 39.79	36.14 ± 1.66	35.53
Abdominal length [Labd]	8.04 - 18.52	16.60 ± 1.05	17.91	15.79 - 17.32	16.81 ± 0.45	16.27	17.49 - 22.17	19.96 ± 1.33	20.52
Thoracic length [Lthor]	11.91 - 19.11	15.99 ± 0.96	15.85	14.89 - 17.09	16.06 ± 0.68	15.85	19.46 - 25.21	21.81 ± 1.65	22.62
Pectoral spine length [Lpect]	18.11 - 28.38	21.65 ± 2.42	27.12	19.56 - 22.10	20.97 ± 0.83	22.10	21.66 - 27.15	24.72 ± 1.73	24.96
Pelvic spine length [Lpelv]	16.10 - 21.26	18.10 ± 1.01	19.05	16.90 - 18.22	17.53 ± 0.42	17.86	17.76 - 25.18	22.86 ± 1.64	22.96
Dorsal spine length [Ldo]	17.84 - 36.27	23.66 ± 3.52	36.27	20.76 - 23.88	22.18 ± 0.90	22.92	17.33 - 27.01	23.76 ± 2.55	17.33
Anal spine length [Lan]	8.01 - 16.91	14.37 ± 1.22	15.72	13.63 - 15.72	14.77 ± 0.67	14.74	11.98 - 18.26	16.23 ± 1.62	13.62
Anus to pelvic-fin origin length [Danpelv]	7.22 - 10.14	8.74 ± 0.60	9.17	8.47 - 9.72	9.00 ± 0.34	9.22	7.78 - 14.10	10.96 ± 1.72	14.10
Anus to pectoral-fin origin length [Danpect]	19.29 - 25.81	23.31 ± 0.93	23.50	22.44 - 25.39	23.92 ± 0.89	23.78	27.89 - 36.62	31.85 ± 2.26	35.43
Anus to anal-fin origin length [Danan]	7.25 - 10.39	8.88 ± 0.52	10.26	8.02 - 9.70	8.94 ± 0.48	8.26	7.97 - 12.79	9.74 ± 1.37	9.07
Anus to tip of snout length [Danmus]	33.84 - 39.68	36.81 ± 0.92	35.96	36.16 - 37.96	37.09 ± 0.58	37.38	44.20 - 50.44	48.09 ± 1.93	49.37
Body width at dorsal-fin origin [lcorDo]	12.64 - 17.31	14.62 ± 0.91	15.54	13.85 - 15.89	15.17 ± 0.61	15.41	16.80 - 22.74	20.06 ± 1.61	21.96
Body width at anal-fin origin [lcorAn]	8.86 - 14.49	12.02 ± 1.06	13.03	10.75 - 12.90	12.06 ± 0.54	12.47	11.72 - 15.34	13.71 ± 1.14	14.95
Body width at eighth postdorsal plate [l8]	6.53 - 11.30	9.34 ± 0.97	10.05	8.13 - 10.07	8.85 ± 0.58	8.52	9.30 - 13.41	11.48 ± 0.94	13.41
Body width at fourteenth postdorsal plate [l14]	2.89 - 4.83	3.96 ± 0.47	4.47	3.51 - 4.61	4.01 ± 0.33	3.85	4.14 - 6.73	5.27 ± 0.75	6.73
Body depth at dorsal-fin origin [Hcor]	6.91 - 11.84	9.16 ± 1.00	9.71	8.60 - 11.08	10.04 ± 0.69	10.24	8.51 - 13.60	11.41 ± 1.41	10.20
Minimum caudal peduncle depth [HminPC]	0.94 - 1.39	1.13 ± 0.10	1.27	1.05 - 1.18	1.11 ± 0.04	1.12	3.88 - 5.59	4.86 ± 0.43	5.36
Head length (HL)	8.96 - 34.90	18.60 ± 7.68	33.57	17.49 - 27.14	23.76 ± 2.63	24.03	6.68 - 11.36	8.32 ± 1.10	11.36
Percents of HL									
Snout length [Lmus]	47.84 - 59.57	53.64 ± 3.05	58.62	43.11 - 55.14	52.04 ± 3.06	55.14	55.48 - 62.82	57.93 ± 1.58	58.80
Nostril to tip of snout length [LnarBM]	32.16 - 44.80	39.16 ± 2.70	43.25	31.05 - 38.37	36.86 ± 1.93	38.37	37.59 - 43.67	40.90 ± 1.51	40.76
Cleithral width [ltet]	71.13 - 95.25	82.81 ± 4.95	87.58	68.32 - 87.40	82.20 ± 4.81	86.23	59.83 - 110.61	97.14 ± 9.54	110.48
Distal end of operculum to tip of snout length [OpercBM]	70.51 - 82.10	77.87 ± 2.11	76.74	67.18 - 80.05	76.51 ± 3.44	80.02	78.57 - 90.63	84.40 ± 2.79	86.80
Maximum orbital diameter [Dmoeil]	18.38 - 28.33	23.47 ± 2.18	18.38	21.56 - 23.70	22.60 ± 0.62	22.43	14.93 - 20.17	17.21 ± 1.20	15.85
Interobital width [Distintorb]	18.80 - 25.60	22.50 ± 1.11	23.41	18.12 - 23.26	21.51 ± 1.29	21.81	30.63 - 38.23	35.58 ± 1.69	37.76
Head depth [Htet]	32.99 - 51.80	40.83 ± 2.85	42.06	35.51 - 44.35	42.21 ± 2.37	43.20	41.17 - 51.05	45.79 ± 2.79	43.49
Head depth at internostril [Hintnar]	23.70 - 37.05	29.69 ± 2.55	32.38	22.64 - 31.62	28.75 ± 2.44	28.80	29.00 - 37.70	33.89 ± 2.22	33.98
Meristic									
Number of premaxillary teeth [Nbdtssup]	21 - 73	40 ± 12	64	35 - 58	47 ± 6	44	20 - 43	29 ± 5	31
Number of dentary teeth [Nbdtsinf]	17 - 64	38 ± 11	64	31 - 48	41 ± 4	39	16 - 39	28 ± 5	24
Number of plates in the lateral series [Nblongit]	28 - 31	30 ± 1	29	30 - 30	30 ± 0	30	24 - 27	26 ± 1	25
Number of lateral abdominal plates [Nbscutvent]	5 - 14	8 ± 2	7	7 - 11	9 ± 1	10	4 - 7	5 ± 1	6

Table I. - Continued 1.

	Harttiella pilosa			*Harttiella parva*			*Harttiella intermedia*		
N	11			6			5		
	range	mean ± sd	holotype	range	mean ± sd	holotype	range	mean ± sd	holotype
Standard length (SL)	22.69 - 40.27	35.05 ± 6.25	39.91	23.73 - 31.27	28.50 ± 2.65	29.54	21.39 - 34.67	27.60 ± 5.81	34.67
Percents of SL									
Head length [Ltet]	22.14 - 27.06	23.74 ± 1.35	23.55	24.27 - 27.82	26.12 ± 1.34	25.22	24.69 - 27.77	26.18 ± 1.12	26.02
Predorsal length [LpreDo]	33.90 - 37.33	35.27 ± 0.93	35.78	37.05 - 38.91	37.72 ± 0.89	37.14	36.37 - 38.76	37.93 ± 1.07	38.62
Postdorsal length [LpostDo]	51.94 - 55.85	53.60 ± 1.45	52.09	49.55 - 53.47	51.77 ± 1.59	51.15	48.90 - 53.44	51.37 ± 1.86	50.74
Caudal peduncle length [LpostAn]	42.34 - 45.46	43.94 ± 0.90	43.72	38.66 - 41.93	40.36 ± 1.06	38.66	40.67 - 43.96	42.35 ± 1.44	40.67
Abdominal length [Labd]	17.21 - 19.28	18.09 ± 0.68	17.94	16.01 - 18.45	17.75 ± 0.91	18.45	16.83 - 20.39	18.43 ± 1.37	20.39
Thoracic length [Lthor]	14.46 - 24.17	18.99 ± 2.35	19.42	19.13 - 23.74	22.47 ± 1.69	23.22	18.52 - 20.97	19.75 ± 0.97	20.45
Pectoral spine length [Lpect]	19.16 - 22.51	21.14 ± 0.98	21.40	22.51 - 25.07	24.00 ± 0.95	24.92	23.16 - 25.12	24.06 ± 0.84	25.12
Pelvic spine length [Lpelv]	19.89 - 21.03	20.55 ± 0.36	20.87	21.09 - 23.05	22.05 ± 0.77	23.05	19.52 - 21.81	20.99 ± 1.02	21.75
Dorsal spine length [Ldo]	19.30 - 22.36	20.98 ± 0.78	20.65	22.08 - 26.61	24.80 ± 1.68	26.61	17.51 - 27.49	23.83 ± 3.74	24.66
Anal spine length [Lan]	14.40 - 18.80	15.73 ± 1.27	16.14	14.50 - 18.11	15.95 ± 1.36	18.11	11.79 - 18.56	16.00 ± 2.54	16.47
Anus to pelvic-fin origin length [Danpelv]	8.34 - 12.20	10.55 ± 1.11	11.05	7.78 - 11.07	9.29 ± 1.42	11.07	10.12 - 12.34	11.31 ± 1.01	12.34
Anus to pectoral-fin origin length [Danpect]	23.50 - 33.53	28.38 ± 2.75	30.04	26.76 - 31.55	29.23 ± 1.81	31.55	28.19 - 31.76	29.16 ± 1.51	31.76
Anus to anal-fin origin length [Danan]	7.08 - 10.59	8.47 ± 1.16	9.60	7.21 - 11.81	9.28 ± 1.81	7.41	6.75 - 9.15	7.79 ± 0.90	7.30
Anus to tip of snout length [Danmus]	40.98 - 45.88	43.32 ± 1.42	43.07	43.91 - 48.54	46.61 ± 1.71	48.54	43.99 - 48.75	46.27 ± 1.90	48.75
Body width at dorsal-fin origin [lcorDo]	16.16 - 19.10	18.09 ± 0.98	18.79	18.51 - 20.92	19.74 ± 1.02	20.01	17.26 - 22.15	20.07 ± 1.81	22.15
Body width at anal-fin origin [lcorAn]	12.09 - 14.80	13.55 ± 0.85	14.31	12.94 - 14.73	13.80 ± 0.72	14.45	11.31 - 14.57	13.03 ± 1.22	14.57
Body width at eighth postdorsal plate [l8]	8.59 - 11.83	10.28 ± 0.98	11.83	10.20 - 12.66	11.00 ± 0.91	12.66	9.63 - 11.94	10.72 ± 0.93	11.94
Body width at fourteenth postdorsal plate [l14]	4.41 - 7.01	5.22 ± 0.75	5.69	4.51 - 6.40	5.59 ± 0.70	6.40	3.72 - 5.28	4.72 ± 0.62	5.05
Body depth at dorsal-fin origin [Hcor]	10.21 - 13.87	11.92 ± 0.97	13.10	9.48 - 11.74	10.34 ± 0.83	10.83	12.22 - 13.46	12.69 ± 0.54	13.04
Minimum caudal peduncle depth [HminPC]	3.05 - 3.74	3.41 ± 0.21	3.68	3.97 - 4.57	4.21 ± 0.21	4.57	3.20 - 3.72	3.46 ± 0.18	3.72
Head length (HL)	6.14 - 9.40	8.25 ± 1.17	9.40	6.50 - 7.96	7.42 ± 0.50	7.45	5.94 - 9.02	7.19 ± 1.33	9.02
Percents of HL									
Snout length [Lmus]	57.33 - 61.76	59.82 ± 1.26	59.26	56.49 - 60.87	58.30 ± 1.46	58.66	55.32 - 60.40	57.70 ± 2.32	55.32
Nostril to tip of snout length [LnarBM]	39.05 - 43.81	41.75 ± 1.44	40.85	40.13 - 43.61	41.56 ± 1.33	40.94	39.41 - 42.86	40.82 ± 1.44	40.69
Cleithral width [ltet]	89.58 - 102.16	95.78 ± 4.04	97.55	94.16 - 105.67	99.93 ± 4.38	104.56	92.90 - 100.50	95.69 ± 3.14	96.90
Distal end of operculum to tip of snout length [OpercBM]	77.85 - 84.32	81.60 ± 1.86	80.96	80.91 - 90.65	84.97 ± 3.46	86.71	80.17 - 86.73	82.71 ± 2.50	82.82
Maximum orbital diameter [Dmoeil]	16.01 - 18.77	17.28 ± 0.89	17.34	16.62 - 18.39	17.58 ± 0.63	18.39	17.07 - 20.12	18.78 ± 1.50	17.07
Interobital width [Distintorb]	32.65 - 38.04	36.06 ± 1.80	36.06	34.42 - 40.00	36.72 ± 2.15	40.00	36.20 - 41.02	38.20 ± 2.03	37.14
Head depth [Htet]	43.82 - 50.62	46.45 ± 1.87	46.60	41.58 - 46.85	43.40 ± 2.11	46.85	44.17 - 49.32	47.02 ± 1.86	46.78
Head depth at internostril [Hintnar]	31.68 - 43.93	36.74 ± 4.00	43.30	31.30 - 40.18	36.14 ± 3.75	37.85	34.32 - 36.30	35.03 ± 0.81	34.37
Meristic									
Number of premaxillary teeth [Nbdtssup]	33 - 46	37 ± 4	33	36 - 53	43 ± 6	53	44 - 59	51 ± 6	52
Number of dentary teeth [Nbdtsinf]	32 - 46	40 ± 5	32	35 - 51	40 ± 6	51	41 - 65	52 ± 9	56
Number of plates in the lateral series [Nblongit]	24 - 27	26 ± 1	26	24 - 25	25 ± 1	25	24 - 25	25 ± 0	24
Number of lateral abdominal plates [Nbscutvent]	5 - 8	6 ± 1	6	5 - 7	6 ± 1	6	5 - 8	6 ± 1	5

Table I. - Continued 2.

	Harttiella lucifer			*Harttiella longicauda*			*Harttiella janmoli*		
N	23			66			80		
	range	mean ± sd	holotype	range	mean ± sd	holotype	range	mean ± sd	holotype
Standard length (SL)	30.99 - 42.68	36.87 ± 3.08	42.68	20.92 - 52.46	36.39 ± 8.26	52.46	22.78 - 47.13	36.88 ± 4.11	47.13
Percents of SL									
Head length [Ltet]	21.81 - 25.49	23.54 ± 0.88	22.75	21.37 - 27.57	24.55 ± 1.34	21.37	22.66 - 30.06	25.54 ± 1.41	24.87
Predorsal length [LpreDo]	33.99 - 38.49	35.95 ± 1.19	35.29	33.07 - 40.57	36.58 ± 1.21	33.07	35.63 - 41.75	38.80 ± 1.17	36.73
Postdorsal length [LpostDo]	50.70 - 56.39	53.75 ± 1.51	53.37	50.47 - 57.85	53.89 ± 1.41	56.73	45.34 - 52.24	48.85 ± 1.33	46.96
Caudal peduncle length [LpostAn]	42.24 - 47.78	44.26 ± 1.48	46.65	39.07 - 49.48	43.61 ± 1.79	46.32	33.73 - 42.58	38.32 ± 1.54	39.23
Abdominal length [Labd]	16.57 - 21.01	18.14 ± 0.92	17.95	12.46 - 21.00	18.40 ± 1.39	17.75	17.55 - 21.46	19.39 ± 0.91	19.07
Thoracic length [Lthor]	19.66 - 23.02	21.12 ± 1.06	22.35	15.85 - 21.40	18.43 ± 0.95	18.38	18.64 - 26.23	22.48 ± 1.62	23.32
Pectoral spine length [Lpect]	20.23 - 25.90	23.09 ± 1.36	24.79	18.92 - 25.91	21.87 ± 1.62	24.13	22.99 - 31.44	27.14 ± 1.54	29.00
Pelvic spine length [Lpelv]	18.76 - 23.16	21.23 ± 1.23	21.93	17.79 - 23.45	19.36 ± 1.17	19.63	21.02 - 31.70	24.67 ± 1.46	24.89
Dorsal spine length [Ldo]	21.24 - 25.94	23.88 ± 1.26	25.94	18.83 - 25.07	21.39 ± 1.50	23.01	21.70 - 28.84	25.25 ± 1.61	24.34
Anal spine length [Lan]	11.97 - 18.15	16.13 ± 1.46	17.03	11.80 - 19.18	15.69 ± 1.43	15.65	15.16 - 25.32	17.77 ± 1.54	19.41
Anus to pelvic-fin origin length [Danpelv]	9.12 - 11.27	10.13 ± 0.63	11.08	7.95 - 12.32	10.44 ± 0.92	11.32	9.57 - 15.38	12.88 ± 0.96	12.09
Anus to pectoral-fin origin length [Danpect]	26.74 - 32.31	29.06 ± 1.57	31.65	23.18 - 30.80	28.46 ± 1.43	28.86	30.45 - 38.64	34.24 ± 1.67	35.77
Anus to anal-fin origin length [Danan]	6.67 - 10.96	8.56 ± 1.17	7.76	6.44 - 12.91	8.33 ± 1.09	6.88	5.66 - 11.77	8.20 ± 1.14	8.47
Anus to tip of snout length [Danmus]	41.78 - 46.67	44.23 ± 1.37	46.51	40.05 - 47.92	44.94 ± 1.66	42.60	46.21 - 52.97	49.81 ± 1.63	49.61
Body width at dorsal-fin origin [lcorDo]	17.04 - 21.61	19.01 ± 1.00	18.13	13.95 - 20.17	17.28 ± 1.11	16.72	20.59 - 25.77	23.46 ± 1.18	21.77
Body width at anal-fin origin [lcorAn]	12.22 - 14.01	13.13 ± 0.48	13.66	9.66 - 13.84	11.99 ± 0.92	13.84	13.26 - 16.91	14.79 ± 0.78	15.85
Body width at eighth postdorsal plate [l8]	8.07 - 11.66	10.39 ± 0.83	10.78	6.37 - 11.61	9.29 ± 1.07	11.61	9.10 - 12.91	11.43 ± 0.72	12.33
Body width at fourteenth postdorsal plate [l14]	3.69 - 5.90	4.95 ± 0.55	5.08	2.61 - 4.88	3.99 ± 0.51	4.88	3.95 - 6.54	5.06 ± 0.50	5.37
Body depth at dorsal-fin origin [Hcor]	9.62 - 12.72	10.66 ± 0.87	10.07	8.17 - 11.57	9.86 ± 0.79	11.08	12.02 - 17.70	14.80 ± 1.14	15.38
Minimum caudal peduncle depth [HminPC]	2.82 - 3.55	3.24 ± 0.21	3.30	1.96 - 2.96	2.53 ± 0.18	2.88	3.97 - 5.26	4.58 ± 0.35	4.73
Head length (HL)	7.72 - 9.71	8.66 ± 0.57	9.71	5.30 - 11.44	8.86 ± 1.74	11.21	6.64 - 11.72	9.38 ± 0.79	11.72
Percents of HL									
Snout length [Lmus]	55.21 - 60.30	58.21 ± 1.36	59.01	54.33 - 65.04	59.05 ± 2.39	56.82	53.64 - 66.74	60.05 ± 2.79	55.20
Nostril to tip of snout length [LnarBM]	38.19 - 43.92	40.93 ± 1.38	39.13	34.84 - 46.74	42.62 ± 2.27	40.32	37.29 - 52.65	41.60 ± 2.55	38.40
Cleithral width [ltet]	93.92 - 107.59	101.65 ± 3.28	103.71	88.25 - 107.89	94.50 ± 4.00	104.91	92.86 - 115.27	104.83 ± 5.24	105.12
Distal end of operculum to tip of snout length [OpercBM]	79.87 - 85.71	83.31 ± 1.66	83.93	77.50 - 100.37	84.86 ± 3.74	81.71	77.70 - 97.41	85.63 ± 4.19	84.90
Maximum orbital diameter [Dmoeil]	14.77 - 18.07	16.64 ± 0.75	16.58	15.57 - 25.51	18.49 ± 2.19	17.31	14.59 - 19.30	16.64 ± 1.02	14.59
Interobital width [Distintorb]	32.12 - 37.24	35.25 ± 1.21	36.35	29.74 - 40.74	33.82 ± 2.03	34.52	32.81 - 43.94	36.04 ± 1.88	33.11
Head depth [Htet]	40.20 - 46.97	44.16 ± 2.01	46.04	37.50 - 48.24	42.51 ± 2.62	44.96	41.60 - 58.50	49.79 ± 3.15	52.05
Head depth at internostril [Hintnar]	30.99 - 43.17	35.34 ± 2.28	33.68	27.99 - 36.53	32.19 ± 1.76	34.17	29.89 - 41.46	34.98 ± 2.79	35.67
Meristic									
Number of premaxillary teeth [Nbdtssup]	25 - 63	46 ± 9	55	21 - 43	32 ± 5	25	23 - 40	31 ± 4	27
Number of dentary teeth [Nbdtsinf]	28 - 67	47 ± 10	52	23 - 41	30 ± 4	23	21 - 44	31 ± 5	28
Number of plates in the lateral series [Nblongit]	25 - 27	26 ± 1	25	25 - 27	26 ± 1	26	23 - 26	25 ± 1	24
Number of lateral abdominal plates [Nbscutvent]	4 - 8	6 ± 1	5	5 - 8	6 ± 1	5	5 - 8	7 ± 1	7

Table I. - Continued 3.

	Harttia guianensis			*Harttia surinamensis*			*Harttia fluminensis*		
N	90			67			24		
	range	mean ± sd	holotype	range	mean ± sd	holotype	range	mean ± sd	holotype
Standard length (SL)	25.20 - 167.00	92.62 ± 31.10	152.36	25.53 - 188.30	104.70 ± 39.54	188.30	67.64 - 151.14	119.06 ± 27.54	151.14
Percents of SL									
Head length [Ltet]	20.72 - 26.63	23.19 ± 1.33	21.03	22.63 - 29.61	24.15 ± 1.38	23.58	22.39 - 24.44	23.30 ± 0.61	23.03
Predorsal length [LpreDo]	29.48 - 35.66	32.10 ± 1.05	31.86	31.65 - 38.74	33.01 ± 1.11	33.30	31.48 - 33.93	32.74 ± 0.61	33.12
Postdorsal length [LpostDo]	53.13 - 60.75	57.35 ± 1.26	58.54	51.74 - 59.98	56.89 ± 1.36	55.92	54.19 - 58.18	56.63 ± 0.94	55.04
Caudal peduncle length [LpostAn]	45.55 - 52.12	48.73 ± 1.29	49.09	45.01 - 49.39	47.67 ± 1.04	45.78	44.97 - 49.87	47.11 ± 1.05	44.97
Abdominal length [Labd]	14.88 - 19.87	17.71 ± 1.03	19.34	14.44 - 19.23	17.51 ± 1.01	18.41	17.09 - 19.99	18.52 ± 0.71	18.94
Thoracic length [Lthor]	14.80 - 19.45	17.30 ± 0.91	17.53	15.85 - 21.90	17.73 ± 0.95	17.55	15.72 - 18.56	17.57 ± 0.75	18.07
Pectoral spine length [Lpect]	18.88 - 29.22	23.14 ± 2.35	24.21	20.08 - 30.05	23.36 ± 2.08	28.20	21.41 - 28.33	24.82 ± 1.86	25.02
Pelvic spine length [Lpelv]	16.34 - 20.63	18.69 ± 0.91	18.46	17.31 - 19.73	18.40 ± 0.56	18.16	17.99 - 19.95	18.84 ± 0.55	19.01
Dorsal spine length [Ldo]	19.29 - 25.07	22.60 ± 1.21	21.91	21.35 - 27.23	25.14 ± 1.24	25.39	23.24 - 27.69	25.01 ± 0.98	23.24
Anal spine length [Lan]	10.83 - 14.29	12.56 ± 0.72	12.73	11.02 - 13.81	12.43 ± 0.66	11.74	12.14 - 14.10	13.16 ± 0.58	13.29
Anus to pelvic-fin origin length [Danpelv]	6.59 - 12.40	10.54 ± 0.91	12.40	7.21 - 11.72	10.63 ± 0.81	11.38	10.32 - 12.16	11.28 ± 0.47	11.93
Anus to pectoral-fin origin length [Danpect]	22.63 - 28.22	25.83 ± 1.15	28.22	24.26 - 27.80	26.10 ± 0.73	26.67	24.93 - 28.07	26.64 ± 0.77	27.12
Anus to anal-fin origin length [Danan]	7.44 - 10.15	8.86 ± 0.54	8.11	6.58 - 9.40	8.38 ± 0.53	8.89	7.94 - 10.00	8.90 ± 0.56	9.07
Anus to tip of snout length [Danmus]	37.16 - 42.41	39.60 ± 1.15	40.82	38.92 - 45.16	40.91 ± 1.21	40.18	39.61 - 41.86	40.74 ± 0.70	41.60
Body width at dorsal-fin origin [lcorDo]	12.30 - 21.51	18.27 ± 1.45	20.64	16.02 - 20.87	18.79 ± 1.07	20.55	17.24 - 21.66	19.54 ± 1.14	21.66
Body width at anal-fin origin [lcorAn]	9.13 - 17.66	14.65 ± 1.39	17.66	9.87 - 17.16	14.93 ± 1.32	16.14	13.79 - 18.10	16.16 ± 1.01	18.10
Body width at eighth postdorsal plate [l8]	8.33 - 14.83	11.82 ± 1.25	14.83	5.52 - 14.46	12.35 ± 1.63	14.13	11.28 - 14.94	13.52 ± 0.99	14.12
Body width at fourteenth postdorsal plate [l14]	3.17 - 6.38	4.89 ± 0.60	6.16	3.60 - 6.88	5.45 ± 0.83	6.48	4.83 - 6.93	5.97 ± 0.64	5.77
Body depth at dorsal-fin origin [Hcor]	6.98 - 10.13	8.25 ± 0.83	9.10	6.74 - 9.93	8.82 ± 0.61	8.71	8.75 - 10.23	9.45 ± 0.37	10.23
Minimum caudal peduncle depth [HminPC]	1.14 - 1.71	1.35 ± 0.11	1.50	1.25 - 1.86	1.44 ± 0.11	1.59	1.40 - 1.81	1.60 ± 0.11	1.81
Head length (HL)	6.50 - 37.70	21.17 ± 6.36	32.04	7.56 - 44.40	24.87 ± 8.72	44.40	16.00 - 34.86	27.64 ± 6.05	34.80
Percents of HL									
Snout length [Lmus]	44.62 - 58.04	54.51 ± 2.03	58.68	51.70 - 61.62	57.02 ± 2.13	59.91	55.25 - 81.68	58.89 ± 5.12	59.02
Nostril to tip of snout length [LnarBM]	32.31 - 45.04	41.04 ± 1.85	45.69	40.21 - 47.97	43.71 ± 1.55	45.50	23.77 - 46.39	42.62 ± 4.27	44.43
Cleithral width [ltet]	69.23 - 110.77	94.10 ± 7.87	110.49	76.06 - 103.94	91.84 ± 5.80	101.13	87.62 - 104.40	96.38 ± 4.37	104.40
Distal end of operculum to tip of snout length [OpercBM]	66.15 - 83.27	78.51 ± 2.47	83.61	72.62 - 84.55	80.54 ± 2.37	81.08	79.53 - 84.43	81.99 ± 1.32	83.88
Maximum orbital diameter [Dmoeil]	19.63 - 25.26	23.37 ± 1.08	22.22	19.11 - 26.67	22.71 ± 1.49	20.72	20.73 - 25.69	22.68 ± 1.43	23.56
Interobital width [Distintorb]	19.54 - 26.26	22.04 ± 1.27	26.03	19.99 - 25.16	22.58 ± 1.07	23.87	21.88 - 26.18	24.25 ± 1.02	26.18
Head depth [Htet]	29.31 - 41.79	35.27 ± 2.23	41.92	23.41 - 41.45	37.19 ± 2.47	34.01	37.31 - 43.30	38.94 ± 1.42	43.30
Head depth at internostril [Hintnar]	23.15 - 33.95	27.61 ± 2.19	31.99	20.77 - 34.70	28.77 ± 2.52	29.73	28.01 - 36.93	31.14 ± 2.52	36.93
Meristic									
Number of premaxillary teeth [Nbdtssup]	15 - 103	76 ± 14	88	10 - 113	78 ± 19	105	63 - 113	93 ± 13	99
Number of dentary teeth [Nbdtsinf]	18 - 104	75 ± 15	97	29 - 116	77 ± 16	88	64 - 115	94 ± 14	105
Number of plates in the lateral series [Nblongit]	27 - 31	29 ± 1	30	27 - 30	29 ± 1	27	29 - 30	29 ± 0	29
Number of lateral abdominal plates [Nbscutvent]	0 - 9	7 ± 1	9	0 - 11	8 ± 2	8	6 - 9	7 ± 1	7

Table I. - Continued 4.

	Harttia guianensis			*Harttia surinamensis*			*Harttia fluminensis*		
N	90			67			24		
	range	mean ± sd	holotype	range	mean ± sd	holotype	range	mean ± sd	holotype
Standard length (SL)	25.20 - 167.00	92.62 ± 31.10	152.36	25.53 - 188.30	104.70 ± 39.54	188.30	67.64 - 151.14	119.06 ± 27.54	151.14
Percents of SL									
Head length [Ltet]	20.72 - 26.63	23.19 ± 1.33	21.03	22.63 - 29.61	24.15 ± 1.38	23.58	22.39 - 24.44	23.30 ± 0.61	23.03
Predorsal length [LpreDo]	29.48 - 35.66	32.10 ± 1.05	31.86	31.65 - 38.74	33.01 ± 1.11	33.30	31.48 - 33.93	32.74 ± 0.61	33.12
Postdorsal length [LpostDo]	53.13 - 60.75	57.35 ± 1.26	58.54	51.74 - 59.98	56.89 ± 1.36	55.92	54.19 - 58.18	56.63 ± 0.94	55.04
Caudal peduncle length [LpostAn]	45.55 - 52.12	48.73 ± 1.29	49.09	45.01 - 49.39	47.67 ± 1.04	45.78	44.97 - 49.87	47.11 ± 1.05	44.97
Abdominal length [Labd]	14.88 - 19.87	17.71 ± 1.03	19.34	14.44 - 19.23	17.51 ± 1.01	18.41	17.09 - 19.99	18.52 ± 0.71	18.94
Thoracic length [Lthor]	14.80 - 19.45	17.30 ± 0.91	17.53	15.85 - 21.90	17.73 ± 0.95	17.55	15.72 - 18.56	17.57 ± 0.75	18.07
Pectoral spine length [Lpect]	18.88 - 29.22	23.14 ± 2.35	24.21	20.08 - 30.05	23.36 ± 2.08	28.20	21.41 - 28.33	24.82 ± 1.86	25.02
Pelvic spine length [Lpelv]	16.34 - 20.63	18.69 ± 0.91	18.46	17.31 - 19.73	18.40 ± 0.56	18.16	17.99 - 19.95	18.84 ± 0.55	19.01
Dorsal spine length [Ldo]	19.29 - 25.07	22.60 ± 1.21	21.91	21.35 - 27.23	25.14 ± 1.24	25.39	23.24 - 27.69	25.01 ± 0.98	23.24
Anal spine length [Lan]	10.83 - 14.29	12.56 ± 0.72	12.73	11.02 - 13.81	12.43 ± 0.66	11.74	12.14 - 14.10	13.16 ± 0.58	13.29
Anus to pelvic-fin origin length [Danpelv]	6.59 - 12.40	10.54 ± 0.91	12.40	7.21 - 11.72	10.63 ± 0.81	11.38	10.32 - 12.16	11.28 ± 0.47	11.93
Anus to pectoral-fin origin length [Danpect]	22.63 - 28.22	25.83 ± 1.15	28.22	24.26 - 27.80	26.10 ± 0.73	26.67	24.93 - 28.07	26.64 ± 0.77	27.12
Anus to anal-fin origin length [Danan]	7.44 - 10.15	8.86 ± 0.54	8.11	6.58 - 9.40	8.38 ± 0.53	8.89	7.94 - 10.00	8.90 ± 0.56	9.07
Anus to tip of snout length [Danmus]	37.16 - 42.41	39.60 ± 1.15	40.82	38.92 - 45.16	40.91 ± 1.21	40.18	39.61 - 41.86	40.74 ± 0.70	41.60
Body width at dorsal-fin origin [IcorDo]	12.30 - 21.51	18.27 ± 1.45	20.64	16.02 - 20.87	18.79 ± 1.07	20.55	17.24 - 21.66	19.54 ± 1.14	21.66
Body width at anal-fin origin [IcorAn]	9.13 - 17.66	14.65 ± 1.39	17.66	9.87 - 17.16	14.93 ± 1.32	16.14	13.79 - 18.10	16.16 ± 1.01	18.10
Body width at eighth postdorsal plate [I8]	8.33 - 14.83	11.82 ± 1.25	14.83	5.52 - 14.46	12.35 ± 1.63	14.13	11.28 - 14.94	13.52 ± 0.99	14.12
Body width at fourteenth postdorsal plate [I14]	3.17 - 6.38	4.89 ± 0.60	6.16	3.60 - 6.88	5.45 ± 0.83	6.48	4.83 - 6.93	5.97 ± 0.64	5.77
Body depth at dorsal-fin origin [Hcor]	6.98 - 10.13	8.25 ± 0.83	9.10	6.74 - 9.93	8.82 ± 0.61	8.71	8.75 - 10.23	9.45 ± 0.37	10.23
Minimum caudal peduncle depth [HminPC]	1.14 - 1.71	1.35 ± 0.11	1.50	1.25 - 1.86	1.44 ± 0.11	1.59	1.40 - 1.81	1.60 ± 0.11	1.81
Head length (HL)	6.50 - 37.70	21.17 ± 6.36	32.04	7.56 - 44.40	24.87 ± 8.72	44.40	16.00 - 34.86	27.64 ± 6.05	34.80
Percents of HL									
Snout length [Lmus]	44.62 - 58.04	54.51 ± 2.03	58.68	51.70 - 61.62	57.02 ± 2.13	59.91	55.25 - 81.68	58.89 ± 5.12	59.02
Nostril to tip of snout length [LnarBM]	32.31 - 45.04	41.04 ± 1.85	45.69	40.21 - 47.97	43.71 ± 1.55	45.50	23.77 - 46.39	42.62 ± 4.27	44.43
Cleithral width [ltet]	69.23 - 110.77	94.10 ± 7.87	110.49	76.06 - 103.94	91.84 ± 5.80	101.13	87.62 - 104.40	96.38 ± 4.37	104.40
Distal end of operculum to tip of snout length [OpercBM]	66.15 - 83.27	78.51 ± 2.47	83.61	72.62 - 84.55	80.54 ± 2.37	81.08	79.53 - 84.43	81.99 ± 1.32	83.88
Maximum orbital diameter [Dmoeil]	19.63 - 25.26	23.37 ± 1.08	22.22	19.11 - 26.67	22.71 ± 1.49	20.72	20.73 - 25.69	22.68 ± 1.43	23.56
Interobital width [Distintorb]	19.54 - 26.26	22.04 ± 1.27	26.03	19.99 - 25.16	22.58 ± 1.07	23.87	21.88 - 26.18	24.25 ± 1.02	26.18
Head depth [Htet]	29.31 - 41.79	35.27 ± 2.23	41.92	23.41 - 41.45	37.19 ± 2.47	34.01	37.31 - 43.30	38.94 ± 1.42	43.30
Head depth at internostril [Hintnar]	23.15 - 33.95	27.61 ± 2.19	31.99	20.77 - 34.70	28.77 ± 2.52	29.73	28.01 - 36.93	31.14 ± 2.52	36.93
Meristic									
Number of premaxillary teeth [Nbdtssup]	15 - 103	76 ± 14	88	10 - 113	78 ± 19	105	63 - 113	93 ± 13	99
Number of dentary teeth [Nbdtsinf]	18 - 104	75 ± 15	97	29 - 116	77 ± 16	88	64 - 115	94 ± 14	105
Number of plates in the lateral series [Nblongit]	27 - 31	29 ± 1	30	27 - 30	29 ± 1	27	29 - 30	29 ± 0	29
Number of lateral abdominal plates [Nbscutvent]	0 - 9	7 ± 1	9	0 - 11	8 ± 2	8	6 - 9	7 ± 1	7

Table I. - End.

	Harttia tuna			*Harttia fowleri*		
N	27			34		
	range	mean ± sd	holotype	range	mean ± sd	holotype
Standard length (SL)	36.93 - 172.21	117.43 ± 33.17	170.95	39.17 - 221.64	140.89 ± 49.13	143.92
Percents of SL						
Head length [Ltet]	23.52 - 28.29	24.97 ± 1.14	25.46	22.07 - 27.09	23.45 ± 0.95	23.52
Predorsal length [LpreDo]	31.86 - 35.40	33.58 ± 0.84	35.40	30.85 - 34.83	32.49 ± 0.91	32.78
Postdorsal length [LpostDo]	53.14 - 57.28	55.44 ± 1.10	53.77	54.04 - 59.36	57.19 ± 1.25	56.81
Caudal peduncle length [LpostAn]	44.04 - 48.66	46.54 ± 1.17	44.04	44.96 - 50.73	47.66 ± 1.29	46.79
Abdominal length [Labd]	14.09 - 20.66	18.34 ± 1.29	20.00	15.54 - 19.91	17.91 ± 1.18	19.03
Thoracic length [Lthor]	16.31 - 20.39	17.63 ± 0.80	16.82	16.63 - 19.57	18.20 ± 0.84	17.26
Pectoral spine length [Lpect]	19.80 - 27.37	23.13 ± 1.94	27.37	19.24 - 37.23	27.99 ± 4.22	21.75
Pelvic spine length [Lpelv]	16.49 - 19.03	17.96 ± 0.66	18.97	17.55 - 28.70	20.18 ± 2.30	17.55
Dorsal spine length [Ldo]	20.10 - 24.50	23.05 ± 1.21	23.30	20.00 - 28.15	24.98 ± 1.78	22.37
Anal spine length [Lan]	10.53 - 14.70	12.37 ± 0.83	14.70	11.90 - 16.79	14.28 ± 1.27	12.15
Anus to pelvic-fin origin length [Danpelv]	9.02 - 12.87	11.10 ± 0.84	12.87	8.04 - 12.01	11.02 ± 0.98	11.42
Anus to pectoral-fin origin length [Danpect]	24.08 - 27.89	26.39 ± 1.01	27.50	24.74 - 29.22	27.32 ± 1.23	27.11
Anus to anal-fin origin length [Danan]	6.72 - 9.85	8.76 ± 0.59	9.09	7.44 - 9.37	8.52 ± 0.46	8.55
Anus to tip of snout length [Danmus]	40.28 - 44.11	41.84 ± 1.02	43.43	38.70 - 43.11	41.24 ± 1.02	40.36
Body width at dorsal-fin origin [IcorDo]	14.76 - 21.79	19.58 ± 1.62	21.57	16.13 - 21.78	18.92 ± 1.22	19.29
Body width at anal-fin origin [IcorAn]	11.21 - 17.31	15.65 ± 1.47	17.16	11.18 - 16.36	14.56 ± 1.45	16.10
Body width at eighth postdorsal plate [I8]	9.07 - 14.90	13.28 ± 1.47	14.90	7.89 - 14.61	11.69 ± 1.32	14.61
Body width at fourteenth postdorsal plate [I14]	3.49 - 7.49	5.80 ± 0.89	6.30	3.27 - 6.69	5.01 ± 0.60	6.69
Body depth at dorsal-fin origin [Hcor]	7.79 - 9.73	8.64 ± 0.47	9.73	8.10 - 10.51	9.54 ± 0.67	8.20
Minimum caudal peduncle depth [HminPC]	1.26 - 1.80	1.43 ± 0.11	1.80	1.17 - 1.59	1.35 ± 0.13	1.38
Head length (HL)	10.42 - 43.52	29.10 ± 8.05	43.52	10.08 - 49.92	32.78 ± 11.16	32.90
Percents of HL						
Snout length [Lmus]	52.11 - 61.03	58.16 ± 2.16	60.89	52.18 - 61.03	56.52 ± 2.01	61.03
Nostril to tip of snout length [LnarBM]	38.68 - 45.88	43.94 ± 1.69	44.58	35.62 - 47.16	42.42 ± 2.21	44.83
Cleithral width [ltet]	72.74 - 102.55	93.29 ± 6.51	101.24	79.96 - 105.49	95.23 ± 5.96	95.41
Distal end of operculum to tip of snout length [OpercBM]	76.98 - 84.44	81.12 ± 1.66	82.86	74.67 - 84.58	79.68 ± 2.24	82.94
Maximum orbital diameter [Dmoeil]	18.37 - 23.97	20.97 ± 1.12	19.12	20.04 - 27.28	23.04 ± 1.65	21.27
Interobital width [Distintorb]	21.09 - 25.67	23.12 ± 1.11	25.67	19.95 - 25.10	22.10 ± 1.24	23.15
Head depth [Htet]	31.96 - 38.77	36.52 ± 1.80	38.07	29.91 - 42.78	39.75 ± 2.37	37.79
Head depth at internostril [Hintnar]	24.66 - 33.47	28.66 ± 1.93	31.07	27.23 - 36.02	31.13 ± 1.99	28.70
Meristic						
Number of premaxillary teeth [Nbdtssup]	45 - 131	91 ± 19	131	45 - 100	79 ± 12	74
Number of dentary teeth [Nbdtsinf]	40 - 129	91 ± 19	129	40 - 83	69 ± 9	65
Number of plates in the lateral series [Nblongit]	29 - 30	29 ± 0	30	29 - 30	29 ± 0	30
Number of lateral abdominal plates [Nbscutvent]	5 - 9	7 ± 1	9	6 - 13	10 ± 2	10

Genetics

To provide further evidence for the assessment of the global diversity of the Guianese Harttiini, the standard 648-bp 5' region of the cytochrome *c* oxidase I (COI) mitochondrial gene used for DNA barcodes was amplified. This DNA marker was sequenced in a total of 42 specimens representing 21 populations and comprising at least one specimen per population. The list of material used for this analysis is provided in table II. Ethanol preserved tissue samples are deposited in MHNG. Total genomic DNA was extracted with the DNeasy Tissue Kit (Qiagen) following the instructions of the manufacturer. The PCR amplifications were carried out using the Taq PCR Core Kit (Qiagen). The primers used were Fish-F1 and Fish-R1 (Ward *et al.*, 2005). The amplifications were performed in a total volume of 50 µl, containing 5 µl of 10x reaction buffer, 1 µl of dNTP mix at 10mM each, 1 µl of each primer at 10 µM, 0.2 µl of *Taq* DNA Polymerase equivalent to 1 unit of Polymerase per tube, and 1 µl of DNA. Cycles of amplification were programmed with the following profile: (1) 3 min. at 94°C (initial denaturing), (2) 35 sec. at 94°C, (3) 30 sec. at 54°C, (4) 50 sec. at 72°C, and (5) 5 min. at 72°C (final elongation). Steps 2 to 4 were repeated 39 times. PCR products were purified with the High Pure PCR Product Purification Kit (Roche). Sequencing reactions were performed with the Big Dye Terminator Cycle Sequencing Ready Reaction 3.1 Kit (Applied Biosystems) following instructions of the manufacturer, and were loaded on an automatic sequencer 3100-Avant Genetic Analyzer (Applied Biosystems, Perkin-Elmer). The sequences were deposited in GenBank, and accession numbers are provided in table II.

The DNA sequences were edited and assembled using BioEdit 7.0.1 (Hall, 1999), and aligned manually since the coding COI gene aligned unambiguously in a single block. The GC content and base composition were computed using the seqinr 2.0-9 package (Charif and Lobry, 2007) in R, and usual tests of homogeneity of nucleotide frequencies and substitution saturation (Xia *et al.*, 2003) were performed using Dambe 4.5.56 (Xia and Xie, 2001). The alignment was secondarily converted into a distance matrix using the Kimura 2 Parameters (K2P) metrics (Kimura, 1980) as implemented in ape 2.5 (Paradis *et al.,* 2004; Paradis, 2006) in R, to evaluate sequence divergence. A Neighbour Joining (NJ) tree (Saitou and Nei, 1987) was reconstructed on this distance matrix to provide a cluster ordination of the species. This ordination did not correspond to a phylogeny, but rather to a group assignment using distances between sequences (whatever their evolutionary history). The NJ algorithm has the advantage over other agglomerative partitioning methods to preserve distances into branch lengths, and consequently to not enforce artificially the grouping of species (e. g. using a mean distance between clusters). To estimate robustness of the groupings, a nonparametric bootstrap

analysis (Efron, 1979) was performed following Felsenstein's (1985) methodology using 9,999 pseudoreplicates. In addition, a levelplot graph allowing a graphical representation of the distance matrix was computed using the lattice 0.18-3 (Sarkar, 2010) and colorRamps 2.3 (Keitt, 2009) packages in R. In a second analysis, the distance matrix was explored by a principal coordinate analysis (PCoA) (Gower, 1966) using Cailliez's (1983) correction for non Euclidian distance matrices, to reveal its structuring onto axes. This analysis provides a tree-free representation of the distance matrix, where the pairwise distances between OTUs are equal to the genetic pairwise distances of the matrix.

Ecology and distribution

To highlight the environmental parameters structuring the different species and populations, four environmental variables and three distributional variables were analyzed. Environmental parameters [pH, conductivity, temperature, and habitat (main channel of rivers or creeks)] and distributional information (latitude, longitude, and altitude) were obtained from the field, the literature (Horeau *et al.,* 1998; Négrel and Lachassagne, 2000; de Mérona, 2005; Sondag *et al.,* 2010), or generously provided by co-workers (B. de Mérona, IRD Cayenne; P. Gaucher, CNRS Guyane; R. Vigouroux, Hydreco Guyane; and O. Tostain, Ecobios Cayenne). Environmental data extracted from the literature were included only if specimens examined by Covain *et al.* (2006) were from exactly the same localities. The final table included 88 rows, corresponding to 19 populations and species, of which nine contained missing values. This dataset was submitted to multivariate analyses using the Non-linear Iterative Partial Least Squares (NIPALS) algorithm (Wold, 1966; Dray *et al.*, 2003) as implemented in ade4 1.4-14. This algorithm allows for PCA analysis on a table with missing data, and does not require the deletion of rows or variables containing missing values. The algorithm is based on successive linear regressions using an iterative procedure (Tenenhaus, 1998) and reconstructs the complete table (i.e. estimation of missing values) for further analyses.

Multi-table analysis

To synthesize the various types of information concerning Harttiini presented above (genetics, morphometry, and distribution-ecology), and identify the possible common structures present within all data sets, the three tables were linked by a multiple co-inertia analysis (MCOA) (Chessel and Hanafi, 1996). Prior to the analysis, all tables were restricted to the subset of populations (n = 19) for which the three types of information were available.

Each of the three reduced tables was reanalyzed separately (PCoA for the genetic data, and PCA for the morphometric and ecological data) to reveal their structuring. Within-population variability was eliminated by the computation of average values for each population. The table reconstructed by the NIPALS algorithm was used for the ecological table. A first assessment of a possible link between the three tables was obtained using the Congruence Among Distance Matrices (CADM) test (Legendre and Lapointe, 2004) as implemented in ape 2.5 in R. The CADM test is a generalization of the Mantel test (Mantel, 1967) to test the null hypothesis of incongruence between several distance matrices. Additionally, an *a posteriori* procedure allows testing for the incongruence of a single distance matrix with respect to the other ones. A Holm's (1979) correction for multiple testing is applied for *a posteriori* tests. Pairwise Mantel correlations of the rank-transformed distances between matrices can also be computed to estimate the strength of the link between each pair of matrices. The CADM test was computed using 9,999 permutations of the three distances matrices. Prior to its computation, Euclidian distances were estimated from the mean populationals' scores of the two PCAs.

MCOA identifies the common structure in all data sets by providing a consensual typology (the compromise) maximizing the link with all tables simultaneously. This link is expressed by the sum of squared covariances between the linear combinations of the variables of each table and the compromise.

Subsequently, in order to interpret the results provided by the MCOA from an evolutionary perspective, MCOA axes and associated variables were submitted to a test of phylogenetic autocorrelation (Abouheif, 1999; Pavoine *et al.*, 2008). This test is equivalent to a Moran's I (Moran, 1950) test of autocorrelation and was designed to detect similarities among adjacent observations in quantitative traits. The test was computed using the adephylo 1.1-0 package (Jombart *et al.,* 2010) in R using 9,999 random permutations. A control for false discovery rate for multiple testing under dependency (Benjamini and Yekutieli, 2001) was applied since all tested variables may be proved to be phylogenetically dependent. The phylogenetic tree used for comparison was obtained from a study currently in progress (Covain *et al.,* 2009a; 2009b; 2009c). The tree topology was computed using probalistic methods on a partitioned data set mixing mitochondrial and nuclear information. The COI marker was not used for this study, providing therefore a relatively independent observation.

RESULTS

Morphometric analysis of all populations and species of Guianese Harttiini

Morphological data were mainly structured on the first two axes of PCA (Fig. 1c) that accounted for 70.34% of the total variation (53.02% for axis 1 and 17.32% for axis 2). The first axis split the Harttiini into three groups (Fig. 1a) corresponding to *Harttiella* representatives on the negative side, followed by representatives of *Harttia*, and finally representatives of the former *Cteniloricaria* except *H. fowleri*. The second axis split *Harttia* representatives on the negative side, from *Harttiella* and the former *Cteniloricaria* representatives. Three morphological groups were consequently recognised and named: *Cteniloricaria*, *Harttia*, and *Harttiella* groups. On the positive side of axis 1, the *Cteniloricaria* group corresponded to high values for maximum orbital diameter, number of plates in the lateral series, caudal peduncle length, and postdorsal length (Fig. 1b). It consisted of representatives of the type species of the genus *C. platystoma,* different populations of *H. maculata* (Corantijn, Suriname, Maroni, and Mana Rivers) and a population from Paru de Oeste River. On the second axis, the *Harttia* group was characterized by high negative values for the number of dentary teeth, number of premaxillary teeth, body width at eighth postdorsal plate, body width at anal-fin origin, and body width at fourteenth postdorsal plate (Fig. 1b).

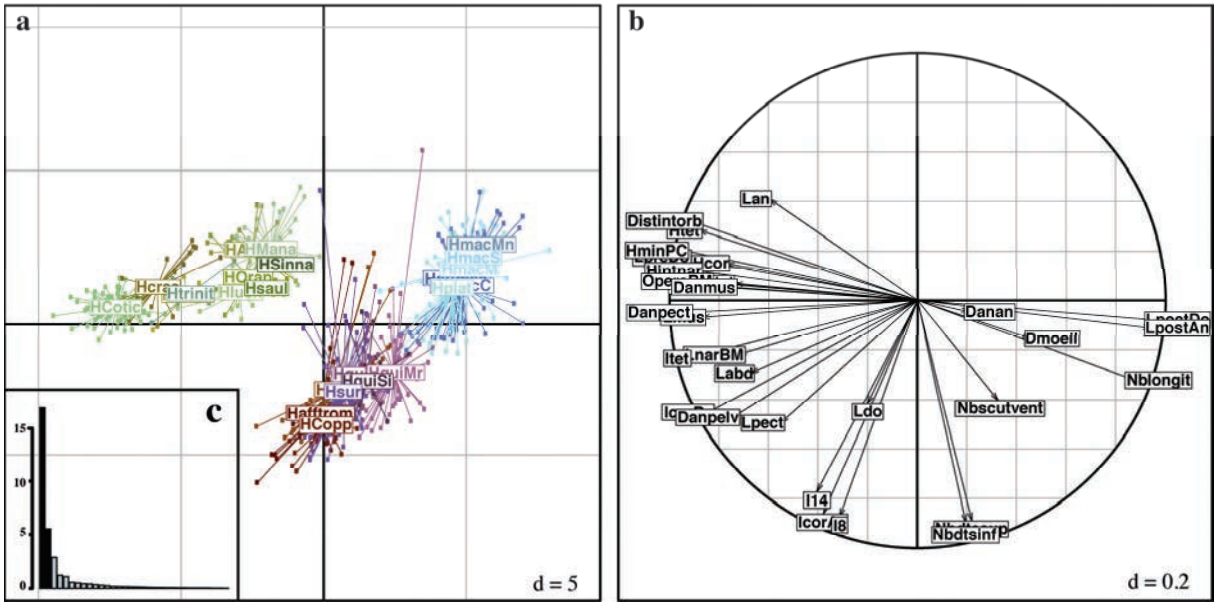


Figure 1. - Principal Components Analysis of the morphometric data table of Guianese Harttiini computed using the correlation matrix on log ratio transformed measurements and untransformed counts. **A**: Projection of the 618 individuals onto the first factorial plane of the PCA; populations and species labelled as in tables II and V, and the list of material. **B**: Correlation circle of the 32 morphometric variables labelled as in table I. Axis 1 horizontal, and axis 2 vertical. **C**: Eigenvalues.

The *Harttia* group comprised representatives of *H. guianensis* (Maroni, Sinnamary, and Approuague Rivers), *H. surinamensis*, *H. fowleri*, and the populations from Coppename and Paru de Oeste rivers. The *Harttiella* group, located on the negative side of the first axis, corresponded to high values for predorsal length, minimum caudal peduncle depth, anus to pectoral-fin origin length, cleithral width, interobital width, head depth at internostril, head depth, snout length, thoracic length, distal end of operculum to tip of snout length, pelvic spine length, body width at dorsal-fin origin, body depth at dorsal-fin origin, anus to tip of snout length, anus to pelvic-fin origin length, head length, nostril to tip of snout length, and abdominal length (Fig. 1b). The *Harttiella* group comprised the type species of the genus, *H. crassicauda* from Nassau Mountain in Suriname, and several populations from French Guiana (Kotika Mountain, Trinité Mountains, Crique Grillon, Crique Aya, Crique Cascade, Crique Coeur Maroni, Crique Limonade, Atachi Bakka Mountains, and Lucifer Mountains). Two morphological tendencies were highlighted by the analysis, with a sub-group made up of *H. crassicauda* plus the populations from Kotika, Atachi Bakka and Trinité Mountains, and the other containing all other populations. The first group corresponded to stockier forms of the genus, whereas the second group assembled slender representatives.

DNA barcode analysis of Guianese Harttiini

The sequence alignment of the 42 barcodes reached a total length of 594 positions including a single ambiguity (Y in position 81 of the COI sequence of *C. maculata* from Suriname River). No insertions, deletions, or stop codons were observed in any sequence. The global base composition was: A = 0.242, T = 0.290, G = 0.180, and C = 0.288. The χ^2 test of heterogeneity of nucleotide frequencies among OTUs failed to reject the null hypothesis (χ^2 = 18.44, p-value = 1) implying that the data set is not at base composition equilibrium. A slight tendency toward AT enrichment was present in the data since the GC content per sequence (Tab. II) was always below 0.5 (mean = 0.468±0.012). In first codon position (GC1) the GC content reached a mean value of 0.538±0.009, versus 0.440±0.00077 in second position (GC2), and 0.426±0.034 in third position (GC3). The maximum in GC content was thus observed in first position, with a mean value above 0.5, whereas a minimum was reached in third position with a significant enrichment in AT bases (0.574). The test on the Index of substitution saturation (Iss) resulted in Iss significantly smaller than Iss.c assuming both a symmetrical and an asymmetrical topology, implying little saturation in the data. The NJ tree reconstruction computed with the K2P distance matrix grouped the different species and populations within three clusters corresponding to the *Harttiella*, *Cteniloricaria*, and *Harttia*

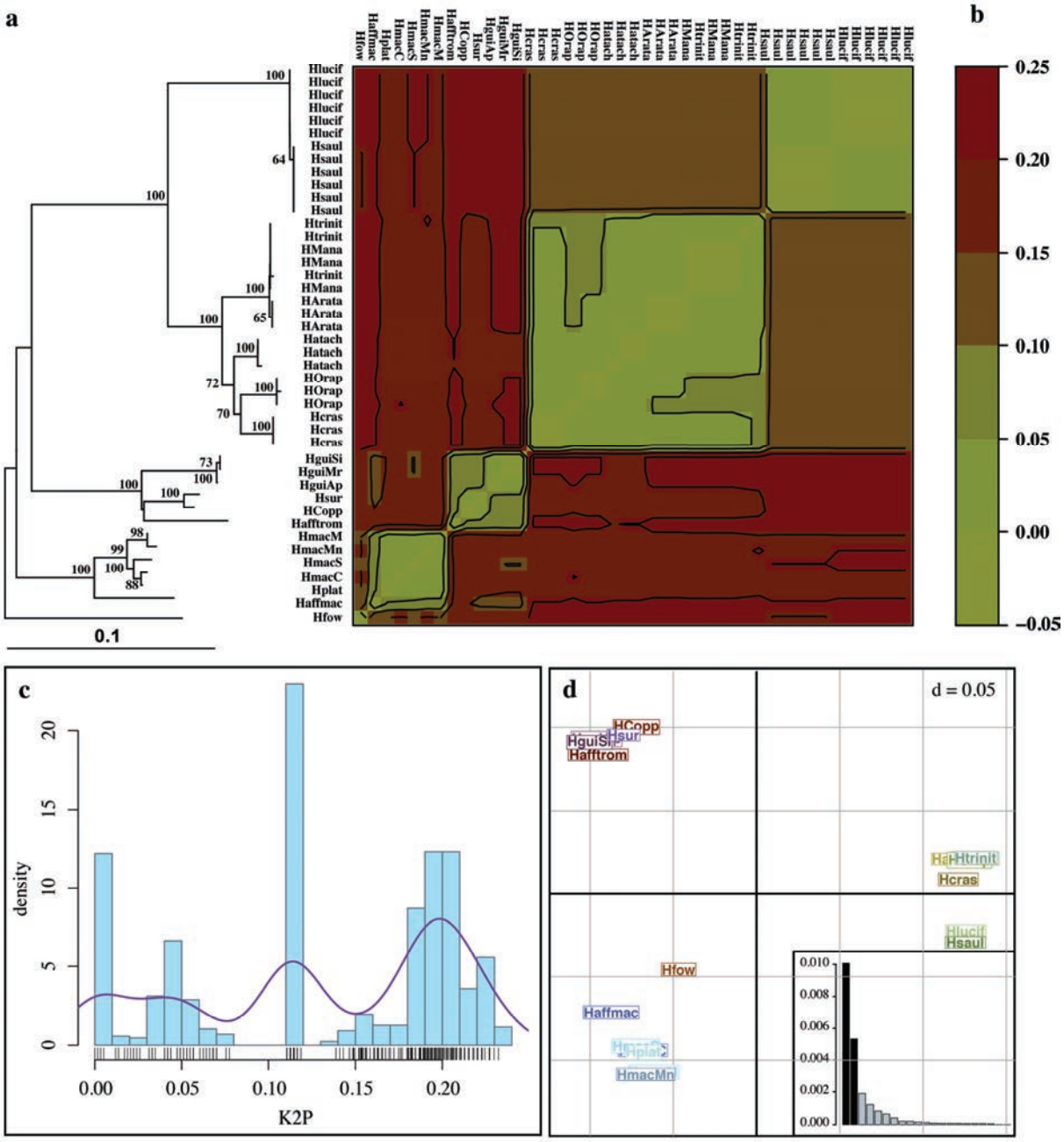


Figure 2. - Analysis of the 42 DNA barcodes of Guianese Harttiini. **A**: NJ tree reconstructed from the K2P distances matrix computed on 594 bases of the mitochondrial COI gene; numbers above branches indicate bootstrap support using 9,999 pseudoreplicates; scale indicates K2P distances; tips labelled as in table II and the list of material. **B**: Levelplot of the ordinated K2P matrix; scale indicates the levels of variation in K2P distances. **C**: Histogram of variation of the K2P distances using 861 paiwise comparisons; scale indicates the frequencies of pairwise comparisons in a definite range. **D**: Principal coordinates analysis of the K2P matrix; taxa labelled as in tables II and V, and the list of material.

groups as previously defined with exception of *H. fowleri* that formed the root of the tree (Fig. 2a). These three groups possessed very strong statistical support (100% bootstraps). Within the *Harttiella* group the total amount of K2P corrected distances varied from 0 to 0.12. In the NJ tree, the first diverging populations comprised representatives from Mana River (Lucifer

Mountains and Crique Cascade) and Maroni River (Crique Limonade), a grouping statistically strongly supported (100% bootstrap). These two populations were genetically almost identical with null K2P distances within-population and a between-population distance of 0.0017. The second well supported (100% bootstrap) diverging group comprised representatives from Approuague River (Crique Cascades), Mana River (Crique Aya), and Sinnamary River (Trinité Mountains). Within-population variation ranged from 0 to 0.003, whereas between-population distances ranged between 0.0017 and 0.005. The last group, also statistically well supported (72.3% bootstrap) comprised populations from Maroni River (Atachi Bakka Mountains and Nassau Mountains) including the type species *H. crassicauda*, and from Orapu River (Crique Grillon). The within-population variations ranged from 0 to 0.0017 whereas between-population variations were comprised between 0.031 and 0.034. The *Harttia* group included all populations of *H. guianensis* (Approuague, Sinnamary, and Maroni Rivers), *H. surinamensis* (Suriname River), and the populations from Coppename and Paru de Oeste Rivers. Within the *H. guianensis* lineage, the K2P distances ranged from 0 to 0.0017, and with other *Harttia*, it diverged by K2P distances ranging from 0.064 (*H. surinamensis*) to 0.077 (Paru de Oeste River). *H. surinamensis* diverged from other representatives by distances ranging from 0.012 (Coppename River) to 0.070 (Paru de Oeste River), while it differed by a mean distance of 0.065 with *H. guianensis*. The specimens from Coppename River diverged from other representatives by distances comprised between 0.012 (*H. surinamensis*) and 0.068 (Paru de Oeste River). The population from the Paru de Oeste possessed the strongest divergence compared to other representatives, ranging from 0.068 (Coppename River) to 0.077 (*H. guianensis* from Approuague River). The *Cteniloricaria* group comprised *C. platystoma* (type species), the different populations of *C. maculata* (Corantijn, Suriname, Maroni and Mana Rivers), as well as an unnamed population from Paru de Oeste River. The latter possessed the strongest divergence from other populations with K2P distances comprised between 0.057 (*C. platystoma*, Essequibo River) and 0.070 (*C. maculata*, Mana River). *C. platystoma* connected within the different populations of *C. maculata* and differed from them by distances varying from 0.003 (Corantijn River) to 0.019 (Maroni River). The within-population variation in *C. maculata* ranged between 0.005 and 0.025. *H. fowleri* connected at the base of the tree, and possessed mean sequence divergences ranging from 0.149 with the *Cteniloricaria* group, and 0.182 and 0.208 in average with the *Harttia* and *Harttiella* groups respectively. In the light of this topology the GC content was explored for the different groups constituted (with exclusion of *H. fowleri*, alone in its own group). Significant differences in mean were recorded for the global GC content (Kruskal-

Table II. - Taxa list, specimen and sequence data for the 42 Harttiini analyzed in this study. Abbreviations of the different species and populations used in the analyses are provided between square brackets. The acronyms of institutions follow Fricke and Eschmeyer (2010).

Species	Catalog Number	Field Number	Locality	GenBank No.	GC content	GC1	GC2	GC3
Harttia guianensis [HguiMr]	MHNG 2643.016	GF00–351	French Guiana, Maroni River drainage, Marouini River	JF292266	0.448	0.535	0.440	0.370
Harttia guianensis [HguiSi]	MHNG 2680.053	RV-21	French Guiana, Sinnamary River drainage, Saut Takari Tanté	JF292267	0.448	0.535	0.440	0.370
Harttia guianensis [HguiAp]	MHNG 2662.091	GF03-160	French Guiana, Approuague River drainage, Crique Arataï	JF292265	0.447	0.535	0.440	0.365
Harttia surinamensis [Hsur]	MHNG 2674.042	SU05-001	Suriname, Suriname River drainage, Gran Rio	JF292264	0.438	0.525	0.440	0.350
Harttia fluminensis [HCopp]	MHNG 2690.013	SU01-445	Suriname, Coppename River drainage, Raleighvallen	JF292263	0.437	0.530	0.440	0.340
Harttia tuna [Hafftrom]	MHNG 2704.029	SU07-644	Brazil, Paru de Oeste River drainage, Four Brothers	JF292262	0.433	0.520	0.435	0.345
Harttia fowleri [Hfow]	MHNG 2643.022	GF99-202	French Guiana, Oyapock River drainage, Crique Gabaret	JF292255	0.497	0.555	0.440	0.495
Cteniloricaria platystoma [Hplat]	MHNG 2650.082	GY04-336	Guyana, Essequibo River drainage, Siparuni River	JF292257	0.465	0.545	0.440	0.410
Cteniloricaria platystoma [HmacC]	MHNG 2672.067	SU05-340	Suriname, Corantijn River drainage, Wonotobo Falls	JF292258	0.465	0.545	0.440	0.410
Cteniloricaria platystoma [HmacS]	MHNG 2674.003	SU05-039	Suriname, Suriname River drainage, Awaradam	JF292259	0.472	0.545	0.440	0.433
Cteniloricaria platystoma [HmacM]	MHNG 2643.014	GF00-092	French Guiana, Maroni River drainage, Marouini River	JF292261	0.472	0.545	0.440	0.430
Cteniloricaria platystoma [HmacMn]	MHNG 2700.054	GF07-265	French Guiana, Mana River drainage, Crique Aya	JF292260	0.473	0.545	0.440	0.435
Cteniloricaria napova [Haffmac]	MHNG 2704.030	SU07-650	Brazil, Paru de Oeste River drainage, Four Brothers	JF292256	0.475	0.565	0.440	0.420
Harttiella crassicauda [Hcras]	MHNG 2674.051	MUS 221	Suriname, Marowijn River drainage, Nassau Mountains in Paramaka Creek	JF292268	0.478	0.530	0.440	0.465
Harttiella crassicauda [Hcras]	MHNG 2674.051	MUS 231	Suriname, Marowijn River drainage, Nassau Mountains in Paramaka Creek	JF292269	0.478	0.530	0.440	0.465
Harttiella crassicauda [Hcras]	MHNG 2679.098	MUS 306	Suriname, Marowijn River drainage, Nassau Mountains in Paramaka Creek	JF292270	0.478	0.530	0.440	0.465
Harttiella pilosa [HOrap]	MHNG 2724.002	GF03-033	French Guiana, Orapu River drainage, Tortue Mountains in Crique Grillon	JF292271	0.475	0.525	0.440	0.460
Harttiella pilosa [HOrap]	MHNG 2682.055	GF06-344	French Guiana, Orapu River drainage, Tortue Mountains in Crique Grillon	JF292272	0.473	0.525	0.440	0.455
Harttiella pilosa [HOrap]	MHNG 2682.055	GF06-343	French Guiana, Orapu River drainage, Tortue Mountains in Crique Grillon	JF292273	0.473	0.525	0.440	0.455
Harttiella parva [Hatach]	MHNG 2723.093	MUS 606	French Guiana, Maroni River drainage, Atachi Bakka Mountains	JF292274	0.468	0.530	0.440	0.435
Harttiella parva [Hatach]	MHNG 2723.093	MUS 607	French Guiana, Maroni River drainage, Atachi Bakka Mountains	JF292275	0.468	0.530	0.440	0.435

Harttiella parva [Hatach]	MHNG 2723.093	MUS 611	French Guiana, Maroni River drainage, Atachi Bakka Mountains	JF292276	0.468	0.530	0.440	0.435
Harttiella intermedia [Htrinit]	MHNG 2713.087	MUS 650	French Guiana, Sinnamary River drainage, Trinité Mountains in Crique Grand Leblond	JF292284	0.473	0.535	0.440	0.445
Harttiella intermedia [Htrinit]	MHNG 2713.087	MUS 651	French Guiana, Sinnamary River drainage, Trinité Mountains in Crique Grand Leblond	JF292285	0.475	0.535	0.440	0.450
Harttiella intermedia [Htrinit]	MHNG 2713.087	MUS 652	French Guiana, Sinnamary River drainage, Trinité Mountains in Crique Grand Leblond	JF292281	0.473	0.535	0.440	0.445
Harttiella lucifer [Hlucif]	MHNG 2721.088	GF10-034	French Guiana, Mana River drainage, Lucifer Mountains in Crique Cascade	JF292291	0.468	0.545	0.440	0.420
Harttiella lucifer [Hlucif]	MHNG 2721.088	GF10-043	French Guiana, Mana River drainage, Lucifer Mountains in Crique Cascade	JF292295	0.468	0.545	0.440	0.420
Harttiella lucifer [Hlucif]	MHNG 2721.088	GF10-037	French Guiana, Mana River drainage, Lucifer Mountains in Crique Cascade	JF292294	0.468	0.545	0.440	0.420
Harttiella lucifer [Hlucif]	MHNG 2721.091	GF10-051	French Guiana, Mana River drainage, Lucifer Mountains	JF292292	0.468	0.545	0.440	0.420
Harttiella lucifer [Hlucif]	MHNG 2721.091	GF10-053	French Guiana, Mana River drainage, Lucifer Mountains	JF292296	0.468	0.545	0.440	0.420
Harttiella lucifer [Hlucif]	MHNG 2721.091	GF10-055	French Guiana, Mana River drainage, Lucifer Mountains	JF292293	0.468	0.545	0.440	0.420
Harttiella lucifer [Hsaul]	MHNG 2712.085	MUS 592	French Guiana, Maroni River drainage, Crique Limonade	JF292290	0.470	0.545	0.440	0.425
Harttiella lucifer [Hsaul]	MHNG 2712.085	MUS 593	French Guiana, Maroni River drainage, Crique Limonade	JF292288	0.470	0.545	0.440	0.425
Harttiella lucifer [Hsaul]	MHNG 2712.085	MUS 591	French Guiana, Maroni River drainage, Crique Limonade	JF292289	0.470	0.545	0.440	0.425
Harttiella lucifer [Hsaul]	MHNG 2712.085	MUS 595	French Guiana, Maroni River drainage, Crique Limonade	JF292286	0.470	0.545	0.440	0.425
Harttiella lucifer [Hsaul]	MHNG 2712.085	MUS 596	French Guiana, Maroni River drainage, Crique Limonade	JF292287	0.470	0.545	0.440	0.425
Harttiella longicauda [HMana]	MHNG 2699.070	GF07-026	French Guiana, Mana River drainage, Crique Aya	JF292280	0.473	0.535	0.440	0.445
Harttiella longicauda [HMana]	MHNG 2699.070	GF07-082	French Guiana, Mana River drainage, Crique Aya	JF292283	0.473	0.535	0.440	0.445
Harttiella longicauda [HMana]	MHNG 2699.070	GF07-111	French Guiana, Mana River drainage, Crique Aya	JF292282	0.473	0.535	0.440	0.445
Harttiella longicauda [HArata]	MHNG 2723.094	MUS 456	French Guiana, Approuague River drainage, Crique Cascades	JF292279	0.475	0.535	0.440	0.450
Harttiella longicauda [HArata]	MHNG 2723.094	MUS 470	French Guiana, Approuague River drainage, Crique Cascades	JF292278	0.475	0.535	0.440	0.450
Harttiella longicauda [HArata]	MHNG 2723.094	MUS 463	French Guiana, Approuague River drainage, Crique Cascades	JF292277	0.475	0.535	0.440	0.450

Wallis test: $\chi^2_{K\text{-}W}$ = 15.8207, p-value = 0.0004), the first position ($\chi^2_{K\text{-}W}$ = 11.9222, p-value = 0.0026), and the third position ($\chi^2_{K\text{-}W}$ = 18.6343, p-value < 0.0001), but no significant difference was highlighted for the second position ($\chi^2_{K\text{-}W}$ = 5.8333, p-value = 0.0541). Concerning the global GC content, a significant difference was detected between *Harttia* and *Cteniloricaria* + *Harttiella* groups, with *Harttia* having a smaller GC content [mean = 0.442±0.007 versus 0.470±0.004 in *Cteniloricaria* (Wilcoxon test: W = 0, p-value = 0.0024) and 0.472±0.003 in *Harttiella* (W = 0, p-value < 0.0001)]. No significant difference was recorded between *Cteniloricaria* and *Harttiella* (W = 69, p-value = 0.4351). In the first position, *Cteniloricaria* possessed a greater GC1 content (mean = 0.548±0.008) than *Harttia* [mean = 0.530±0.003 (W = 0, p-value = 0.0017)] and *Harttiella* [mean = 0.537±0.007 (W = 146.5, p-value = 0.003)], whereas no difference in GC1 was found between *Harttia* and *Harttiella* (W = 48, p-value = 0.0789). In the third position, GC3 content was highly variable between groups with a significantly smaller GC3 in *Harttia* [mean = 0.357±0.013 versus 0.423±0.011 in *Cteniloricaria* (W = 0, p-value = 0.0025), and 0.439±0.016 in *Harttiella* (W = 0, p-value < 0.0001)]. Moreover, the GC3 content was significantly lower in *Cteniloricaria* than in *Harttiella* (W = 37.5, p-value = 0.0153).

Assuming the ordination of the different species and populations reinforced by lineage-specific variations in GC contents, the matrix was reordinated and a levelplot reconstructed (Fig. 2b). Three levels of variation were recorded in the matrix corresponding to within species (between population), between species, and between genera levels. The within species level (light green) ranged from 0 to 0.026 (mean = 0.003±0.005). The between species within genera level (green to khaki to brown) ranged from 0.031 to 0.119 (mean = 0.088±0.033) and possessed the widest range of variation with 2 maxima (Fig. 2c). The first one was located at a mean value of 0.047±0.011, and the second at 0.113±0.002. The between genera level (dark red to light red) ranged from 0.139 to 0.232 (mean = 0.197±0.019).

The PCoA computed from the K2P distances matrix (Fig. 2d) splits the Harttiini along the two first axes that accounted for 71.24% of the total inertia. The first principal coordinate that explained 46.59% of the total inertia splits *Harttiella* in positive scores from *Harttia* and *Cteniloricaria* in negative scores. The second principal coordinate (24.64% of the total inertia) splits *Harttia* (positive scores) from *Cteniloricaria* (negative scores). The position of *H. fowleri*, close to *Cteniloricaria*, was in contradiction with the morphology that grouped it among *Harttia* representatives.

Analysis of the ecology and distribution of Guianese Harttiini

The PCA computed using the NIPALS revealed structures of the ecological and distributional data on the first axis (Fig. 3c) that explained 41.11% of the total variation. This axis splits the Harttiini into two groups (Fig. 3a) with representatives of *Harttiella* in negative values, and representatives of *Cteniloricaria* and *Harttia* gathered together rather in positive values. The single specimen of *Cteniloricaria* from Mana River drainage (Crique Aya) was grouped with representatives of *Harttiella* due to the fact that it was collected together with *Harttiella* representatives, and that if formed the unique known specimen from this drainage. Three variables were strongly correlated with the first axis (Fig. 3b): the altitude, the type of biotope and the temperature. High altitude, creek, and low temperature characterized *Harttiella*, which are inhabitants of small creeks in mountainous areas where the water is cooler, whereas *Harttia* and *Cteniloricaria* are representative of the main stream of rivers, in lowlands, where the water is warmer.

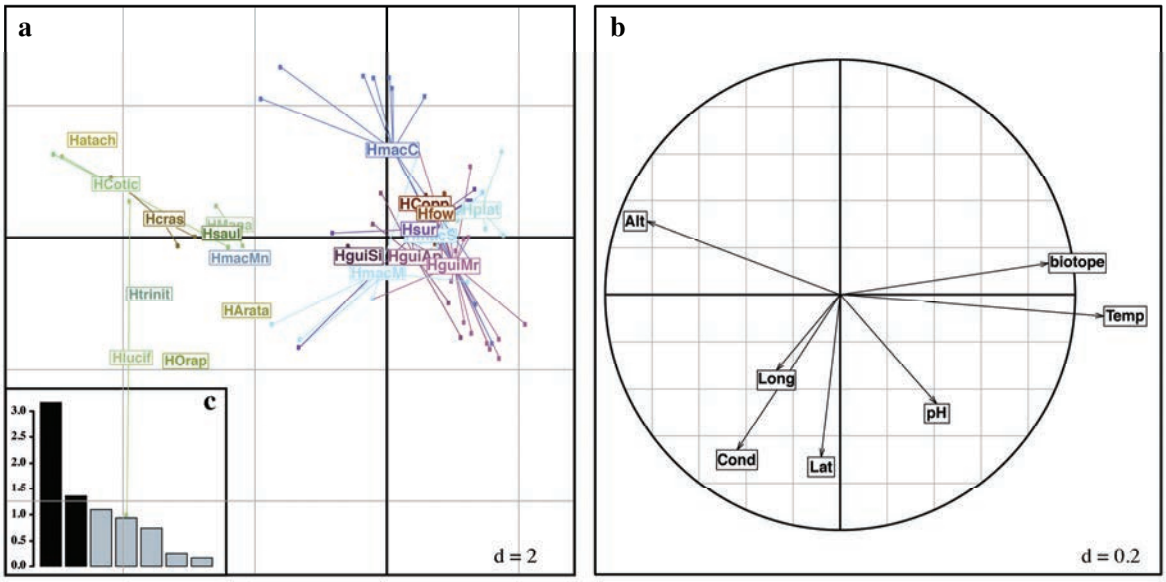


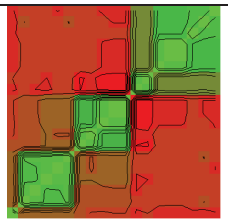
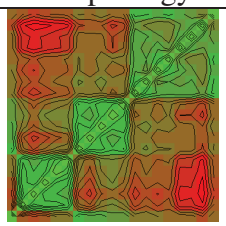
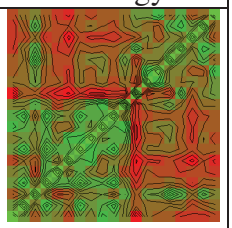
Figure 3. - Principal Components Analysis of the ecology-distribution data of Guianese Harttiini using the NIPALS algorithm for missing data. **A**: Projection of the 88 individuals onto the first factorial plane of the PCA; populations and species labelled as in tables II and V, and the list of material. **B**: Correlation circle of the 4 ecological and 3 distributional variables: biotope: type of biotope 1 = creek, 2 = river; Temp: temperature in degrees Celsius; pH: potential Hydrogen; Cond: conductivity in µS.cm^{-1}; Lat: latitude in decimal degrees; Long: longitude in decimal degrees; Alt: altitude in meters above sea level. Axis 1 horizontal, and axis 2 vertical. **C**: Eigenvalues.

Multiple co-inertia analysis of morphology, genetics and ecology of Guianese Harttiini

The results obtained from the three previous analyses seemed to imply that a common structure was shared between the three types of information (morphology, genetics, and ecology), in particular considering the first axis. The three tables were consequently reduced to 19 common species and populations and submitted to preliminary analyses prior to the multi-table analysis. A first assessment of the relationships between morphology, genetics, and ecology was performed using the CADM test. Prior to its computation, mean population scores obtained from the PCA of the morphological and ecological data were converted into distances matrices using the canonical metrics. The K2P matrix was used as is. A first visual representation of the common structure present within each table was obtained by levelplots of the three distances matrices (Tab. III). The structure of the information was mainly organized into three blocs corresponding to the three groups previously defined (Tab. III, columns Genetic and Morphology). The ecological data set (Tab. III, column Ecology) was organized into two blocs corresponding to the splitting between *Harttiella* and *Harttia* + *Cteniloricaria* despite important background noise. The global CADM test showed a strong and significant correlation between all distance matrices (p-value = 0.0001; W = 0.666). *A posteriori* tests did not detect any conflicting matrix, since each of them displayed significant correlations with respect to the other matrices (Tab. III). Pairwise Mantel correlations highlighted that the genetic data were more correlated to the morphometric (Mantel = 0.596, p-value = 0.0001) and ecological data (Mantel = 0.509, p-value = 0.0001) than were the latter to morphometric data (Mantel = 0.393, p-value = 0.0007). The first plane of MCOA accounted for 74.11% of the total co-structure (54.74 % for axis 1 and 19.37 % for axis 2) (Fig. 4c). MCOA statistics provided in table III showed that the amount of variation explained by MCOA axes is quite equivalent to those obtained in the separated analyses: 99.46% ((0.481+0.252)/(0.486+0.251) = 0.733/0.737) of the genetic data structure, 99.39% of the morphological data structure, and 93.05% of the ecological data structure were recovered by the first two axes. The contribution of each table to the quantity maximized by MCOA (i.e. sum of squared covariances between the linear combinations of the variables of each table and the compromise) is also presented (Cov^2 in Tab. III). The associated correlations (Cos^2 in Tab. III) showed that the first two axes of the compromise are strongly linked to each separated table except for the second axis of ecological data (0.957 and 0.953 for the genetic data, 0.922 and 0.908 for the morphometric data, and 0.868 and 0.324 for the ecological data). The first axis of MCOA aligned the *Harttiella* group (negative scores) followed by *Harttia* then

Cteniloricaria groups (positive scores). The second axis splits *Harttia* from *Cteniloricaria*, but poorly characterized *Harttiella*. The projection of genetic, morphological and ecological

Table III. - Main characteristics of the multi-table analysis computed on the restricted data set (n = 19). Genetic: genetic data table; Morphology: morphometric data table; Ecology: ecology-distribution data table. Levelplot: graphical representation of the structure of each data set converted into distances matrix: K2P distances for the genetic data, and Euclidian distances for the morphometric and ecology-distribution data. CADM: test of congruence among distances matrices. Mantel.mean: correlation of each matrix with respect to the two other matrices. p-value: significance of the test for $\alpha = 0.05$ using Holm's correction. Mantel correlations: pairwise Mantel correlations of the rank-transformed distances between matrices. MCOA: multiple co-inertia analysis. Inertia: maximum inertia projected onto the two first axes of the simple analyses (eigenvalues of the PCoA for the genetic data, and eigenvalues of PCAs for the morphometric and ecology-distribution data tables). Co-inertia: inertia of the three tables projected onto the two first multiple co-inertia axes. Cos^2: correlation between the scores of each table and the synthetic variable of same rank (axes 1 and 2). Cov^2: squared covariance between the scores of each table and the synthetic variable of same rank (maximized by the analysis); note that Cov^2 provides the contribution of each table to the compromise established by the multiple co-inertia analysis.

	Genetic	Morphology	Ecology
Levelplot			
CADM			
Mantel.mean	0.553	0.494	0.451
p-value	0.0003	0.0003	0.0003
Mantel correlations			
Genetic	1.000	0.596	0.509
Morphology	0.596	1.000	0.393
Ecology	0.509	0.393	1.000
MCOA			
Inertia			
Axis 1	0.486	0.608	0.458
Axis 2	0.251	0.215	0.175
Co-Inertia			
Axis 1	0.481	0.586	0.456
Axis 2	0.252	0.232	0.133
Cos^2			
Axis 1	0.957	0.922	0.868
Axis 2	0.953	0.908	0.324
Cov^2			
Axis 1	0.461	0.541	0.396
Axis 2	0.241	0.211	0.043

information onto MCOA axes (Fig. 4a) illustrate the most important differences between the three types of information (dots) and the compromise established by the MCOA (labels). These differences mainly concerned the second axis, and particularly the specimen of *C.*

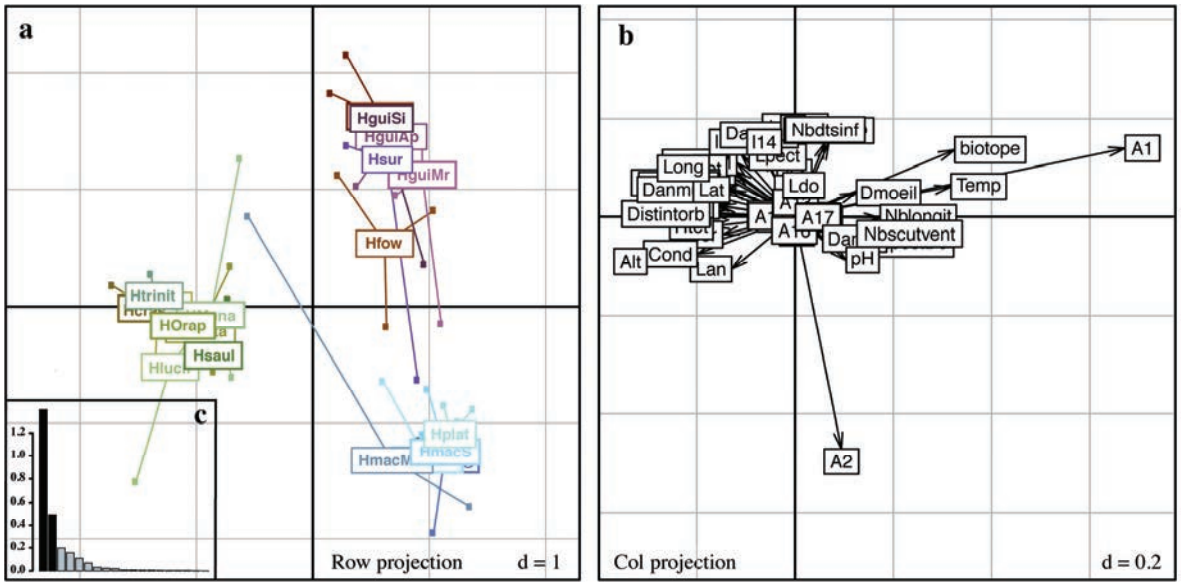


Figure 4. - Multiple co-inertia analysis. Projection of data coordinates of preliminary analyses (PCoA of genetic data and PCAs of morphological and ecology-distribution data) onto axes 1 and 2 of the multiple co-inertia analysis. **A**: Reference structure (labels) and superimposed normalized individuals' scores of preliminary analyses (dots) in the multiple co-inertia plane; populations and species labelled as in tables II and V, and the list of material. **B**: Coordinates of the variables in the first multiple co-inertia plane (labelled as in Tab. IV). **C**: Eigenvalues of the multiple co-inertia analysis.

maculata from Crique Aya. The second unstable position between the three tables and the compromise concerned *H. fowleri* which stayed distinct but close to *Harttia*. Correlations with MCOA axes (Fig. 4b) allow identification of the most important variables. On axis 1 these variables corresponded, in decreasing order of scores (absolute values for negative scores), to: the altitude, conductivity, longitude, interobital width, minimum caudal-peduncle depth, pre-dorsal length, head depth, head depth at internostril, anus to tip of snout length, anus to pectoral-fin origin length, thoracic length, pelvic-spine length, distal end of operculum to tip of snout length, body depth at dorsal-fin origin, snout length, head length, cleithral width, and latitude in negative values, and to the: first principal coordinate of PCoA, biotope, temperature, number of plates in the lateral series, postdorsal length, caudal-peduncle length, number of lateral abdominal plates, and maximum orbital diameter in positive values. On the second axis the variables with greater scores corresponded to the second principal coordinate of the PCoA in negative values, and in decreasing order to the: body width at anal-fin origin, body width at eighth postdorsal plate, number of dentary teeth, number of premaxillary teeth, and anus to pelvic-fin origin length in positive values (Tab. IV).

The Abouheif's tests identified a significant positive phylogenetic autocorrelation for the first two axes of MCOA (C-mean$_1$ = 0.8284, p-value = 0.0001; C-mean$_2$ = 0.6907, p-value = 0.0001). No significant phylogenetic dependence was recovered on other axes that were

Table IV. - Tests against phylogenetic dependence of the variables constituting the different data sets. Variables: A1 to A5: five first eigenvalues of the PCoA of the K2P distances matrix; Ltet to Nbscutvent: morphometric variables labelled as in table 1; biotope to Long: ecology-distribution variables labelled as in figure 3. Axis 1: scores of the different variables onto the first multiple co-inertia axis; Axis 2: scores of the different variables onto the second multiple co-inertia axis; C-mean: Abouheif's measures of local autocorrelation corresponding to the degree to which related species are close from each other in a given trait; p-value: uncorrected significance of the Abouheif's test for $\alpha = 0.05$; C p-value: corrected p-value using the control for false discovery rate for multiple testing under dependency.

Variable	Axis 1	Axis 2	C-mean	p-value $_{(X \geq Xobs.)}$	C p-value $_{(X \geq Xobs.)}$
A1	0.6719	0.1392	0.8457	0.0001	0.0011
A2	0.0944	-0.4704	0.7086	0.0001	0.0011
A3	-0.0010	0.0635	0.5478	0.0021	0.0115
A4	-0.0061	0.0314	0.4317	0.0005	0.0032
A5	0.0153	0.0018	0.4128	0.0060	0.0312
Ltet	-0.1406	0.0875	0.6830	0.0001	0.0011
Labd	-0.1216	0.0914	0.4795	0.0009	0.0052
LpreDo	-0.1691	0.0421	0.8059	0.0001	0.0011
LpostDo	0.1613	-0.0538	0.7392	0.0001	0.0011
LpostAn	0.1594	-0.0345	0.7275	0.0001	0.0011
Lmus	-0.1443	0.0819	0.7518	0.0001	0.0011
LnarBM	-0.1187	0.1103	0.6680	0.0001	0.0011
Lthor	-0.1549	0.0219	0.6527	0.0002	0.0018
Lpect	-0.0355	0.0950	0.2661	0.0383	0.1842
Lpelv	-0.1540	0.0222	0.6340	0.0002	0.0018
Ldo	0.0177	0.0370	0.0265	0.4021	1.0000
Lan	-0.1288	-0.1052	0.6790	0.0001	0.0011
ltet	-0.1392	0.0988	0.7495	0.0001	0.0011
lcorDo	-0.1088	0.1260	0.6606	0.0005	0.0032
lcorAn	0.0004	0.1574	0.5890	0.0002	0.0018
Hcor	-0.1473	-0.0354	0.4916	0.0010	0.0057
Htet	-0.1668	-0.0186	0.7478	0.0002	0.0018
HminPC	-0.1721	0.0082	0.8049	0.0001	0.0011
Hintnar	-0.1666	0.0244	0.7646	0.0001	0.0011
Dmoeil	0.1256	0.0487	0.5301	0.0005	0.0032
Distintorb	-0.1733	0.0055	0.8190	0.0001	0.0011
OpercBM	-0.1536	0.0690	0.7490	0.0001	0.0011
Danpelv	-0.0727	0.1403	0.5536	0.0006	0.0037
Danpect	-0.1565	0.0643	0.7682	0.0001	0.0011
Danan	0.0565	-0.0438	0.3310	0.0135	0.0666
Danmus	-0.1625	0.0552	0.7657	0.0001	0.0011
l8	-0.0042	0.1550	0.5470	0.0003	0.0024
l14	-0.0623	0.1259	0.5276	0.0008	0.0048
Nbdtssup	0.0688	0.1493	0.6088	0.0005	0.0032
Nbdtsinf	0.0613	0.1510	0.5865	0.0005	0.0032
Nblongit	0.1736	-0.0024	0.8200	0.0001	0.0011
Nbscutvent	0.1263	-0.0340	0.5362	0.0003	0.0024
biotope	0.3254	0.1362	0.6626	0.0003	0.0024
Temp	0.3174	0.0612	0.5407	0.0005	0.0032
pH	0.1044	-0.0868	-0.2344	0.9420	1.0000
Cond	-0.1991	-0.0745	0.2276	0.0796	0.3735
Alt	-0.3015	-0.0899	0.4825	0.0026	0.0139
Lat	-0.1332	0.0550	-0.0730	0.6673	1.0000
Long	-0.1745	0.0984	0.3917	0.0079	0.0400

consequently discarded from further interpretation. Results of tests conducted on the different variables constituting the three initial tables are provided in table IV. As expected, axes of the PCoA describing the structure of the genetic data were found to be significantly positively autocorrelated with the phylogeny, and particularly axes 1 (A1) and 2 (A2) displaying the strongest scores (in absolute values) on axes 1 and 2 of the MCOA respectively. Twenty nine morphometric variables out of 32 were found to be significantly phylogenetically dependent. Only three variables: the dorsal-spine length, the pectoral-spine length, and the anus to anal-fin origin length displayed variations independent from the phylogeny. Concerning the ecological and distributional variables, two ecological variables (type of biotope and temperature), and two geographical variables (altitude and longitude) were found to be positively linked to the evolutionary history of Harttiini.

TAXONOMIC IMPLICATIONS

Based on these results, the systematics of Guianese Harttiini is revised. Due to the very strong genetic, morphological and ecological groupings, three valid genera are here recognized: *Cteniloricaria*, *Harttiella*, and *Harttia* (their diagnosis is presented later on in this chapter). Several populations within these three genera represent new taxa, and one synonymy is highlighted. For diagnoses of new species and redescriptions of formerly described species, all variables were submitted to an analysis of variance between species, and significant differences in mean were evaluated using the Tukey's Honest Significant Differences (HSD) post-hoc test using a 95% confidence interval. This single-step multiple comparison procedure allows to find which means are significantly different from one another. Prior to the analysis of variance, individuals' measurements were rank-transformed by species to reduce problems related to small samples.

Cteniloricaria Isbrücker and Nijssen, 1979

Cteniloricaria Isbrücker and Nijssen, in Isbrücker, 1979: 91. Type species: *Loricaria platystoma* Günther, 1868. Type by original designation. Gender: Feminine.

Cteniloricaria is distinguished from all other Guianese Harttiini by 30 morphometric variables (Tab. V) among which, six possessed very strong loadings onto PCA axes (Fig. 1b). *Cteniloricaria* is characterized by a slender appearance with a greater postdorsal length

Table V. - Tukey's Honest Significant Differences post-hoc tests between genera (black dots) and within genera-between species (grey dots) for all morphometric variables. Presence of dots refers to significant differences in mean for the genus under consideration (or the species of a given genus), against all other genera (or species belonging to the same genus). Variables labelled as in table I. Labels refer to the labels used in the analyses for the different populations of a species and the different species.

Genus/species	Labels	Ltet	LpreDo	LpostDo	LpostAn	Labd	Lthor	Lpect	Lpelv	Ldo	Lan	Danpelv	Danpect	Danan	Dammus	lcorDo	lcorAn	l8	l14	Hcor	HminPC	Lmus	LnarBM	ltet	OpercBM	Dmoeil	Distintorb	Hfet	Hintnar	Nbdtssup	Nbdtsinf	Nblongit	Nbscutvent
Cteniloricaria	-	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●
Harttiella	-	●	●	●	●	●	●		●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Harttia	-	●	●	●	●	●	●		●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●
C. platystoma	Hplat, HmacC, HmacS, HmacM, HmacMn		●	●	●				●				●			●		●		●			●				●	●		●			
C. napova	Haffmac		●	●	●				●				●			●		●		●			●				●	●		●			
Hl. crassicauda	Hcras			●	●								●								●												
Hl. pilosa	Horap																																
Hl. parva	Hatach																																
Hl. intermedia	Htrinit																																
Hl. lucifer	Hlucif, Hsaul																																
Hl. longicauda	Hmana, Harata, Hsinna								●									●	●		●						●		●				
Hl. janmoli	Hcotic			●	●			●	●			●	●		●	●				●	●												
H. guianensis	HguiMr, HguiSi, HguiAp				●										●							●	●					●					
H. surinamensis	Hsur	●																●															
H. fluminensis	HCopp																				●						●						
H. tuna	Hafftrom	●	●	●																						●							
H. fowleri	Hfow																																●

representing on average 61.53±1.14% of SL versus 56.91±1.36 in *Harttia* (Tukey HSD, p-value < 0.0001) and 51.18±2.96 in *Harttiella* (HSD, p-value < 0.0001), a longer caudal peduncle [mean = 51.69±1.16% of SL versus 47.88±1.39 (HSD, p-value < 0.0001) and 40.73±3.41 (HSD, p-value < 0.0001) in *Harttia* and *Harttiella* respectively], a greater anus to anal-fin origin length [mean = 8.89±0.52% of SL versus 8.67±0.57 (HSD, p-value = 0.004) and 8.49±1.27 (HSD, p-value < 0.0001) in *Harttia* and *Harttiella* respectively], larger eye with a greater maximum orbital diameter [mean = 23.40±2.11% of HL versus 22.80±1.50 (HSD, p-value = 0.0016) and 17.38±1.68 (HSD, p-value < 0.0001) in *Harttia* and *Harttiella* respectively], more numerous plates in the lateral series [mean = 30±1 versus 29±1 (HSD, p-value < 0.0001) and 25±1 (HSD, p-value < 0.0001) in *Harttia* and *Harttiella* respectively], and a greater number of lateral abdominal plates [in mean 8±2 versus 7±2 (HSD, p-value = 0.0111) and 6±1 (HSD, p-value < 0.0001) in *Harttia* and *Harttiella* respectively]. The following combination of characters also differentiates the genus: abdomen completly covered with medium sized rhombic plates, these plates becoming more numerous and decreasing in size toward the head; abdominal cover reaching gill opening, not organized in rows, and complete around 70 mm SL; presence of a black crescent in the caudal fin.

Within the *Cteniloricaria* group strong genetic divergences and morphological structures were found with significant differences in PCA scores for the first axis ($\chi^2_{K\text{-}W}$ = 13.7128, p-value = 0.0175), but not for the second ($\chi^2_{K\text{-}W}$ = 8.4508, p-value = 0.1331). No significant differences in shape (W = 1118, p-value = 0.1383), nor in genetics (K2P = 0.003) were recorded between *C. platystoma* from Essequibo drainage [described from Suriname (Günther, 1868), but subsequently restricted to Guyana (Boeseman, 1971)] and *C. maculata* from Corantijn River (type locality, Sipaliwini River). As a consequence, *C. maculata* falls here into the synonymy of *C. platystoma*. This latter therefore includes all populations from the Essequibo in Guyana to the Sinnamary River in French Guiana, including Mana River (new record). All barcoded populations previously recorded as *C. maculata* fell within the usual range of populational variation of COI barcodes (≤ 0.03). Populations from French Guiana nevertheless displayed stronger differences in genetics (0.017 to 0.026) and shape (W = 2930, p-value = 0.0161) with respect to Western populations. A shift between morphology and genetics was also observed with populations from Maroni, Mana, and Suriname Rivers exhibiting similar appearance ($\chi^2_{K\text{-}W}$ = 0.1398, p-value = 0.9325), whereas genetically the latter was more closely related to populations from Corantijn and Essequibo Rivers (in mean 0.013 versus 0.025 K2P divergence). A population from Paru de Oeste River displayed strong genetic differences of specific level (K2P distances > 0.05), but displayed few morphometric

differences compared to *C. platystoma*. Only 12 morphometric variables out of 32 distinguished significantly both species (Tab. V). The colour pattern also distinguished the population from Paru de Oeste River from the previous species.

Cteniloricaria platystoma (Günther, 1868)
(Supplementary material S1)

Loricaria platystoma Günther, 1868: 478. Type locality: Surinam (?). Lectotype: BMNH 1866.8.14.124, designated by Isbrücker (1979: 113).

Oxyloricaria platystoma (Günther, 1868): Regan 1904: 298. *Parasturisoma platystoma* (Günther, 1868): Boeseman 1971: 37.

Cteniloricaria platystoma (Günther, 1868): Isbrücker 1979: 91; Isbrücker 1980: 89; Burgess 1989: 440; Isbrücker 2001: 26, 29; Isbrücker 2002: 15; Ferraris in Reis *et al.* 2003: 331; Ferraris 2007: 233; Vari *et al*. 2009: 39.

Harttia platystoma (Günther, 1868): Eigenmann 1912: 251; Rapp Py-Daniel and Oliveira 2001: 80, Provenzano *et al.* 2005: 521; Covain *et al.* 2006: 17.

Parasturisoma maculata Boeseman, 1971: 33, pl. 5. Type locality: Sipaliwini, near airstrip, upper Corantijn River basin, Surinam. Holotype: RMNH 26381.

Harttia maculata (Boeseman, 1971): Rapp Py-Daniel and Oliveira 2001: 80; Provenzano *et al.* 2005: 521; Covain *et al.* 2006: 9.

Cteniloricaria maculata (Boeseman, 1971): Isbrücker 1979: 91; Burgess 1989: 440; Le Bail *et al.* 2000: 268; Isbrücker 2001: 26, 30; Isbrücker 2002: 15; Ferraris in Reis *et al.* 2003: 331; Ferraris 2007: 233.

Morphometric and meristic data are provided in table I, and GenBank accession numbers for barcodes in table II. Twelve morphometric variables distinguish *C. platystoma* from its congener (Tab. V). *Cteniloricaria platystoma* is distinguished from *C. napova* n. sp. by a greater postdorsal length (mean = 61.60±1.14% of SL versus 60.73±0.74; HSD, p-value = 0.006), longer caudal peduncle (mean = 51.74±1.14% of SL versus 51.03±0.90; HSD, p-value = 0.0191), and pelvic-fin spines (mean = 18.10±1.01% of SL versus 17.53±0.42; HSD, p-value = 0.0342), a wider body at eighth postdorsal plate (mean = 9.34±0.97% of SL versus 8.85±0.58; HSD, p-value = 0.0348), a greater nostril to tip of snout length (mean =

39.16±2.70% of HL versus 36.86±1.93; HSD, p-value = 0.009), and interorbital width (mean = 22.50±1.11% of HL versus 21.51±1.29; HSD, p-value = 0.0025). K2P distances to congeneric species ranged from 0.056 to 0.070 according to the population. Its colouration makes it difficult to observe in its natural habitat (Supplementary material S2). The background colour of the dorsal surface is brown with darker indistinct marbling forming black transverse bands toward the tail. Limits of plates are well defined and appear darker, particularly in the anterior region. Areas with golden to bronze shimmers are present below the eyes, eye copper-coloured. A black crescent is present in the caudal fin, sometimes extending toward the lower lobe making it almost black. A black colouration may be also present in the anterior and uppermost part of the dorsal fin. All fins but anal possess dark punctuation on rays forming stripes. The lower surface is yellowish tan. The teeth are not numerous for a Harttiini (around 40 on each jaw), pedunculated, and arranged in a single, comblike row. Sexual dimorphism has never been reported despite a large sampling effort. It could be therefore different from what is commonly reported for other Harttiini. Indeed, certain specimens exhibit much longer pectoral and dorsal fins, the pectoral spines sometimes bearing short but more developed odontodes on their external surface, compared to others of the same size collected at the same place. Such specimens may represent males, which typically exhibit this type of feature in other species. If it is confirmed, the lectotype of *C. platystoma* represents thus a male specimen, whereas the holotype of *C. maculata* corresponds to a female. This is a widespread species distributed in almost all Atlantic coastal drainages from Essequibo in Guyana to Sinnamary in French Guiana (Fig. 5). It is an inhabitant of the main channel of rivers where it colonizes rocky and sandy areas, in fast flowing waters. The species is locally abundant, particularly in its western distribution where it forms the only representative of the Harttiini. When it is sympatric with other Harttiini such as *Harttia surinamensis* or *H. guianensis*, its occurrence becomes scarcer, probably due to competitive exclusion, and it is more frequently observed in the marginal areas of its prefered biotopes, or even in forest creeks.

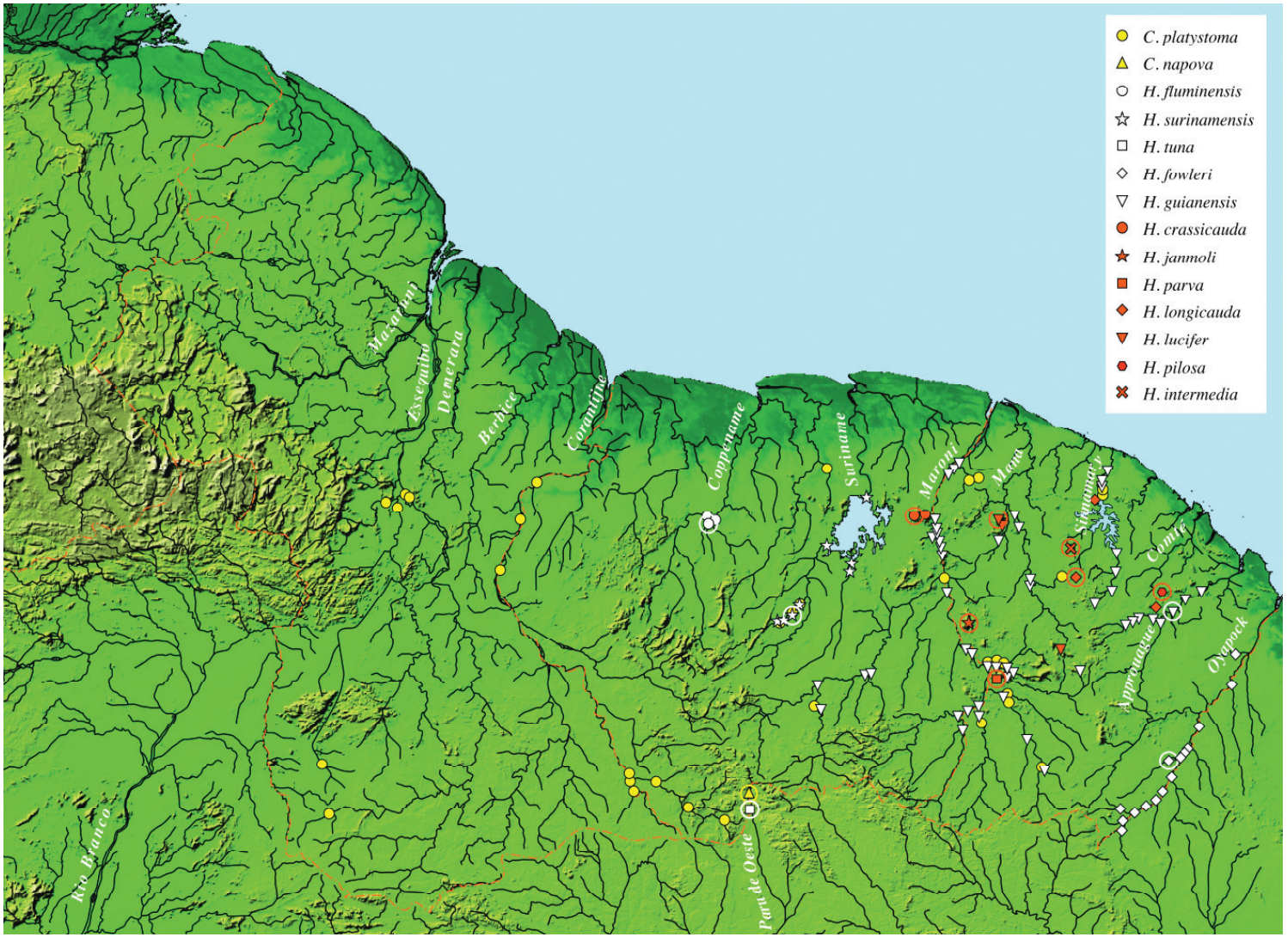


Figure 5. - Geographic distribution of Guianese Harttiini; circled symbols refer to type localities. The map takes into account present results and updates previous records and known distributions accordingly.

***Cteniloricaria napova* Covain and Fisch-Muller, new species**

(Figs. 6, 5, Tabs. I, II)

Holotype

MZUSP 108146 (ex MHNG 2704.030, specimen SU07-667), 113.20 mm SL, Sipaliwini Savannah in Trio Amerindian territory at the Suriname-Brazil border, Four Brothers Mountains in a tributary of the Paru de Oeste River, gift of the Trio tribe in Sipaliwini, 20-21 Oct. 2007.

Paratypes

MHNG 2704.030 (6); MZUSP 108147 (2, ex MHNG 2704.030); MNHN 2011-0017 (2, ex MHNG 2704.030); National Zoological Collection of Suriname (NZCS) NZCS F7071 (1, ex MHNG 2704.030), same data as holotype.

Diagnosis

C. napova is distinguished from *C. platystoma*, its only congener, by its distinctly spotted colour pattern versus indistinctly marbled, and its specific barcode sequence (JF292256). Additionally, it is distinguished by a greater predorsal length (mean = 30.91±0.38% of SL versus 29.91±0.90 in *C. platystoma*; HSD, p-value < 0.0001), anus to pectoral-fin origin length (mean = 23.92±0.89% of SL versus 23.31±0.93; HSD, p-value = 0.0429), body width at dorsal-fin origin (mean = 15.17±0.61% of SL versus 14.62±0.91; HSD, p-value = 0.0228), body depth at dorsal-fin origin (mean = 10.04±0.69% of SL versus 9.16±1.00; HSD, p-value = 0.0012), head depth (mean = 42.21±2.37% of HL versus 40.83±2.85; HSD, p-value = 0.029), and more numerous premaxillary teeth (mean = 47±6 versus 40±12; HSD, p-value = 0.0099).

Description

Morphometric and meristic data in table I, and GenBank accession number in table II.

General aspect of fish slender and depressed, especially posterior to dorsal fin. Head triangular in dorsal view, with sides straight and snout slightly rounded. Eye large, orbit round, smooth, without notch. Odontodes very short, making fish rather smooth. Snout tip naked.

Mouth elliptic with large upper and lower lips. One buccal papilla. Surface of lips papillose, papillae numerous. Distal margin of the lower lip fringed with minute triangular papillae. Maxillary barbel minute. Teeth numerous (> 40 per jaw), pedunculated and arranged in a single, comblike row. Subpreopercle large and triangular densely covered by odontodes. Abdomen completely covered by medium to small rhombic plates between lateral abdominal plates. Plates reaching gill opening, decreasing in size and becoming more numerous toward pelvic girdle. Abdominal plates not or poorly organized in rows. Throat not covered. Two large preanal plates. Seven to 11 (modally 7) lateral abdominal plates, plates keeled but not sharp. Thirty plates in median lateral series, plates keeled, coalescing in last nine to ten plates. Caudal peduncle becoming slightly more compressed in the last 10 plates.

Figure 6. - *Cteniloricaria napova*, holotype, MZUSP 108146, 113.20 mm SL, Sipaliwini Savannah in Trio Amerindian territory at the Suriname-Brazil border, Four Brothers Mountains in a tributary of the Paru de Oeste River.

Posterior margin of dorsal fin straight, generally with first and second branched ray longest. Dorsal and pectoral fins with i,6 rays. Pectoral spine longer than branched rays, reaching beyond pelvic-fin origin. Pelvic fin with i,5 rays; spine longer, reaching to or slightly

beyond anal-fin origin. Anal fin with i,4 rays, spine longer. Caudal fin deeply forked with i,12,i rays.

Colouration

In alcohol, background colour of dorsal surface of head and body tan with numerous distinct small equally spaced dark spots. Anterior part of body, between pectoral fins, darker. Dark shading extending on the sides, but not onto the back, anterior of the pelvic fins. Ventral surface uniformly pale yellowish, appearing greyish in the transparent portion of the abdominal region due to dark pigmentation of internal organs. Dorsal-fin rays yellowish tan with numerous dark brown spots arranged in bands, with a black blotch on the tip. Caudal fin with a dark crescent in its middle part and dark stripes on its lower and upper lobes. Pectoral, pelvic, and anal fins lighter, with indistinct dark markings.

Sexual dimorphism

Unknown. Maybe reminiscent of *C. platystoma* (see above).

Distribution and habitat

Known from upper Paru de Oeste River (Fig. 5).

Etymology

The species group name *napova* is from the Amerindian Trio-Wayana meaning thank you. It honours the Trio people from Sipaliwini who offered us these fish. Name used in apposition.

Harttiella Boeseman, 1971

Harttiella Boeseman, 1971: 25. Type species: *Harttia crassicauda* Boeseman, 1953. Type by original designation. Gender: Feminine.

Harttiella is distinguished from all other Guianese Harttiini by 30 morphometric variables (Tab. V) among which, 18 possessed very strong loadings onto PCA axes (Fig. 1b). *Harttiella* is differentiated from other Guianese Harttiini by: a longer head respectively to its size [mean = 25.00±1.51% of SL versus 23.70±1.35 (HSD, p-value < 0.0001) and 21.13±1.18 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively]; greater predorsal length [mean = 37.56±1.66% of SL versus 32.64±1.10 (HSD, p-value < 0.0001) and 29.99±0.91

(HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively], abdominal length [mean = 18.89±1.30% of SL versus 17.83±1.09 (HSD, p-value < 0.0001) and 16.61±1.02 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively], thoracic length [mean = 20.78±2.27% of SL versus 17.61±0.92 (HSD, p-value < 0.0001) and 15.99±0.94 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively], pelvic-spine length [mean = 22.11±2.60% of SL versus 18.75±1.26 (HSD, p-value < 0.0001) and 18.06±0.99 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively], anal-spine length [mean = 16.58±1.76% of SL versus 12.80±1.02 (HSD, p-value < 0.0001) and 14.40±1.19 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively], anus to pelvic-fin origin length [mean = 11.36±1.60% of SL versus 10.76±0.89 (HSD, p-value = 0.0003) and 8.76±0.59 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively], anus to pectoral-fin origin length [mean = 31.09±3.13% of SL versus 26.26±1.12 (HSD, p-value < 0.0001) and 23.35±0.94 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively], and anus to tip of snout length [mean = 47.03±2.92% of SL versus 40.55±1.35 (HSD, p-value < 0.0001) and 36.83±0.90 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively]; a wider body at dorsal-fin origin [mean = 20.25±2.89% of SL versus 18.78±1.39 (HSD, p-value < 0.0001) and 14.66±0.90 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively]; a deeper body at dorsal-fin origin [mean = 12.13±2.38% of SL versus 8.75±0.83 (HSD, p-value < 0.0001) and 9.22±1.00 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively], and deeper caudal peduncle [mean = 3.75±0.99% of SL versus 1.41±0.14 (HSD, p-value < 0.0001) and 1.13±0.09 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively]; a longer snout [mean = 59.39±3.34% of HL versus 56.32±2.95 (HSD, p-value < 0.0001) and 53.52±3.07 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively]; a wider head [mean = 99.62±6.94% of HL versus 93.77±6.74 (HSD, p-value < 0.0001) and 82.76±4.93 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively]; a greater distance from the distal end of operculum to tip of snout [mean = 84.71±3.67% of HL versus 79.87±2.53 (HSD, p-value < 0.0001) and 77.77±2.25 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively], and interorbital width [mean = 35.29±2.12% of HL versus 22.53±1.34 (HSD, p-value < 0.0001) and 22.43±1.15 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively]; a deeper head [mean = 46.09±4.12% of HL versus 36.94±2.72 (HSD, p-value < 0.0001) and 40.93±2.83 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively], and greater head depth at internostril [mean = 34.16±2.83% of HL versus 28.90±2.62 (HSD, p-value < 0.0001) and 29.62±2.55 (HSD, p-value < 0.0001) in *Harttia* and *Cteniloricaria* respectively].

The following combination of characters also differentiates the genus: abdomen naked with exception of lateral abdominal plates and, rarely, preanal plates; small size (largest known specimen reached 52.46mm SL); body densely covered by odontodes; subpreopercle not exposed; lateral plates not keeled.

Within the *Harttiella* group, significant morphological structures were recovered by axes one and two of the PCA ($\chi^2_{K\text{-}W}$ = 181.7766, p-value < 0.0001; $\chi^2_{K\text{-}W}$ = 137.4764, p-value < 0.0001), as well as deep genetic divergences (0 ≤ K2P ≥0.119). Two main morphological tendencies were highlighted on both axes by the morphometric study, with on one hand stockier forms constituting a first group named the *crassicauda* group, and on the other hand slender representatives forming a second group named the *longicauda* group (W = 37, p-value < 0.0001; W = 1321, p-value < 0.0001). The *crassicauda* group included the type species *H. crassicauda* and populations from the Kotika, Atachi Bakka and Trinité Mountains. The four populations constituting the *crassicauda* group were morphologically significantly distinct ($\chi^2_{K\text{-}W}$ = 56.3771, p-value < 0.0001; $\chi^2_{K\text{-}W}$ = 15.6406, p-value = 0.0013 for axis 1 and 2 respectively), and possessed deep genetic divergences (0.031 ≤ K2P ≥0.051) of interspecific level for the three barcoded populations. These four populations therefore constitute distinct species. Surprisingly, the population from Trinité Mountains that belonged to the *crassicauda* group, displayed almost no genetic divergence with populations from Crique Aya, and Cascades (from 0.003 to 0.005 K2P divergence respectively) both belonging to the *longicauda* group, whereas it showed strong morphological differences to them (W = 7, p-value = 0.0004; W = 31, p-value = 0.0027 for axes 1 and 2). The genetic divergence between Aya and Cascades was 0.0017 implying populational variations. Little morphometric variation was nevertheless recorded for the first axis but not for the second ($\chi^2_{K\text{-}W}$ = 6.5204, p-value = 0.0384; $\chi^2_{K\text{-}W}$ = 0.5922, p-value = 0.7437), and included three populations: Crique Aya (Mana drainage), Crique Cascades (Approuague drainage), and Crique Coeur Maroni (Sinnamary drainage). Within the *longicauda* group, the populations from Lucifer massif (Mana drainage) and Crique Limonade (Maroni drainage) possessed very similar mitochondrial signature (0 ≤ K2P ≥0.002) as well as little morphological differentiation on the first axis (W = 14, p-value = 0.0077; W = 38, p-value = 0.3917). These two populations correspond thus to a single species that possesses the strongest genetic divergence with congeneric representatives (0.112 ≤ K2P ≥0.119). The population from Crique Grillon (Orapu drainage) appeared genetically closer to representatives of the *crassicauda* group (mean K2P divergence to *crassicauda* group = 0.039 versus 0.090 with representatives of the *longicauda* group). All genetic variations corresponded to the between species level (> 0.03). Significant

morphological tendencies were also highlighted by the PCA between the population from Orapu and those from Aya, Cascades, and Coeur Maroni creeks (W = 222, p-value = 0.0408; W = 122, p-value = 0.0005 for the two first axes). Nevertheless, this population appeared morphologically close to the forms from Lucifer massif and Crique Limonade (W = 131, p-value = 0.8848 for axis 1, W = 188, p-value = 0.023 for axis 2).

Harttiella crassicauda (Boeseman, 1953)
(Supplementary material S3)

Harttia crassicauda Boeseman, 1953: 10, Figs. 1b, 2. Type locality: Nassau Mountains, in creek, Suriname. Holotype: RMNH 19418 (largest of 15 specimens), not separated from paratypes.

Harttiella crassicauda (Boeseman, 1953): Boeseman 1971:11; Isbrücker 1980:89; Burgess 1989:439; Le Bail *et al.* 2000:276; Isbrücker 2001:27; Isbrücker 2002:16; Ferraris in Reis *et al.* 2003:336; Ferraris 2007:242; Vari *et al*. 2009: 39.

Morphometric and meristic data are provided in table I, and GenBank accession numbers for barcodes in table II. Four morphometric variables significantly characterize *H. crassicauda* (Tab. V). *Harttiella crassicauda* is distinguished from all other congeneric species by a smaller postdorsal length [mean = 48.05±0.92 % of SL versus 48.85±1.33 < mean < 53.89±1.41% of SL in all other congeners; (HSD, p-values < 0.0261)], a shorter caudal peduncle [mean = 36.14±1.66 % of SL versus 38.32±1.54 < mean < 43.94±0.90% of SL in all other congeners; (HSD, p-values < 0.0001], and a deeper caudal peduncle [mean = 4.86±0.43% of SL versus 2.53±0.18 < mean < 4.58±0.35% of SL in all other congeners; (HSD, p-values < 0.0015)]. It is also distinguished from all other congeneric species except *H. janmoli* n. sp. by a greater (smaller compared to *H. janmoli*) anus to pectoral-fin origin length [mean = 31.85±2.26% of SL versus 28.38±2.75 < mean < 29.23±1.81% of SL in other congeners; (HSD, p-values < 0.0067), and mean = 34.24±1.67% of SL in *H. janmoli* (HSD, p-value = 0.0179)]. K2P distances to congeneric species ranged between 0.031 and 0.119 according to the species. No differences (K2P = 0) were recorded between the two barcoded populations of Paramaka Creek. The general appearance of the species is broad, with a triangular head, and a short and thick caudal peduncle. The background colouration in dorsal view is brown, generally with five narrow dark brown transverse bands posterior to dorsal-fin

insertion. Some indistinct dark spotting may also be present between the postdorsal bands. The anterior part of the body is indistinctly marbled, conferring the species camouflage with the substrate (Supplementary material S4-A). Fin rays also have darker markings, more or less forming stripes. Ventral surface is lighter. The sexual dimorphism consists in the hypertrophy of odontodes on the entire body, and particularly on the S-shaped pectoral-fin spines and around the snout in males.

Harttiella crassicauda is only known from Nassau Mountains in Suriname (Fig. 5), where it occurs in the upper reaches of Paramaka Creek, a tributary of Marowijne River, at an altitude up to 250 m above mean sea level. It has not been collected in streams to the north (Anjoemara Creek) or to the south (Gran Creek) of Paramaka Creek. The reaches with *H. crassicauda* were shallow (mainly <50 cm water depth), but with year-round running water (e.g. *H. crassicauda* was not collected in the extreme headwaters of Paramaka Creek which fall dry in the long dry season September-November). The bottom substrate consisted of bedrock, boulders, pebbles, gravel and sometimes large-grain sand. The water was clear (Secchi transparency >200 cm in deep pools at the edge of the plateau), slightly acidic (pH 5.1-6.9), with low conductivity (23-28 μS/cm), variable current velocity (0-70 cm/s), and relative low temperature (22.2-23.2°C) (Mol *et al.*, 2007). The upper reaches of Paramaka Creek had no aquatic vegetation except for some clumps of filamentous red algae (mainly *Batrachospermum* spp.) and stands of the emergent *Thurnia sphaerocephala* at the edge of the plateau. Other fish species of these high-altitude streams in Nassau Mountains included: *Rivulus* cf. *igneus*, *Synbranchus marmoratus*, *Callichthys callichthys*, *Lithoxus* spp, an unindentified trichomycterid catfish, and a new *Guyanancistrus* species (see this volume).

The extremely limited distribution of *H. crassicauda* in a single creek on a single mountain, coupled with the small population sizes, make it highly vulnerable. Urgent measures should be taken to protect this species and its immediate environment which is directly endangered by mining activities in Nassau Mountains.

Harttiella pilosa Covain and Fisch-Muller, new species

(Figs. 7, 5, Tabs. I, II)

Holotype

MHNG 2724.004 (ex MHNG 2682.055, specimen GF06-338), 39.91 mm SL, French Guiana, Tortue Mountains, Orapu River drainage in Crique Grillon at the ONF camp, Covain *et al.*, 8 Nov. 2006.

Paratypes

MNHN 2011-0018 (4, ex MHNG 2682.055); MHNG 2682.055 (4); NZCS F7072 (1, ex MHNG 2682.055); same data as holotype. MHNG 2724.002 (1), French Guiana, Tortue Mountains, Orapu River drainage in Crique Grillon at the ONF camp, Vigouroux *et al*., 7 Nov. 2003.

Figure 7. - *Harttiella pilosa*, holotype, MHNG 2724.004, 39.91 mm SL, French Guiana, Tortue Mountains, Orapu River drainage in Crique Grillon at the ONF camp.

Diagnosis

Harttiella pilosa is distinguished from all other *Harttiella* by its hispid appearance, versus smooth to velvety in congeneric species, and its specific barcode sequences (JF292271, JF292272, JF292273). No morphometric variable strictly distinguishes *H. pilosa* from all other congeners (Tab. V). It can be distinguished from species belonging to the *crassicauda* group by a shorter head [mean = 23.74±1.35% of SL versus 25.54±1.41 < mean < 26.18±1.34% of SL; (HSD, p-values < 0.0033)], and from other species of the *longicauda* group by a deeper body at dorsal-fin origin [mean = 11.92±0.97% of SL versus 9.86±0.79 < mean < 10.66±0.87% of SL; (HSD, p-values < 0.0069)].

Description

Morphometric and meristic data in table I, and GenBank accession numbers in table II.

A member of the *longicauda* group. General aspect of fish small, slender and hairy, especially anterior to dorsal-fin origin. Caudal peduncle, long and slender. Anterior margin of head rounded in dorsal view. Eye small, orbit round, without notch. Odontodes short and thick, densely covering body making fish rather hispid or slightly spiny particularly in males. Snout tip naked.

Mouth elliptic with large and thick upper and lower lips. One buccal papilla. Surface of lips papillose, with numerous papillae. Distal margin of the lower lip fringed with minute triangular papillae. Maxillary barbel minute. Teeth numerous (≈ 40 per jaw), pedunculated and arranged in a single, comblike row. Subpreopercle not visible in ventral view. Abdomen naked except for lateral abdominal plates, and sometimes preanal plates in larger specimens. Six to 8 (modally 6) lateral abdominal plates, plates keeled but not sharp. Twenty-six plates in median lateral series, plates not coalescing.

Dorsal-fin origin more or less in front of pelvic-fin insertion. Dorsal and pectoral fins with i,6 rays. Pectoral spine longer than soft rays, reaching slightly beyond pelvic-fin origin. Pelvic fin with i,5 rays; spine longer, reaching anal-fin origin. Anal fin with i,4 rays, spine longer. Caudal fin truncated with i,12,i rays.

Colouration

In alcohol, background colour of dorsal surface of head and body greyish brown with 4 to 5 dark saddles posterior to dorsal-fin origin. On side of body, saddles have form of indistinct blotches. Anterior part of body darker. Ventral surface lighter. Lower caudal peduncle dingy off-yellow with dark marks. Black blotch at base of anal and pelvic fins. Fin

rays yellowish tan with dark brown spots. Fins membranes hyaline. Caudal fin with indistinct dark markings.

In life, background colour of dorsal surface reddish brown, with black postdorsal bands (Supplementary material S4-B).

Sexual dimorphism

Males with longer, thicker and more widely spaced apart odontodes on the head surface, and on pectoral spines, and with a deeper snout, particularly at the level of internostril.

Distribution and habitat

Only known from type locality. *Harttiella pilosa* was collected in Crique Grillon, a tributary of Orapu River in Tortue Mountains (Fig. 5), at an altitude of approximately 200 m above mean sea level. The portion of the river in which *H. pilosa* was found was immediately upstream of a 30 m high waterfall (*H. pilosa* was not collected downstream of the waterfall). That portion of the river was approximately 10 m wide with shallow (5-40 cm) water and a bottom substrate consisting of gravel, pebbles, boulders, bed rock and sand mainly constituted of iron hydroxide (Edwin Gnos, MHNG, pers. comm.). Leaf litter and large woody debris were also abundant. The water was clear (Secchi transparency >40 cm), slightly acidic (pH 6.5), with low conductivity (52 μS/cm), variable current (0-70 cm/s), and relatively low temperature (24 °C). The reach had no aquatic macrophytes, but clumps of filamentous red algae were observed on rocky bottom substrate. The fish community included: *Bryconops affinis*, *Hemigrammus unilineatus*, *Melanocharacidium blennioides*, *Helogenes marmoratus*, *Pseudopimelodus raninus*, *Ancistrus* cf. *leucostictus*, *Lithoxus planquettei*, *Guyanancistrus* aff. *brevispinis*, *Krobia guianensis*, and *Crenicichla* sp.

Etymology

The species group name *pilosa* is from Latin *pilosus* meaning hairy, and makes reference to the unusual aspect of males' head.

***Harttiella parva* Covain and Fisch-Muller, new species**

(Figs. 8, 5, Tabs. I, II)

Holotype

MNHN 2011-0019 (ex MHNG 2723.093, specimen MUS-607), 29.54 mm SL, French Guiana, Atachi Bakka Mountains, Maroni River drainage, Gaucher, June 2009.

Paratypes

MNHN 2011-0020 (2, ex MHNG 2723.093); MHNG 2723.093 (3), same data as holotype.

Figure 8. - *Harttiella parva*, holotype, MNHN 2011-0019, 29.54 mm SL, French Guiana, Atachi Bakka Mountains, Maroni River drainage.

Diagnosis

Harttiella parva is distinguished from all other *Harttiella* by its small size with sexual dimorphism expressed around 25mm SL (versus around 30mm SL in congeneric species), by the distinct banded colour pattern of the caudal fin (versus blotched or indistinct banded pattern in congeneric species), and by its specific barcode sequences (JF292274, JF292275, JF292276). *Harttiella parva* does not show unique morphometric tendencies distinguishing it from all other congeneric species (Tab. V). It is distinguished from other congeners except *H. intermedia* n. sp. by a caudal peduncle: shorter compared to the species belonging to the *longicauda* group [mean = 40.36±1.06% of SL versus 43.61±1.79 < mean < 44.26±1.48% of SL; (HSD, p-values < 0.0002)], and longer compared to the other species of the *crassicauda* group [mean = 40.36±1.06% of SL versus 36.14±1.66 < mean < 38.32±1.54% of SL; (HSD, p-values < 0.0281)]. It is distinguished from *H. intermedia* by a smaller body depth at dorsal-fin origin [mean = 10.34±0.83% of SL versus 12.69±0.54; (HSD, p-value = 0.0041)].

Description

Morphometric and meristic data in table I, and GenBank accession numbers in table II.

A member of the *crassicauda* group. General aspect of fish small and stocky, with a short, broad and thick caudal peduncle. Head rounded in dorsal view. Eye small, orbit round, without notch. Odontodes short, densely covering body conferring fish a velvety aspect. Snout tip naked.

Mouth elliptic with large upper and lower lips. One buccal papilla. Surface of lips papillose, with numerous papillae. Distal margin of the lower lip fringed with minute triangular papillae. Maxillary barbel minute. Teeth numerous (≈ 40 per jaw), pedunculated and arranged in a single, comblike row. Subpreopercle not visible in ventral view. Abdomen naked except for lateral abdominal plates. Five to 7 (modally 6) lateral abdominal plates, plates keeled but not sharp. Twenty four to 25 (modally 25) plates in median lateral series, plates not coalescing.

Dorsal fin originates more or less in front of pelvic-fin insertion. Dorsal and pectoral fins with i,6 rays. Pectoral spine longer than soft rays, S shaped, reaching slightly beyond pelvic-fin origin. Pelvic fin with i,5 rays; spine longer, reaching beyond anal-fin origin. Anal fin with i,4 rays, spine longer. Caudal fin truncated with i,12,i rays.

Colouration

In alcohol, background colour of dorsal surface of head and body brownish tan with 4 thin postdorsal dark bands. Anterior part of body darker. Ventral surface lighter. Fin rays yellowish tan with dark brown spots forming stripes. Fin membranes hyaline. Uppermost part of dorsal fin sometime with a small black blotch. Caudal fin with distinct dark stripes (usually 4) becoming larger toward distal margin. Last stripe forming a large black band at tail extremity.

Sexual dimorphism

Males with long and thicker odontodes on the external surface of pectoral spines.

Distribution and habitat

Only known from type locality (Fig. 5), a small forest creek in Atachi Bakka Mountains.

Etymology

The species group name *parva* is from Latin *parvus* meaning small, and makes reference to the size of the species.

***Harttiella intermedia* Covain and Fisch-Muller, new species**

(Figs. 9, 5, Tabs. I, II)

Holotype

MNHN 2011-0021 (ex MHNG 2713.087 specimen MUS-650), 34.67 mm SL, French Guiana, Sinnamary River drainage, Tabular Mountain of Trinité massif, Crique Grand Leblond, 4°36’35’’N, 53°21’33’’W, alt. 320m, Tostain and Ravet, 6 Oct. 2009.

Paratypes

MNHN 2011-0022 (2, ex MHNG 2713.087); MHNG 2713.087 (2), same data as holotype.

Figure 9. - *Harttiella intermedia*, holotype, MNHN 2011-0021, 34.67 mm SL, French Guiana, Sinnamary River drainage, Tabular Mountain of Trinité massif, Crique Grand Leblond.

Diagnosis

Harttiella intermedia is distinguished from all other *Harttiella* by its stocky body shape reminiscent of the *crassicauda* group, and by its mitochondrial barcode signature typical for the *longicauda* group (JF292281, JF292284, JF292285). No unique morphometric data characterize *H. intermedia* (Tab. V). It is distinguished from *H. parva* plus representatives of the *longicauda* group except *H. pilosa* by a deeper body at dorsal-fin origin [mean = 12.69±0.54% of SL versus 9.86±0.79 < mean < 10.66±0.87% of SL; (HSD, p-values < 0.0041)], and from *H. janmoli* n. sp. by a shallower body at dorsal-fin origin [mean =

12.69±0.54% of SL versus 14.80±1.14% of SL; (HSD, p-value = 0.015)]. It is distinguished from *H. pilosa* by a longer head [mean = 26.18±1.12% of SL versus 23.74±1.35% of SL; (HSD, p-value = 0.0031)], and from *H. crassicauda* by a longer caudal peduncle [mean = 42.35±1.44% of SL versus 36.14±1.66% of SL; (HSD, p-value < 0.0001)].

Description

Morphometric and meristic data in table I, and GenBank accession numbers in table II.

A member of the *crassicauda* group. General aspect of fish small and stocky, with a short, broad and thick caudal peduncle. Head rounded to slightly triangular in dorsal view. Eye small, orbit round, without notch. Odontodes short, densely covering body, conferring fish with a velvety aspect. Snout tip naked.

Mouth elliptic with large upper and lower lips. One buccal papilla. Surface of lips papillose, with numerous papillae. Distal margin of the lower lip fringed with minute triangular papillae. Maxillary barbel minute. Teeth numerous (≈ 50 per jaw), pedunculated and arranged in a single, comblike row. Subpreopercle not visible in ventral view. Abdomen naked except for lateral abdominal plates. Five to 8 (modally 6) lateral abdominal plates, plates keeled but not sharp. Twenty four to 25 (modally 25) plates in median lateral series, plates not coalescing.

Dorsal-fin origin more or less in front of pelvic-fin insertion. Dorsal and pectoral fins with i,6 rays. Pectoral spine longer than branched rays, straight, reaching beyond pelvic-fin origin. Pelvic fin with i,5 rays; spine longer, reaching beyond anal-fin origin. Anal fin with i,4 rays, spine longer. Caudal fin truncated with i,12,i rays.

Colouration

In alcohol, background colour of dorsal surface of head and body brownish tan with 5 thick postdorsal dark bands. Anterior part of body darker. Ventral surface lighter. Fin rays yellowish tan with indistinct dark brown spots more or less forming stripes. Fins membranes hyaline. Caudal fin with usually four indistinct dark stripes. Tip of caudal fin whitish. Some specimens with a basicaudal spot.

Sexual dimorphism

Unknown. Probably similar to that observed in *H. crassicauda* (see above).

Distribution and habitat

Only known from type locality (Fig. 5), in headwaters of Crique Grand Leblond on the Tabular Mountain of the Trinité Massif. The species was collected with representatives of *Ituglanis nebulosus*, *Rivulus igneus*, *R. lungi*, and *R.* aff. *breviceps*.

Etymology

The species group name *intermedia* is from the Latin *intermedius* meaning intermediary, making reference to the contradiction between morphometry and genetics.

***Harttiella lucifer* Covain and Fisch-Muller, new species**

(Figs. 10, 5, Tabs. I, II)

Holotype

MNHN 2011-0023 (ex MHNG 2721.088 specimen GF10-034), 42.68 mm SL, French Guiana, Mana River drainage, Lucifer Mountains, West of Crique Cascade, 4°47'44.7''N, 53°55'49.4''W, alt. 450 m, Montoya-Burgos and Fischer, 10 Feb. 2010.

Paratypes

MNHN 2011-0024 (4, ex MHNG 2721.088); MHNG 2721.088 (4); NZCS F7073 (1, ex MHNG 2721.088), same data as holotype. MNHN 2011-0025 (3, ex MHNG 2721.091), MHNG 2721.091 (3), NZCS F7074 (1, ex MHNG 2721.091), French Guiana, Mana River drainage, headwater of a creek in Lucifer massif flowing toward Citron, 4°45'54''N 53°56'14.9''W, alt. 365 m., Montoya-Burgos and Fischer, 11 Feb. 2010. MNHN 2011-0026 (4, ex MHNG 2712.085 specimens), MHNG 2712.085 (4), French Guiana, Maroni River drainage, Galbao Mountains in a tributary of Crique Limonade, 3°35'56.6''N 53°15'12.6''W, alt. 202 m., Tostain, 18 Mar. 2008.

Figure 10. - *Harttiella lucifer*, holotype, MNHN 2011-0023, 42.68 mm SL, French Guiana, Mana River drainage, Lucifer Mountains, West of Crique Cascade.

Diagnosis

No unique character distinguishes *Harttiella lucifer* from all other congeneric species (Tab. V) except its barcode sequence, the most divergent of all *Harttiella* representatives (JF292286 to JF292296). Compared to congeneric species of the *crassicauda* group except *H. intermedia*, *H. lucifer* possesses a longer caudal peduncle [mean = 44.26±1.48% of SL versus 36.14±1.66 < mean < 40.36±1.06% of SL; (HSD, p-values < 0.0001)]. Compared to other representatives of the *longicauda* group, *H. lucifer* is characterized by a greater cleithral width [mean = 101.65±3.28% of HL versus 94.50±4.00 < mean < 95.78±4.04% of HL; (HSD, p-

values < 0.002)]. It is distinguished from *H. intermedia* by a smaller predorsal length [mean = 35.95±1.19% of SL versus 37.93±1.07% of SL; (HSD, p-value = 0.0049)].

Description

Morphometric and meristic data in table I, and GenBank accession numbers in table II.

A member of the *longicauda* group. General aspect of fish small, flat, and slender, with a long and slender caudal peduncle. Body wider in its anterior part. Head large, short and rounded to slightly triangular in dorsal view. Eye small, orbit round, without notch. Odontodes short, densely covering body conferring fish a velvety aspect. Snout tip naked.

Mouth elliptic with large upper and lower lips. One buccal papilla. Surface of lips papillose, with numerous papillae. Distal margin of the lower lip fringed with minute triangular papillae. Maxillary barbel minute. Teeth numerous (≈ 45 per jaw), pedunculated and arranged in a single, comblike row. Subpreopercle not visible in ventral view. Abdomen naked except for lateral abdominal plates. Four to 8 (modally 6) lateral abdominal plates, plates keeled but not sharp. Twenty five to 27 (modally 26) plates in median lateral series, plates not coalescing.

Dorsal-fin origin more or less in front of pelvic-fin insertion. Dorsal and pectoral fins with i,6 rays. Pectoral spine longer than soft rays, slightly curved, reaching beyond pelvic-fin origin. Pelvic fin with i,5 rays; spine longer, reaching beyond anal-fin origin. Anal fin with i,4 rays, spine longer. Caudal fin truncated with i,12,i rays.

Colouration

In alcohol, background colour of dorsal surface of head and body variable, from dark brown to reddish brown or light tan, with 5 thick postdorsal dark bands. Anterior part of body darker. Ventral surface lighter, yellowish tan. Fin rays yellowish tan with indistinct dark brown spots more or less forming stripes. Fin membranes hyaline. Caudal fin with indistinct dark stripes (2). Distal caudal-fin margin yellowish.

Sexual dimorphism

Males with larger head and thickened pectoral spines, bearing hypertrophied odontodes.

Distribution and habitat

Occurs in mountainous areas in the Lucifer and Galbao massifs in Central French Guiana (Fig. 5). In the Lucifer Mountains, the species has been collected with representatives of *Rivulus igneus* and *Ituglanis* sp.

Etymology

The species group name *lucifer* refers to the type locality. A name used in apposition.

***Harttiella longicauda* Covain and Fisch-Muller, new species**

(Figs. 11, 5, Tabs. I, II)

Holotype

MNHN 2011-0027 (ex MHNG 2699.070 specimen GF07-049), 52.46 mm SL, French Guiana, Trinité Mountains, Mana River drainage, in a tributary of Crique Baboune, Crique Aya around 100m in front of Aya Camp, 4°36'11''N, 53°25'04''W, alt. 122 m, Montoya-Burgos and Melki, 28 Nov. – 4 Dec. 2007.

Paratypes

MNHN 2011-0028 (23, ex MHNG 2699.070); MHNG 2699.070 (23); NZCS F7075 (2, ex MHNG 2699.070); ANSP 190961 (2, ex MHNG 2699.070); MZUSP 108148 (2, ex MHNG 2699.070), same data as holotype. MNHN 2011-0029 (2, ex MHNG 2699.098); MHNG 2699.098 (2), French Guiana, Trinité Mountains, Mana River drainage, in a tributary of Crique Aya at foot of the inselberg, N4°36'33'' W53°24'46'', alt. 149 m, Montoya-Burgos and Melki, 28 Nov. – 4 Dec. 2007. MNHN 2011-0030 (8, ex MHNG 2723.094); MHNG 2723.094 (8), French Guiana, Balenfois Mountains, Approuague River drainage, around 1 km upstream of Nouragues camp, Crique Cascades, Gaucher, Feb. 2008. MHNG 2723.095 (1, ex MHNG 2643.030), French Guiana, Sinnamary River drainage, at mouth of Crique Coeur Maroni, Le Bail *et al.*, 15 Oct. 1982 or 2 Feb. 1983.

Diagnosis

Harttiella longicauda is distinguished from all other congeneric species except *H. pilosa* by the frequent presence of few small preanal plates (versus no preanal plates), and by its specific barcode sequences (JF292277, JF292278, JF292279, JF292280, JF292282,

JF292283). It can be distinguished from *H. pilosa* by having the pectoral girdle wider than pelvic girdle (versus pectoral girdle approximately as wide as the pelvic girdle). Additionally six unique morphometric variables distinguish *H. longicauda* from all other congeners (Tab. V). *Harttiella longicauda* possesses shorter pelvic spines [mean = 19.36±1.17% of SL versus 20.55±0.36 < mean < 24.67±1.46% of SL; (HSD, p-values < 0.0045)]; a smaller body width at eighth postdorsal plate [mean = 9.29±1.07% of SL versus 10.28±.98 < mean < 11.48±0.94% of SL; (HSD, p-values < 0.0238)], body width at fourteenth postdorsal plate [mean = 3.99±0.51% of SL versus 4.72±0.62 < mean < 5.59±0.70% of SL; (HSD, p-values < 0.0198)], minimum caudal peduncle depth [mean = 2.53±0.18% of SL versus 3.24±0.21 < mean < 4.86±0.43% of SL; (HSD, p-values < 0.0001)], interorbital width [mean = 33.82±2.03% of HL versus 35.25±1.21 < mean < 38.20±2.03% of HL; (HSD, p-values < 0.03)], and head depth at internostril [mean = 33.19±1.76% of HL versus 33.89±2.22 < mean < 36.74±4.00% of HL; (HSD, p-values < 0.0083)].

Description

Morphometric and meristic data in table I, and GenBank accession numbers in table II.

General aspect of fish small, flat, and slender, with a long and slender caudal peduncle. Body wider in its anterior part. Head large, short and rounded in dorsal view. Eye small, orbit round, without notch. Odontodes short, densely covering body conferring fish a velvety aspect. Snout tip naked.

Mouth elliptic with large upper and lower lips. One buccal papilla. Surface of lips papillose, with numerous papillae. Distal margin of the lower lip fringed with minute triangular papillae. Maxillary barbel minute. Teeth numerous (≈ 45 per jaw), pedunculated and arranged in a single, comblike row. Subpreopercle not visible in ventral view. Abdomen naked except for few rhombic preanal plates, and lateral plates. Five to 8 (modally 6) lateral abdominal plates. Twenty-five to 27 (modally 26) plates in median lateral series, plates not coalescing.

Dorsal-fin origin more or less in front of pelvic-fin insertion. Dorsal and pectoral fins with i,6 rays. Pectoral spine longer than branched rays, slightly curved, reaching pelvic-fin origin. Pelvic fin with i,5 rays; spine longer, reaching anal-fin origin. Anal fin with i,4 rays, spine longer. Caudal fin truncated with i,12,i rays.

Figure 11. - *Harttiella longicauda*, holotype, MNHN 2011-0027, 52.46 mm SL, French Guiana, Trinité Mountains, Mana River drainage, in a tributary of Crique Baboune, Crique Aya around 100 m in front of Aya Camp.

Colouration

In alcohol, background colour of dorsal surface of head and body variable, from dark brown tan to greyish tan, with 5 thick postdorsal dark bands. Anterior part of body darker. Population from Crique Cascade, Approuague River drainage, with dark spots or vermiculations on head. Ventral surface lighter, yellowish tan. Often with a dark blotch at

anal-fin origin. Fin rays yellowish tan with indistinct dark brown spots more or less forming stripes. Fins membranes hyaline. Caudal fin with indistinct, poorly defined, dark stripes.

Sexual dimorphism

Males with a wider head and thickened pectoral spines bearing hypertrophied odontodes.

Distribution and habitat

Occurs in mountainous areas in the Trinité and Balenfois massifs in Northern French Guiana (Fig. 5). In the Trinité Mountains, the species has been collected with representatives of *Guyanancistrus* aff. *brevispinis*, *Krobia itanyi*, *Rhamdia quelen*, *Ancistrus* cf. *leucostictus*, *Lithoxus planquettei*, *Characidium fasciadorsale*, *Melanocharacidium* cf. *dispilomma*, and *Rineloricaria* aff. *stewarti*.

Etymology

The species group name *longicauda* is from Latin *longus* meaning long, and *cauda* meaning tail. The name makes reference to the shape of the caudal peduncle.

***Harttiella janmoli* Covain and Fisch-Muller, new species**

(Figs. 12, 5, Tabs. I, II)

Holotype

MNHN 2011-0031 (ex MHNG 2695.059), 47.13 mm SL, French Guiana, Maroni River drainage, Kotika Mountain, 3°57'16''N, 54°10'50''W, alt. 515 m., Tostain, 5 Sept. 2007.

Paratypes

MNHN 2011-0032 (35, ex MHNG 2695.059); MHNG 2695.059 (36); NZCS F7076 (2, ex MHNG 2695.059); ANSP 190962 (2, ex MHNG 2695.059); MZUSP 108149 (2, ex MHNG 2695.059); RMNH.PISC.37459 (1, ex MHNG 2695.059); RMNH.PISC.37460 (1, ex MHNG 2695.059), same data as holotype.

Figure 12. - *Harttiella janmoli*, holotype MNHN 2011-0031, 47.13 mm SL, French Guiana, Maroni River drainage, Kotika Mountain.

Diagnosis

Harttiella janmoli is distinguished from all other congeneric species by its dark brown colouration with a large transverse postdorsal saddle corresponding to the position in congeners of the third and fourth bands posterior to dorsal-fin origin (versus brownish colouration normally with five postdorsal bands). Additionally 10 morphometric variables strictly characterize *H. janmoli* (Tab. V). *Harttiella janmoli* possesses longer pectoral spines [mean = 27.14±1.54% of SL versus 21.14±0.98 < mean < 24.06±0.84% of SL; (HSD, p-values < 0.0001)], pelvic spines [mean = 24.67±1.46% of SL versus 19.36±1.17 < mean <

22.86±1.64% of SL; (HSD, p-values < 0.0001)]; a greater anus to pelvic-fin origin length [mean = 12.88±0.96% of SL versus 9.29±1.42 < mean < 11.31±1.01% of SL; (HSD, p-values < 0.012)], anus to pectoral-fin origin length [mean = 34.24±1.67% of SL versus 28.38±2.75 < mean < 31.85±2.26% of SL; (HSD, p-values < 0.0001)], and anus to tip of snout length (mean = 49.81±1.63% of SL versus 43.32±1.42 < mean < 48.09±1.93% of SL; (HSD, p-values < 0.0002)]; a wider body at dorsal-fin origin [mean = 23.46±1.18% of SL versus 17.28±1.11 < mean < 20.06±1.61% of SL; (HSD, p-values < 0.0001)]; and a deeper body at dorsal-fin origin [mean = 14.80±1.14% of SL versus 9.86±0.79 < mean < 11.41±1.41% of SL; (HSD, p-values < 0.015)].

Description

Morphometric and meristic data in table I.

A representative of the *crassicauda* group. General aspect of fish small and stocky, with a short, large, thick and flattened caudal peduncle. Head large, short and rounded in dorsal view. Eye small, orbit round, without notch. Odontodes short, densely covering body conferring fish a velvety aspect. Snout tip naked.

Mouth elliptic with large and thick upper and lower lips. One buccal papilla. Surface of lips papillose, with numerous papillae. Distal margin of the lower lip fringed with minute triangular papillae. Maxillary barbel minute. Teeth not numerous (≈ 30 per jaw), pedunculated and arranged in a single, comblike row. Subpreopercle not visible in ventral view. Abdomen naked except for lateral abdominal plates. Five to 8 (modally 7) lateral abdominal plates. Twenty-four to 26 (modally 25) plates in median lateral series, plates not coalescing.

Dorsal-fin origin more or less in front of pelvic-fin insertion. Dorsal and pectoral fins with i,6 rays. Pectoral spine longer than branched rays, slightly curved to S-shaped, reaching beyond pelvic-fin origin. Pelvic fin with i,5 rays; spine longer, reaching beyond anal-fin origin. Anal fin with i,4 rays, spine longer. Caudal fin truncated with i,12,i rays.

Colouration

In alcohol, background colour of dorsal surface of head and body dark brown, with usually 4 thick postdorsal dark bands, bands in the position of the third and fourth of congeners merged into a large black transverse saddle on the caudal peduncle. Anterior part of body darker, almost black in certain areas. Ventral surface lighter, yellowish tan. Often with a dark blotch at anal-fin origin. Fin rays yellowish tan with indistinct dark brown spots more or

less forming stripes. Fins membranes hyaline, becoming lighter toward distal margins. Caudal fin with poorly defined dark stripes. Medial part of caudal fin with a lighter yellowish band.

Sexual dimorphism

Males with a wider head and thickened pectoral spines bearing hypertrophied odontodes. Body more densely covered by odontodes than in females, especially on head.

Distribution and habitat

Only known from type locality in French Guiana, in a small forest creek of the Kotika Mountains at an altitude of 515 m (Fig. 5).

Etymology

The species group name *janmoli* honours the Dutch ecologist Jan H. Mol for his strong personal investment in the knowledge and protection of *Harttiella*, especially in Suriname where he recovered the highly vulnerable *H. crassicauda*.

Harttia Steindachner, 1877

Harttia Steindachner, 1877: 668. Type species: *Harttia loricariformis* Steindachner, 1877. Type by monotypy. Gender: Feminine.

Harttia is significantly distinguished from all other Guianese Harttiini by 29 morphometric variables (Tab. V) among which, six possessed very strong loadings onto PCA axes (Fig. 1b). *Harttia* is diagnosed from other Guianese Harttiini by a wider body at anal-fin origin [mean = 14.98±1.44% of SL versus 13.50±1.43 (HSD, p-value < 0.0001) and 12.02±1.03 (HSD, p-value < 0.0001) in *Harttiella* and *Cteniloricaria* respectively], at eighth postdorsal plate [mean = 12.28±1.50% of SL versus 10.59±1.29 (HSD, p-value < 0.0001) and 9.30±0.95 (HSD, p-value < 0.0001) in *Harttiella* and *Cteniloricaria* respectively], and at fourteenth postdorsal plate [mean = 5.27±0.81% of SL versus 4.76±0.77 (HSD, p-value < 0.0001) and 3.96±0.46 (HSD, p-value < 0.0001) in *Harttiella* and *Cteniloricaria* respectively]; a greater nostril to tip of snout length [mean = 42.45±2.47% of HL versus 41.74±2.23 (HSD, p-value < 0.0001) and 38.98±2.71 (HSD, p-value < 0.0001) in *Harttiella* and *Cteniloricaria* respectively]; and more numerous premaxillary [mean = 80±17 versus 34±8 (HSD, p-value < 0.0001) and 40±12 (HSD, p-value < 0.0001) in *Harttiella* and

Cteniloricaria respectively] and dentary teeth [mean = 78±17 versus 33±8 (HSD, p-value < 0.0001) and 39±10 (HSD, p-value < 0.0001) in *Harttiella* and *Cteniloricaria* respectively]. The following combination of characters also differentiates the genus: abdomen partially to wholly covered by very small, rhombic, plates without particular organization. Abdominal plating sometimes restricted to preanal and lateral abdominal plates. Body large, flattened covered by very short odontodes conferring a rather smooth aspect to the species. Subpreopercle exposed. Lateral plates keeled and coalescing toward the end of caudal peduncle. Caudal peduncle becoming more compressed between the eighth and fourteenth postdorsal plates.

Within the *Harttia* group, very strong morphological structures were found with significant differences in PCA scores on the two first axis ($\chi^2_{K\text{-}W}$ = 128.9601, p-value < 0.0001; $\chi^2_{K\text{-}W}$ = 44.2382, p-value < 0.0001). Deep genetic divergences were also recovered with K2P distances ranging between 0 and 0.19. Considering the different populations of *H. guianensis*, slight differences in shape were found on axis 1 ($\chi^2_{K\text{-}W}$ = 35.4856, p-value < 0.0001; $\chi^2_{K\text{-}W}$ = 0.1685, p-value = 0.9192), and almost no differences in genetics (0 < K2P < 0.0017). The three populations of *H. guianensis* (Maroni, Sinnamary, and Approuague drainages) therefore correspond to a single, morphologically relatively plastic, species. Significant differences between populations were highlighted and characterized in Covain *et al.* (2006), and are not repeated herein. Significant differences in shape were also recorded between *H. surinamensis* and the populations from Coppename and Paru de Oeste Rivers ($\chi^2_{K\text{-}W}$ = 17.9322, p-value = 0.0001; $\chi^2_{K\text{-}W}$ = 14.1004, p-value = 0.0009), whereas slight genetic differences of populational level were obtained between *H. surinamensis* and the population from Coppename River (K2P = 0.012), and deep divergences of between species level between *H. surinamensis* and the population of Paru de Oeste River (K2P = 0.07). Nevertheless no morphometric differences were found between Coppename and Paru de Oeste populations (W = 358, p-value = 0.5305; W = 280, p-value = 0.415), even though these two populations diverged from a K2P distance of 0.068. Moreover, significant differences in shape were recovered by both axes between *H. surinamensis* and the population of Coppename River on one hand (W = 1138, p-value = 0.0027; W = 1175, p-value = 0.0008), and the population of Paru de Oeste on the other hand (W = 1344, p-value = 0.0002; W = 1209, p-value = 0.0111). These three populations represent distinct species, with the one from Coppename River sharing the morphology of the species from Paru de Oeste, and possessing a mitochondrial signal close to the one of *H. surinamensis*. *Harttia fowleri* does not possess strong morphometric differences compared to other *Harttia* (W = 2002, p-value = 0.9877; W

= 2498, p-value = 0.0298). Nevertheless, it possesses the strongest genetic divergences, with K2P distances ranging between 0.176 and 0.190.

Harttia guianensis Rapp Py-Daniel and Oliveira, 2001
(Supplementary material S5)

Harttia guianensis Rapp Py-Daniel and Oliveira, 2001:88, Fig. 6. Type locality: Approuague River, Saut Athanase, 4°11'N, 52°19'W, French Guiana. Holotype: MNHN 1998-0395.

Harttia guianensis Rapp Py-Daniel and Oliveira, 2001: Isbrücker 2001:27; Isbrücker 2002:16; Ferraris in Reis *et al.* 2003:335; Provenzano *et al*. 2005:521; Covain *et al.* 2006:9; Ferraris 2007:241; Vari *et al*. 2009:29.
Harttia surinamensis not Boeseman, 1971: Boujard *et al*. 1997:141; Le Bail *et al.* 2000:274.

Morphometric and meristic data are provided in table I, and GenBank accession numbers for barcodes in Table II. *Harttia guianensis* is distinguished from congeneric species by five morphometric variables (Tab. V). *Harttia guianensis* possesses a longer caudal peduncle [mean = 48.73±1.29% of SL versus 46.54±1.17 < mean < 47.67±1.04% of SL; (HSD, p-values < 0.0002)]; a smaller anus to tip of snout length [mean = 39.60±1.15% of SL versus 40.74±0.70 < mean < 41.84±1.02% of SL; (HSD, p-values < 0.0001)]; a shorter snout [mean = 54.51±2.03% of HL versus 56.52±2.01 < mean < 58.89±5.12% of HL; (HSD, p-values < 0.0001)]; and a smaller nostril to tip of snout length [mean = 41.04±1.85% of HL versus 42.42±2.21 < mean < 43.94±1.69% of HL; (HSD, p-values < 0.0003)], and head depth [mean = 35.27±2.23% of HL versus 36.52±1.80 < mean < 39.75±2.37% of HL; (HSD, p-values < 0.0254)]. K2P distances to congeneric species ranged from 0.064 to 0.183 according to the population. Its colouration confers it camouflage with rocks in its natural habitat, making it difficult to observe (Supplementary material S6-A). The background colour of the dorsal surface is yellowish tan to beige. A dark, almost black, marbling covers the dorsal surface and five black postdorsal bands are present. In juveniles, this pattern exhibits greater contrast and the head appears greenish with a golden area on the supra-occipital and between the eyes; eye copper coloured. A large black basicaudal blotch is present. The caudal fin is deeply forked and has the distal ends of upper and lower lobes black, and the medial part bright yellow. A black blotch is also often present on the tip of the dorsal fin. All paired fins and dorsal fin

possess dark spots on rays forming distinct stripes. The lower surface is yellowish tan. The abdominal plating is restricted to lateral abdominal plates (5 to 8, modally 7) and to the preanal plates. Two large quadrangular plates are present immediately in front of the anus and are bordered by smaller plates up to the pelvic-fin insertion. *Harttia guianensis* has usually 29 plates in the lateral series, these plates are keeled and coalescing toward the 20th to 22nd plates. The caudal peduncle becomes much more compressed after the confluence of plates. The head is large, with a large elliptic mouth with papillose lips. The subpreopercle is well exposed, triangular, and covered by odontodes. The teeth are numerous (around 80 on each jaw), pedunculated, and arranged in two staggered, comblike rows. In males, the sexual dimorphism consists in the hypertrophy of odontodes on the upper surface of the thickened pectoral spines, on the snout margin, and on keels of the lateral plates. The sexual dimorphism is seasonal. Evers and Seidel (2005) reported that breeding males lost the hypertrophied odontodes of the pectoral spines, at least five days after breeding. This species occurs in coastal drainages of French Guiana and Suriname, from the Approuague River to the Maroni/Marowijn River (Fig. 5). It is an inhabitant of the main channel of rivers, where it colonizes rocky and sandy areas in fast flowing waters. The species is locally very abundant, and is often syntopic with *C. platystoma*, except in Approuague River.

Harttia surinamensis Boeseman, 1971

(Supplementary material S7)

Harttia surinamensis Boeseman, 1971: 28, pl. 3. Type locality: Grandam, Gran Rio, upper Suriname River, Surinam. Holotype: RMNH 26388 (188.30 mm specimen, holotype not separated from paratypes).

Harttia surinamensis Boeseman, 1971: Isbrücker 1980:90; Burgess 1989:439; Langeani *et al.* 2001:141; Rapp Py-Daniel and Oliveira 2001:80; Isbrücker 2001:27; Isbrücker 2002:16; Ferraris in Reis *et al.* 2003:335; Provenzano *et al.* 2005:521; Covain *et al.* 2006:9; Ferraris 2007:242; Vari *et al*. 2009:39.

Morphometric and meristic data are provided in table I, and GenBank accession number for barcode in table II. Two morphometric variables strictly characterized *H. surinamensis* (Tab. V). *Harttia surinamensis* is distinguished from all congeneric species except *H. tuna* n. sp. by a longer head (shorter compared to *H. tuna*) [mean = 24.15±1.38% of SL versus 23.19±1.33 <

mean < 23.45±0.95% of SL; (HSD, p-values < 0.0364), and mean = 24.97±1.14% of SL in *H. tuna* (HSD, p-value = 0.0033)]; a wider body at eighth postdorsal plate compared to *H. guianensis* and *H. fowleri* [mean = 12.35±1.63% of SL versus 11.69±1.32 < mean < 11.82±1.25% of SL; (HSD, 0.0113< p-values < 0.0325)], and narrower compared to *H. tuna* and *H. fluminensis* n. sp. [mean = 12.35±1.63% of SL versus 13.28±1.47 < mean < 13.52±0.97% of SL; (HSD, 0.001< p-values < 0.007)]. K2P distances to congeneric species ranged between 0.064 and 0.176. Its colouration is reminiscent of the substrate, making it difficult to observe in its natural habitat (Supplementary material S6-B). The background colour of the dorsal surface is yellowish tan. Dark marbling covers the dorsal surface and five indistinct postdorsal bands are present, the last three toward the tail being more clearly marked. The eyes are copper coloured. A large deep-black band covers the basal one third of the caudal-fin surface. The caudal fin is deeply forked with the distal end of the lower lobe black, and the medial part bright yellow. A blackish thin band is present in the yellow part of the caudal fin. A black blotch is also often present on the tip of the dorsal fin. All paired fins and dorsal fin possess dark spots on the rays forming distinct stripes. The lower surface is yellowish tan. The abdominal plating is complete in specimens > 150 mm SL, but otherwise usually incomplete, and made of small granular platelets without particular organization. Ontogenetic development of the abdominal cover starts with the appearance (around 80 mm SL) of small granular platelets at the border of the preanal plates. The number of platelets increases then slowly with fish size, until establishing a connection between the preanal plates and the lateral abdominal plates (around 90 mm SL). The number of platelets then continues to increase in the preanal area, along the pelvic-fin insertion, and along the lateral abdominal plates. When the region delimited by the preanal plates, and the pelvic-fin insertion is almost wholly plated, a second transverse arch of platelets crosses the abdomen starting from the insertion of pelvic spines, or the first lateral abdominal plates (around 110 mm SL). The transverse arch becomes thicker with the increasing number of platelets. The region between the arch and the preanal area is eventually covered, and a medial row of platelets appears on the abdomen (around 140 mm SL). The number of platelets along the lateral abdominal plates continues to increase with the size of the fish, as well as in the middle part of the abdomen, making the medial row thicker. The regions delimited by the medial row, and the left and right series of lateral abdominal plates are then little by little covered by platelets, and the convergence is obtained around 180 mm SL. A stage of this developmental pattern is present in almost all specimens of *H. surinamensis*, but the size to which the abdomen appears wholly platted is highly variable among individuals. *Harttia surinamensis* has usually 29 plates in the

lateral series, these plates keeled and coalescing toward the 19th to 22nd plates. The caudal peduncle becomes abruptly more compressed after the confluence of plates. The head is large, with a large elliptic mouth with papillose lips. The subpreopercle is well exposed, triangular, and covered by odontodes. The teeth are numerous (approximately 80 on each jaw), pedunculated, and arranged in two staggered, comblike rows. Sexual dimorphism consists of the hypertrophy of odontodes on the upper surface of the thickened pectoral spines in mature males. *Harttia surinamensis* is restricted to the Suriname River (Fig. 5) where it frequents the main channel over rocky and sandy bottoms, in fast flowing waters.The species is locally very abundant, and is often syntopic with *C. platystoma*.

***Harttia fluminensis* Covain and Fisch-Muller, new species**

(Figs. 13, 5, Tabs. I, II)

Holotype

MHNG 2724.003 (ex MHNG 2690.013, specimen SU01-458), 151.14 mm SL, Suriname, Coppename River at Raleighvallen, Mol, 30 Nov. 2006.

Paratypes

MHNG 2690.013 (14); MNHN 2011-0033 (2, ex MHNG 2690.013); same data as holotype. MHNG 2690.012 (6); NZCS F7077 (1, ex MHNG 2690.012), Suriname, Coppename River at Raleighvallen, Mol, 29 Nov. 2006.

Diagnosis

Harttia fluminensis is distinguished from all congeners except *H. tuna* n. sp. and *H. trombetensis* by an incomplete abdominal cover, restricted to preanal and abdominal lateral plates with a row of platelets joining these two series of plates (versus no row of platelets making junction between preanal and lateral abdominal plates), and by its specific barcode sequence (JF292263). It can be distinguished from *H. tuna* by a deeper head [37.31-43.30, mean 38.94±1.42% of HL, versus 31.96-38.77, mean 36.52±1.80% of HL (HSD, p-value < 0.0001)], and from *H. trombetensis* by colour pattern of caudal fin (a large dark band at base of caudal fin, versus a dark rounded blotch). Additionally *H. fluminensis* is distinguished from all other congeneric species by two morphometric variables (Tab. V). *Harttia fluminensis* possesses a greater minimum caudal peduncle depth [mean = 1.60±0.11% of SL versus

1.35±0.13 < mean < 1.43±0.11% of SL; (HSD, p-values < 0.0001)]; and a greater interorbital width [mean = 24.25±1.02% of HL versus 22.04±1.27 < mean < 23.12±1.11% of HL; (HSD, p-values < 0.028)].

Figure 13. - *Harttia fluminensis*, holotype, MHNG 2724.003, 151.14 mm SL, Suriname, Coppename River at Raleighvallen.

Description

Morphometric and meristic data in table I, and GenBank accession numbers in table II.

General aspect of fish flat and broad, with a thick caudal peduncle before confluence of lateral keels. Head large, short and triangular to slightly rounded in dorsal view. Eye large, orbit more or less round, without notch. Odontodes very short, conferring fish a smooth aspect. Snout tip naked.

Mouth elliptic with large and thick upper and lower lips. Surface of lips papillose, with numerous papillae. Distal margin of the lower lip fringed with minute triangular papillae. Maxillary barbel minute. Teeth numerous (≈ 90 per jaw), pedunculated and arranged in two staggered, comblike rows. One buccal papilla. Subpreopercle well exposed in ventral view, triangular, and covered by odontodes. Abdomen naked except for preanal plates, lateral abdominal plates, and a row of platelets making junction between previous series of plates. Six to 9 (modally 7) lateral abdominal plates, plates keeled but not sharp. Two large preanal plates. Twenty-nine to 30 (modally 29) plates in median lateral series. Lateral plates keeled, coalescing between 7^{th} and 9^{th} last postdorsal plates. Caudal peduncle abruptly compressed after confluence of lateral plates.

Dorsal-fin origin more or less in front of pelvic-fin insertion. Dorsal and pectoral fins with i,6 rays. Pectoral spine longer than soft rays, slightly curved, reaching much beyond pelvic-fin origin. Pelvic fin with i,5 rays; spine longer, just reaching anal-fin origin. Anal fin with i,4 rays, spine shorter. Caudal fin forked with i,12,i rays.

Colouration

In alcohol, background colour of dorsal surface of head and body dark brown tan, with 5 to 6 indistinct postdorsal dark bands and dark marbling. Ventral surface lighter, yellowish tan. Abdomen whitish. A large dark band at base of caudal fin representing 1/3 of the fin surface. Distal two-thirds of caudal fin lighter with a thinner dark band. Tip of lower lobe black. Fin rays yellowish tan with distinct dark brown spots forming stripes. Tip of dorsal fin with a black blotch. Fins membranes hyaline, except paired fins reddish anteriorly.

Sexual dimorphism

Males with a wider head and thickened pectoral spines bearing hypertrophied odontodes.

Distribution and habitat

Only known from the Coppename River drainage in Suriname (Fig. 5), where it frequents the main channel over rocky and sandy bottom, in fast flowing waters.

Etymology

The species group name *fluminensis* is from Latin *flumen* meaning river, and makes reference to the ecology of *Harttia* that represents a group of rheophilic fish from the main channel of rivers.

***Harttia tuna* Covain and Fisch-Muller, new species**

(Figs. 14, 5, Tabs. I, II)

Holotype

MZUSP 108150 (ex MHNG 2704.029, specimen SU07-660), 170.95 mm SL, Sipaliwini Savannah in Trio Amerindian territory at the Suriname-Brazil border, Four Brothers Mountains in a tributary of the Paru de Oeste River, gift of the Trio tribe in Sipaliwini, 20-21 Oct. 2007.

Paratypes

MHNG 2704.029 (20); MZUSP 108151 (2, ex MHNG 2704.029); MNHN 2011-0034 (2, ex MHNG 2704.029); NZCS F7078 (2, ex MHNG 2704.029), same data as holotype.

Diagnosis

Harttia tuna is distinguished from all other congeneric species except *H. fluminensis* and *H. trombetensis* by an incomplete abdominal cover restricted to preanal and abdominal lateral plates with a row of platelets joining these two series of plates (versus no row of platelets making junction between preanal and lateral abdominal plates), and by its specific barcode sequence (JF292262). It can be distinguished from *H. fluminensis* by a shallower head [31.96-38.77, mean 36.52±1.80% of HL, versus 37.31-43.30, mean 38.94±1.42% of HL; (HSD, p-value < 0.0001)], and from *H. trombetensis* by the colour pattern of the caudal fin (a dark rounded blotch at base of caudal fin, versus a large dark band). Four morphometric variables strictly characterize *H. tuna* (Tab. V). *Harttia tuna* possesses a longer head [mean = 24.97±1.14% of SL versus 23.19±1.33 < mean < 24.15±1.38% of SL; (HSD, p-values < 0.0032)]; a greater predorsal length [mean = 33.58±0.84% of SL versus 32.10±1.05 < mean <

33.01±1.11% of SL; (HSD, p-values < 0.017)]; a smaller postdorsal length [mean = 55.44±1.10% of SL versus 56.63±0.94 < mean < 57.35±1.26% of SL; (HSD, p-values < 0.0157)]; and a smaller orbital diameter [mean = 20.97±1.12% of HL versus 22.68±1.43 < mean < 23.37±1.08% of HL; (HSD, p-values < 0.0006)].

Figure 14. - *Harttia tuna*, holotype, MZUSP 108150, 113.20 mm SL, Sipaliwini Savannah in Trio Amerindian territory at the Suriname-Brazil border, Four Brothers Mountains in a tributary of the Paru de Oeste River.

Description

Morphometric and meristic data in table I, and GenBank accession number in table II.

General aspect of fish flat and broad, with a thick caudal peduncle before confluence of lateral keels. Head large, short and triangular to slightly rounded in dorsal view. Eye large, orbit more or less round, without notch. Odontodes very short, conferring fish a smooth aspect. Snout tip naked.

Mouth elliptic with large and thick upper and lower lips. Surface of lips papillose, with numerous papillae. Distal margin of the lower lip fringed with minute triangular papillae. Maxillary barbel minute. Teeth numerous (≈ 90 per jaw), pedunculated and arranged in two staggered, comblike rows. One buccal papilla. Subpreopercle well exposed in ventral view, triangular, and covered by odontodes. Abdomen naked except for preanal plates, lateral abdominal plates, and a row of platelets making junction between previous series of plates. Six to 9 (modally 7) lateral abdominal plates, plates keeled but not sharp. Two medium sized preanal plates. Twenty-nine to 30 (modally 29) plates in median lateral series. Lateral plates keeled, coalescing between 7^{th} and 9^{th} last postdorsal plates. Caudal peduncle abruptly compressed after confluence of lateral plates.

Dorsal-fin origin more or less in front of pelvic-fin insertion. Dorsal and pectoral fins with i,6 rays. Pectoral spine longer than branched rays, slightly curved, reaching much beyond pelvic-fin origin. Pelvic fin with i,5 rays; spine longer, just reaching anal-fin origin. Anal fin with i,4 rays, spine shorter. Caudal fin forked with i,12,i rays.

Colouration

In alcohol, background colour of dorsal surface of head and body greyish tan, with 6 to 7 indistinct postdorsal darker bands and brownish poorly defined spots and marbling. A large black quadrangular area below eyes. Ventral surface lighter, yellowish. Abdomen whitish. A large dark band at base of caudal fin representing 1/3 of fin surface. External part of caudal fin lighter with a thinner brownish band. Tip of lower lobe black. Fin rays yellowish tan with distinct dark brown spots forming stripes. Tip of dorsal fin with a black blotch. Fins membranes hyaline.

Sexual dimorphism

Males with a larger head and thickened pectoral spines bearing hypertrophied odontodes.

Distribution and habitat

Known from upper Paru de Oeste River (Fig. 5).

Etymology

The species group name *tuna* is from the Amerindian Trio-Wayana meaning river, water. It refers to *H. fluminensis* which has a name with the same meaning, because of their extreme morphological resemblance. A name used in apposition.

Harttia fowleri (Pellegrin, 1908)

(Supplementary material S8)

Oxyloricaria fowleri Pellegrin, 1908: 126. Type locality: Rivière Camopi (Guyane française). Holotype: MNHN 1901-0372.

Harttia fowleri (Pellegrin, 1908): Boeseman 1971:9; Rapp Py-Daniel and Oliveira 2001:81; Provenzano *et al.* 2005:521; Covain *et al.* 2006:9.

Cteniloricaria fowleri (Pellegrin, 1908): Isbrücker 1979:91; Burgess 1989:440; Le Bail *et al.* 2000:266; Isbrücker 2001:26, 30; Isbrücker 2002:15; Ferraris in Reis *et al.* 2003:331; Ferraris 2007:233; Vari *et al*. 2009:39.

Morphometric and meristic data are provided in table I, and GenBank accession number for barcode in table II. Only one morphometric variable distinguishes *H. fowleri* from all congeneric species (Tab. V). *Harttia fowleri* possesses more numerous lateral abdominal plates [mean = 10±2 versus 7±1 < mean < 8±2; (HSD, p-values < 0.0001)]. K2P distances to congeneric species ranged between 0.176 and 0.190. Its colouration mimics the substrate. The background colour of the dorsal surface is reddish tan (Supplementary material S6-C). Sparse dark marbling covers the head surface and 5 to 8 (modally 6) distinct postdorsal bands are present. The eyes are golden to copper-coloured. In juveniles, this colour pattern is more contrasted over a rather greenish background dorsal colour (Supplementary material S6-D). A large deep black basicaudal blotch is present. The caudal fin is deeply forked with the distal end of the lower and upper lobes black, and the medial part yellowish. A black blotch is also present on the tip of the dorsal fin. All paired-fins and dorsal-fin spines are covered with dark spots. The surface colour of paired fins is reddish anteriorly, rather yellowish further posteriorly, and blackish toward their extremity. The lower surface of body is yellowish tan.

The abdominal plating is complete in specimens greater than 120 mm SL, made of small granular platelets without particular organization. Abdominal plating reaches gill opening with the throat not covered, and the anterior margin V-shaped. Ontogenetic development of the abdominal cover is similar to that of *H. surinamensis*, begins at a smaller size and is always complete in adults. *Harttia fowleri* usually has 29 plates in the lateral series, these plates are keeled and coalescing toward the 19th to 21st plates. The caudal peduncle becomes abruptly more compressed after the confluence of plates. The head is wide, with a large elliptic mouth with papillose lips. The subpreopercle is well exposed, triangular, and covered by odontodes. The teeth are numerous (around 75 on each jaw), pedunculated, and arranged in a single, comblike row. The sexual dimorphism is unknown despite a large sampling effort, but could be reminiscent of what can be observed in other *Harttia*. Some specimens bear thicker pectoral spines with few, well visible odontodes. Moreover, such specimens, suspected to be males, also possess longer pectoral and pelvic-fin spines which are prolonged into soft extensions (not filamentous). This species is restricted to the Oyapock/Oiapoque River drainage in French Guiana and Brazil (Fig. 5). This is the largest species of the group within the Guianas, and specimens greater than to 220mm SL are not unusual. It is an inhabitant of the main channel where it colonizes rocky and sandy areas, in fast flowing waters. The species is locally abundant.

DISCUSSION

This global assessment of the diversity of Harttiini within the Guianas unambiguously demonstrates that the richness of this group was greatly underestimated until now. No fewer than 9 new taxa are presented here increasing the total number of known species to 14 (more than twice the number previously recorded). The Harttiini show strong morphological trends supporting the validity of three genera: *Harttiella*, *Cteniloricaria* and *Harttia*. This division into three entities was also strongly supported by the COI barcodes, with distinct lineage-specific patterns in GC contents and deep genetic divergences between genera (mean = 0.197 K2P distance). Notably, the high divergences between genera found here are greater than reported elsewhere. Ward *et al.* (2009), in a review about the campaign of DNA barcoding in fishes, reported a mean value of 0.1619±0.0004 for the K2P variation within family (= between genera) based on the sequencing of 1,677 specimens belonging to 546 species and 273 genera, most of them representing Australian marine forms. In another study conducted

on freshwater fishes from Canada, Hubert *et al.* (2008) reported a between genera variation of 0.1538±0.0001 based on the sequencing of 1,360 specimens belonging to 190 species and 85 genera. Our results relating to between and within species levels perfectly corroborate previous findings, with within species K2P distance variation reaching 0.0027±0.0005 in this study versus 0.0035±0.0001 in Ward *et al*. (2009) and 0.0027±0.0001 in Hubert *et al*. (2008). The within genera divergences reached 0.0878±0.0333 in this study and 0.0811±0.0004 and 0.0837±0.0003 in Ward *et al*. (2009) and Hubert *et al*. (2008), respectively. Contrasting slightly with these results, Valdez-Moreno *et al*. (2009) reported a variation of 0.1357±0.0007 at between genera level, and 0.051±0.0008 and 0.0045±0.0001 at within genera and within species levels respectively, in the COI sequences of freshwater fishes from Mexico and Guatemala (results obtained based on 427 specimens representing 61 species and 36 genera). These authors hypothesised a more recent origin of freshwater fishes compared to their marine counterparts to explain differences with Ward *et al*.'s results. Nevertheless, the hypothesis of a younger origin of freshwater fish species is not supported by our results, nor by the study of Hubert *et al.* (2008). The latter, assuming the hypothesis that the fragmentation of freshwater ecosystems leads to stronger genetic structure among populations and to deeper divergence among haplotypes in freshwater fishes than in marine ones (Ward *et al*. 1994), pointed out that the pattern of variation in distances was strikingly similar between both groups (freshwater and marine fishes). Although they detected geographic structure in their data, they concluded that the higher geographic structure in freshwater fishes was not necessarily reflected in deeper intra and interspecific divergence. They nevertheless admitted that the Canadian freshwater fish fauna could be relatively recent given that most of the rivers and lakes were colonized after the glacial retreat at the end of the Pleistocene. The deep differences between genera and the surprisingly similar levels of variation between and within species observed in our data may thus be explained by the fact that Guianese Harttiini represents an ancient lineage, but its diversification within the Guianas could be relatively recent.

Hebert *et al*. (2004) suggested that divergent specimens could be flagged as putative species if they showed 10-fold the mean intraspecific differentiation for the group under study. Ward (2009) demonstrated that this statement was correct, even thought rather conservative especially considering cryptic speciation. Ward (2009) refined the approach of Hebert *et al*. (2004), and based on the analysis of 1,088 species of fish, proposed that specimens with divergences greater than 2% were likely to be different species with a probability greater than 0.95. This threshold applied to a great majority of our data, since all

but two species exhibit interspecific variations greater than 0.027 (10x within species distance here of 0.0027) leading to a distinct barcoding gap between species (Meyer and Paulay, 2005). Only *Harttiella intermedia* shares identical barcode sequences with its congener *H. longicauda*, representing less than 6% of all species assignment. Different explanations have been proposed to explain such phenomena (Hebert *et al*., 2003; Meyer and Paulay, 2005; Hubert *et al*., 2008; Ward *et al.,* 2009). Introgressive hybridization and poor taxonomy were often put forward. Nevertheless Hubert *et al*. (2008) pointed out that the establishment of reciprocal monophyly between two sister taxa was also a function of time, given that fixation of a new coalescent follows the line of descent. When not enough time passed to split sister species, one may obtain a paraphyletic grouping with one species nested within a second one (then the coalescent of the first species is contained within the coalescent of the second) or a polyphyletic grouping, both species sharing the same coalescent (Meyer and Paulay, 2005). *Harttiella intermedia* may consequently represent a vicariant form of the latter resulting from a founder effect. Both species being present within the Sinnamary basin, a small population derived from *H. longicauda* may have been quite recently isolated in the Trinité Massif. Following the example of the East African lacustrine cichlid species flock, evolution of morphology in a small isolated population can occur very quickly, before enough time has passed to genetically differentiate the species. *Harttiella intermedia* could therefore represent rather a very recently emerging species whose morphology evolved very quickly making it perfectly distinct from *H. longicauda*. The second problem with the global threshold used here concerned the lineage including *Harttia surinamensis*. The 2% threshold used does not allow recognition of *H. surinamensis* and *H. fluminensis* as distinct species whereas these two entities are clearly morphologically diagnosable. Conversly, this threshold allowed the discovery of two pairs of cryptic species: *Harttia fluminensis* and *H. tuna*, and *Harttiella lucifer* and *H. longicauda*. These two pairs of species are indeed very difficult to distinguish morphologically but the amount of genetic divergence accumulated by both pairs of species left no doubt about their validity. This case of morphological stasis where the ancestral shape of the group was maintained almost identically in the two species while a significant amount of mutation has accumulated in their respective COI genes, contrasts with the case of *H. intermedia* and *H. longicauda*. A last unexpected result was the amount of divergence observed in *Harttia fowleri*. While it appears morphologically very close to Guianese *Harttia*, it possesses smaller genetic divergences with *Cteniloricaria*. Moreover, the NJ unrooted tree obtained here placed *H. fowleri* outside *Harttia* and *Cteniloricaria* at the base of the tree.

Despite the problems generally encountered in highly diversified lineages, the COI barcode approach has proven to be a relevant and powerful tool to assess the global diversity of Harttiini within the Guianas. Moreover, the significant lineage dependence highlighted in GC content, particularly GC1 and GC3, allows envisaging their use directly in a multivariate framework for explanatory or discrimination purposes.

The unifying structure provided by the multi-table approach including genetics, morphometry, and ecology-distribution establishes the link between all types of data, and provided a graphical output allowing recognition of congruence and incongruence between tables. Unsurprisingly, the morphology and genetics were highly congruent, and few variations were observable on the factorial map, the most unstable species between preliminary representations being *H. fowleri*. On the other hand, ecological and distributional data displayed stronger differences. In all respects, the quality of the obtained consensus allowed a detailed exploration of the data. Indisputably, the greatest advantage of the MCOA is the unification of the different variables contained in the different data set within the same analysis. This allows a graphical exploration of those variables and highlights unrevealed associations between them onto co-inertia axes. Indeed, strong correlations were found between an intraphenotypic component composed of genetics and morphology, and an extraphenotypic component made of ecological and distributional variables. Moreover the tests against phylogenetic dependence, first on MCOA axes and secondarily on all variables, allow the interpretation of these associations in an evolutionary perspective. The evolution of Harttiini within the Guianas was thus shaped by (or oriented toward) adaptations to a definite type of biotope. Indeed, *Cteniloricaria* and *Harttia* are members of the rheophilic fauna inhabiting the main stream of rivers, a biotope strongly exposed to the sunlight. These ecological parameters were tightly linked to morphological adaptations such as an increase in size of the caudal peduncle revealing adaptation toward better abilities for swimming (Watson and Balon, 1984), and to an increase in the number of plates providing further protection in these rocky and turbulent biotopes. The increase in size of the eye may imply that these fish are more active by day (higher temperature of the biotope due to higher exposition to the sunlight), thus representing diurnal loricariids, a family of catfishes usually considered as nocturnal. Moreover, *Harttia* possesses strong tendencies toward having a wider caudal peduncle, making it an even more powerful and more efficient swimmer, as well as having more numerous teeth, thereby increasing its ability to grasp algae that grows over rocks. These strong ecomorphological trends probably enable it to exploit its immediate environment more effectively than *Cteniloricaria*. This probably explains the relative scarcity of the latter

when both genera are sympatric. This complex relationship nevertheless deserves further research to better characterize the ecomorphological trends shaping these two genera. In contrast, *Harttiella* evolved adaptations to mountainous forest creeks, a biotope characterised by its cool temperature due to altitude and probably to tree shade, and its greater conductivity due to the small size of the streams (less water compared to the river) and to the abundant dissolved organic matter issued from the decomposition of the constantly falling dead leaves. These adaptations include dwarfism since all *Harttiella* represent dwarf species of Harttiini, the largest specimen presently known being the holotype of *H. longicauda* (52.46 mm SL), as well as changes in shape. These include a tendency for the species to be rather thickset with broader, longer and deeper head characteristics, and a shorter, broader and thicker caudal peduncle. The eye is small in *Harttiella,* perhaps due to the abundant forest coverage restricting sunlight or to nocturnal habits. An evolutionary trend was also detected in the longitudinal dispersion of Harttiini, *Harttiella* being rather distributed in the eastern part of the Guianas, and *Cteniloricaria* in the western part. Even though an evolutionary gradient is revealed, the areas of dispersal overlap between the three genera. However, this distribution may reflect the capture effort which as been more intense in eastern Guianas. Excluding *H. fowleri*, restricted to the extreme east of the Guianas, and following a gradient from west to east, *Cteniloricaria* is distributed from the Essequibo to the Sinnamary, *Harttia* from the Coppename to the Approuague, and *Harttiella* from the Maroni to the Approuague. *Harttiella* possesses thus the smallest distribution of all Harttiini within the Guianas, as well as the greatest number of species. This implies very limited distribution for several of its representatives, most of them being distributed in patches, particularly in the Maroni system. All members of the *crassicauda* group are restricted to few or even single creeks of a single mountain, making them highly vulnerable. The small size of populations coupled with a potential absence of gene flow within these species (each genetic signal being unique for the time being along the Maroni River for example) may threaten them with extinction in case of severe damage to their immediate environment. This makes them species of conservation interest for the definition of protected areas, and urgent measures should be taken to protect the species, several being directly affected by mining activities. Only some members of the *longicauda* group seem to have a wider distribution that includes several river systems.

A last result provided by the MCOA may be noted. The fact that principal coordinates computed from the decomposition of the K2P matrix were highly correlated with the MCOA axes, and that these axes were under phylogenetic dependence, implies that the distance matrix contained a significant amount of phylogenetic signal. Moreover the significance of

the test of substitution saturation implies that the COI gene is a good candidate for the reconstruction of a phylogeny of Guianese Harttiini. The NJ K2P distance tree obtained herein could therefore be very close to the topology reconstructed using robust phylogenetic methods. If the position of *H. fowleri* in the NJ tree corresponds to its phylogenetic position, *Harttia* could represent a paraphyletic assemblage. Since all *Harttiella* and *Cteniloricaria* appear to form monophyletic groups in the present topology, the assignment of Guianese representative of *Harttia* to that genus should be reconsidered. A genetic comparison to the type species, *H. loricariformis* from the Paraíba do Sul River in Southeast Brazil would clear up this uncertainty.

The multi-table approach, initially devoted for the study of ecological patterns, has already proven to be relevant in the study of synchrony in the temporal variability of aquatic communities (Bady *et al*., 2004), or to the contribution of molecular markers to the structures of populations (Jombart *et al*., 2006). In this study, the MCOA also revealed its ability to extract the evolutionary trends shaped through time in a tribe of poorly differentiated catfishes. Still rarely used, this type of approach should be considered more widely in an evolutionary framework to provide stronger prerequisites for a correct estimation of the underlying forces driving the evolution of the groups under study.

KEY TO THE SPECIES OF GUIANESE HARTTIINI

1a. – Minimum caudal peduncle depth 0.94-1.9% of SL **2**

1b. - Minimum caudal peduncle depth 2.0-5.6% of SL ***Harttiella* 8**

2a. - Presence of a complete abdominal cover in specimens greater than 70 mm SL made of medium sized rhombic plates; caudal fin with a large median black crescent: ***Cteniloricaria* 3**

2b. - Absence of a complete abdominal cover; when present, abdominal cover restricted to lateral abdominal plates, preanal plates or made of small granular platelets, cover not complete in specimens smaller than 120 mm SL; caudal fin often with a black basicaudal blotch ***Harttia* 4**

3a. – Colour pattern of dorsal surface of body distinctly spotted ***C. napova* (Paru de Oeste River)**

3b. – Colour pattern of dorsal surface of body indistinctly blotched or marbled ***C. platystoma* (Essequibo to Sinnamary Rivers)**

4a. – Abdominal cover constituted of small granular platelets on the abdomen **5**

4b. - Abdominal cover restricted to preanal and lateral plates; a row of platelets may join these two series **6**

5a. – Presence of a large basicaudal spot; teeth arranged in a single row ***H. fowleri*** **(Oyapock River)**

5b. – Presence of a large basicaudal band; teeth arranged in two staggered rows ***H. surinamensis*** **(Suriname River)**

6a. – Presence of small granular platelets between lateral abdominal plates and base of pectoral fins; in adults, presence of a row of platelets joining preanal to lateral abdominal plates **7**

6b. – Absence of small granular platelets between lateral abdominal plates and base of pectoral fins; in adults, absence of a row of platelets joining preanal to lateral abdominal plates ***H. guianensis*** **(Maroni/Marowijne to Approuague Rivers)**

7a. – Head depth representing 37.3-43.3% of HL ***H. fluminensis*** **(Coppename River)**

7b. – Head depth representing 32.0-38.8% of HL ***H. tuna*** **(Paru de Oeste River)**

8a. – Minimum caudal peduncle depth representing 7.3-15.8% of caudal peduncle length ***crassicauda*** **group 9**

8b. – Minimum caudal peduncle depth representing 4.3-8.4% of caudal peduncle length ***longicauda*** **group 12**

9a. – Colour pattern of caudal fin distinctly banded ***H. parva*** **(Atachi Bakka Mt)**

9a. – Colour pattern of caudal fin not distinctly banded **10**

10a. – Dorsal surface with usually 5 well separated dark bands posterior to dorsal-fin origin **11**

10b. – Dorsal surface with usually 4 postdorsal bands, 3rd band appearing as a large black transverse saddle ***H. janmoli*** **(Kotika Mt.)**

11a. - Caudal peduncle length more than 40 % of SL ***H. intermedia*** **(Trinité Mt.)**

11b. - Caudal peduncle length less than 40 % of SL ***H. crassicauda*** **(Nassau Mt.)**

12a. – Hispid appearance of mature males, width of pectoral and pelvic girdles almost equivalent ***H. pilosa*** **(Tortue Mt.)**

12b. – Smooth appearance of mature males, pectoral girdle much wider than pelvic girdle **13**

13a. Pelvic spine just reaching anal-fin origin ***H. longicauda***

13a. Pelvic spine reaching beyond anal-fin origin ***H. lucifer***

Acknowledgments. - We are grateful to Philippe Keith, Romain Causse and Patrice Pruvost, MNHN; Martien van Oijen and Koos van Egmond, RMNH; Ronald Vonk and Hielke Praagman, ZMA; James Maclaine, BMNH; Mark Sabaj Perez and John Lundberg, ANSP for the loan of type and complementary material and welcome in collections; Philippe Gaucher, CNRS Guyane; Olivier Tostain, Ecobios Cayenne; Régis Vigouroux and Philippe Cerdan, Hydreco Guyane; Michel Jégu and Bernard de Mérona, IRD; François Meunier and Yves Fermon, MNHN; Hélène Pagesy, CNRS; Alexandre Fort, Pedro Hollanda Carvalho, Claude Weber and Alain Merguin, MHNG; Maël Dewynter, ONF Guyane; Frédéric Melki, Biotope France; Kenneth Wan Tong You, Paramaribo; Paul Ouboter, NZCS; Calvin Bernard, CSBD; Vincent Piron and Colin Niel, Parc de la Guyane; the North Rupununi District Development Board (NRDDB); the Association Arataï; and the Iwokrama organization for their field and logistic assistance; the G. and A. Claraz Foundation for their financial support for the missions in Suriname in 2001, 2005, 2007 and 2008, and in French Guiana in 2006; the Académie Suisse des Sciences Naturelles (ScNat) for their financial support for the missions in Guyana 2004 and French Guiana 2006; The Constantin Topali Found for their financial contribution to the acquisition of field material for the mission Suriname 2007, and lab material in 2009; André Huser, Sallmann-Fehr AG for the gift of gill nets for the mission in Suriname in 2005 and 2007; the Guyana Environmental Protection Agency, and Ministry of Amerindian affairs; the French Guiana Diren ,and Préfecture; and the Surinamese Ministry of Agriculture, Animal Husbandry and Fisheries for the different necessary authorizations and collecting permits. Part of this project was supported by the Fond National Suisse de la Recherche Scientifique (JIMB 3100A0–104005). The pictures and figures were finalized by Philippe Wagneur and Florence Marteau, MHNG. Preliminary results of this study were presented in 4ème Rencontres de l'Ichtyologie en France (RIF), Paris, 2009; the 6ème symposium national de morphométrie et évolution des formes (SMEF), Montpellier, 2009; and the 13th Evolutionary Biology Meeting at Marseilles, 2009 with the financial support of the Société Académique de Genève. John A. Hollier, MHNG, improved language usage and style on the earliest version of the manuscript. This manuscript benefited from useful and constructive comments from Carl J. Ferraris, Smithsonian Institution, Robert D. Ward, CSIRO Marine and Atmospheric Research, and Pierre Sagnes, University of Lyon. This work represents part of the PhD dissertation of the first author, who would particularly acknowledge Denis Duboule, University of Geneva, Sonia Fisch-Muller, MHNG, and Stéphane Dray, University of Lyon for their supervision.

REFERENCES

ABOUHEIF E., 1999. - A method to test the assumption of phylogenetic independence in comparative data. *Evol. Ecol. Res*., 1: 895-909.

AITCHINSON J., 1986. - The Statistical Analysis of Compositional Data. 416 p. London: Chapman & Hall.

ALBRECHT G., 1978. - Some comments on the use of ratios. *Syst. Zool*, 27: 67-71.

ATCHLEY W.R. & ANDERSON D., 1978. - Ratios and the statistical analysis of biological data. *Syst. Zool*., 27: 71-78.

ATCHLEY R., GASKINS C.T. & ANDERSON D., 1976. - Statistical properties of Ratios. I. Empirical results. *Syst. Zool*., 25: 137-148.

BADY P., DOLÉDEC S., DUMONT B. & FRUGET J.F., 2004. - Multiple co-inertia analysis: a tool for assessing synchrony in the temporal variability of aquatic communities. *C.R. Biol*., 327: 29-36.

BAKKER J.P. & LANJOUW J., 1949. - Indrukken van de natuurwetenschappelijke expeditie naar Suriname 1948-49. *Tijdschr. K. Ned. Aardr. Gen*., 66: 538-557.

BENJAMINI Y. & YEKUTIELI D., 2001. - The control of the false discovery rate in multiple testing under dependency. *Ann. Stat*., 29: 1165-1188.

BOESEMAN M., 1953. - Scientific results of the Surinam expedition 1948-1949. Part II. Zoology, No. 2. The Fishes (I). *Zool. Med*., 32: 1-24.

BOESEMAN M., 1971. - The "comb-toothed" Loricariinae of Surinam, with reflections on the phylogenetic tendencies within the family Loricariidae (Siluriformes, Siluroidei). *Zool. Verh.,* 116: 1-56.

BOUJARD T., PASCAL M., MEUNIER J.F. & LE BAIL P.-Y. (eds.), 1997. - Poissons de Guyane. Guide écologique de l'Approuague et de la réserve des Nouragues. 219 p. Paris: INRA, Hydrobiologie et Aquaculture.

BURGESS W.E., 1989. - An atlas of freshwater and marine catfishes. A preliminary survey of catfishes. 784 p. Neptune City: T. F. H. Publications, Inc.

CAILLIEZ F., 1983. - The analytical solution of the additive constant problem. *Psychometrika*, 48: 305-310.

CHARIF D. & LOBRY J.R., 2007. - Seqin R 1.0-2: a contributed package to the R project for statistical computing devoted to biological sequences retrieval and analysis. *In*: Structural Approaches to Sequence Evolution: Molecules, Networks, Populations (Bastolla U., Porto M., Roman H.E. & Vendruscolo M., eds), pp. 207-232. New York: Springer Verlag.

CHESSEL D. & HANAFI M., 1996. - Analyses de la co-inertie de K nuages de points. *Rev. Stat. Appl*., 44: 35-60.

CORRUCINI S., 1977. - Correlation properties of Morphometric ratios. *Syst. Zool*., 26: 211-214.

COVAIN R. & FISCH-MULLER S., 2007. - The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: Loricariidae): a practical key and synopsis. *Zootaxa*, 1462: 1-40.

COVAIN R., LE BAIL P.-Y., SAGNES P. & FISCH-MULLER S., 2006. - Les espèces du genre *Harttia* (Siluriformes : Loricariidae) en Guyane française : morphologie, taxinomie et distribution. *Cybium*, 30: 3-18.

COVAIN R., DRAY S., FISCH-MULLER S. & MONTOYA-BURGOS J.I., 2008. - Assessing phylogenetic dependence of morphological traits using co-inertia prior to

investigate character evolution in Loricariinae catfishes. *Mol. Phylogenet. Evol.*, 46: 986-1002.
COVAIN R., FISCH-MULLER S., MONTOYA-BURGOS J.I., MOL J. LE BAIL P-Y. & DRAY S., 2009a. - Les Harttiini (Siluriformes, Loricariidae) des Guyanes : phylogénie et évolution des formes ou variation sur un même thème. *In* : Quatrièmes Rencontres de l'Ichtyologie en France, p. 40. Paris: SFI, IRD.
COVAIN R., FISCH-MULLER S., MONTOYA-BURGOS J.I., MOL J. LE BAIL P-Y. & DRAY S., 2009b. - Phylogeny and evolution of shapes: a new approach to predict phylogenetic positions of missing taxa. *In :* Sixième Symposium national de Morphométrie et Évolution des Formes, p. 41. GRD 2474 CNRS "Morphométrie et Evolution des Formes", Montpellier.
COVAIN R., FISCH-MULLER S., MONTOYA-BURGOS J.I., MOL J. LE BAIL P-Y. & DRAY S., 2009c. - Extracting phylogenetic signal in morphometric data to complete molecular phylogenies: a case study on Harttiini (Siluriformes, Loricariidae) from the Guianas. *In:* Thirteenth Evolutionary Biology Meeting at Marseilles, p. 80. AEEB, Marseille.
DODSON P., 1978. - On the use of ratios in growth studies. *Syst. Zool*., 27: 62-67.
DRAY S. & DUFOUR A.B., 2007. - The ade4 package: implementing the duality diagram for ecologists. *J. Stat. Soft*., 22(4): 1-20. .

DRAY S., PETTORELLI N. & CHESSEL D., 2003. - Multivariate analysis of incomplete mapped data. *Trans. GIS*, 7:411-422.

EFRON B., 1979. - Bootstrap methods: another look at the jackknife. *Ann. Stat*., 7: 1-26.
EIGENMANN C.H., 1912. - The freshwater fishes of British Guiana, including a study of the ecological grouping of species and the relation of the fauna of the plateau to that of the lowland. *Mem. Carneg. Mus.*, 5: 1-578, 103 pls.
EVERS H.G. & SEIDEL I., 2005. - Catfish Atlas Vol. 1: South American Catfishes of the Families Loricariidae, Cetopsidae, Nematogenyidae and Trichomycteridae. 943 p. Melle: Mergus Verlag Gmbh.
FELSENSTEIN J., 1985. - Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39: 783-791.
FERRARIS Jr C.J., 2003. - Subfamily Loricariinae (Armored catfishes). *In*: Check List of the Freshwater Fishes of South and Central America (CLOFFSCA) (Reis R.E., Kullander S.O. & Ferraris C. J., eds), pp. 330-350. Porto Alegre: EDIPUCRS.

FERRARIS Jr C.J., 2007. - Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. *Zootaxa*, 1418: 1-628.

FRICKE R. & ESCHMEYER W.N., 2010. - A guide to Fish Collections in the Catalog of Fishes database. On-line version of 15 Jan. 2010. http://research.calacademy.org/redirect?url=http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp
GOWER J.C., 1966. - Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika*, 53: 325-338.

GÜNTHER A., 1868. - Diagnoses of some new freshwater fishes from Surinam and Brazil, in the collection of the British Museum. *Ann. Mag. Nat. Hist*., ser. 4, 1: 475-481.
HALL T.A., 1999. - BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.*, 41: 95-98.

HEBERT P.D.N., CYWINSKA A., BALL S.L. &. DE WAARD J.R., 2003. - Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B*., 270: 313-322.

HEBERT P.D.N., STOECKLE M.Y., ZEMLAK T.S. & FRANCIS C.M., 2004. - Identification of birds through DNA barcodes. *PLoS Biol.,* 2:1657-1663.

HILLS M., 1978. - On ratios - A response to Atchley, Gaskins, and Anderson. *Syst. Zool*., 27: 61-62.

HOLM S., 1979. - A simple sequentially rejective multiple test procedure. *Scand. J. Stat*., 6: 65-70.

HOREAU V., CERDAN P., CHAMPEAU A. & RICHARD S., 1998. - Importance of aquatic invertebrates in the diet of rapids-dwelling fish in the Sinnamary River, French Guiana. *J. Trop. Ecol*., 14: 851-864.

HUBERT N., HANNER R., HOLM E., MANDRAK N.E., TAYLOR E., BURRIDGE M., WATKINSON D., DUMONT P., CURRY A., BENTZEN P., ZHANG J., APRIL J. & BERNATCHEZ L., 2008. - Identifying Canadian freshwater fishes through DNA barcodes. *Plos One*, 3(6): e2490. Doi:10.1371/journal.pone.0002490.

ISBRÜCKER I.J.H., 1973. - Redescription and figures of the South American mailed catfish *Rineloricaria lanceolata* (Günther, 1868) (Pisces, Siluriformes, Loricariidae). *Beaufortia*, 278: 75-89.

ISBRÜCKER I.J.H., 1979. - Descriptions préliminaires de nouveaux taxa de la famille des Loricariidae, poissons-chats cuirassés néotropicaux, avec un catalogue critique de la sous-famille nominale (Pisces, Siluriformes). *Rev. Fr. Aquariol*., 5: 86-116.

ISBRÜCKER I.J.H., 1980. - Classification and catalogue of the mailed Loricariidae (Pisces, Siluriformes). *Versl. tec. Geg., Inst. taxon. Zoöl. (zoöl. Mus.)*, 22: 1-181.

ISBRÜCKER I.J.H., 2001. - Nomenklator der Gattungen und Arten der Harnischwelse, Familie Loricariidae Rafinesque, 1815 (Teleostei, Ostariophysi). *Datz Harnischwelse*, 2: 25-32.

ISBRÜCKER I.J.H., 2002. -Nomenclator of the 108 genera with 692 species of the mailed catfishes, family Loricariidae Rafinesque, 1815 (Teleostei, Ostariophysi). *Cat Chat, Journal of the catfish study group (UK)*, 3: 11-30.

ISBRÜCKER I.J.H., SEIDEL I., MICHELS J.P., SCHRAML E. & WERNER A., 2001. - Diagnose vierzehn neuer Gatungen der Familie Loricariidae Rafinesque, 1815 (Teleostei, Ostariophysi). *In:* Harnischwelse 2 (Stawikowski R., ed.), pp. 17-24. Stuttgart, Germany: Die Aquarien und Terrarien Zeitschrift, Sonderheft, Eugen Ulmer.

JOMBART T., MOAZAMI, GOUDARZI K., DUFOUR A.B. & LALOË D., 2006. - Fréquences alléliques et cohérence entre marqueurs moléculaires : des outils descriptifs. *Actes BRG*, 6: 25-39.

JOMBART T., BALLOUX F. & DRAY S., 2010. - Adephylo: new tools for investigating the phylogenetic signal in biological traits. *Bioinformatics*, 26: 1907-1909.

KEITT T., 2009. - ColorRamps: builds color tables. R package version 2.3. http://CRAN.R-project.org/package=colorRamps.

KIMURA M., 1980. - A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*., 15: 111-120.

LANGEANI F., OYAKAWA O.T. & MONTOYA-BURGOS J.I., 2001. - New species of *Harttia* (Loricariidae, Loricariinae) from the São Francisco Basin. *Copeia*, 2001: 136-142.

LE BAIL P.-Y., KEITH P. & PLANQUETTE P., 2000. - Atlas des poissons d'eau douce de Guyane. Tome 2, fascicule II: Siluriformes. 307 p. Paris: M.N.H.N./S.P.N.

LEGENDRE P. & LAPOINTE F.J., 2004. - Assessing congruence among distance matrices: single malt Scotch whiskies revisited. *Aust. N.Z. J. Stat*., 46: 615-629.

LÉVÊQUE C., OBERDORFF T., PAUGY D., STIASSNY M.L.J. & TEDESCO P.A., 2008. - Global diversity of fish (Pisces) in freshwater. *Hydrobiologia,* 595: 547-567.

MANTEL N., 1967. - The detection of disease clustering and a generalized regression approach. *Cancer Res.*, 27: 209-220.

MÉRONA B. de, 2005. - Le Fleuve, le Barrage et les Poissons. Le Sinnamary et le Barrage de Petit-Saut en Guyane française. 135 p. Paris: Editions IRD.

MEYER C.P. & PAULAY G., 2005. - DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol.,* 3: 2229-2238.

MOL J.H. & OUBOTER P.E., 2004. - Downstream effects of erosion from small-scale gold mining on the instream habitat and fish community of a small neotropical rainforest stream. *Conserv. Biol.*, 18: 201-214.

MOL J.H., WAN TONG YOU K., VREDE I., FLYNN A., OUBOTER P. & VAN DER LUGT F., 2007. - Fishes of Lely and Nassau Mountains, Suriname. *In:* A Rapid Biological Assessment of the Lely and Nassau Plateaus, Suriname (with additional information on the Brownsberg Plateau) (Alonso L.E. & J.H. Mol, eds), pp. 107-118. RAP Bulletin of Biological Assessment 43. Arlington, USA: Conservation International.

MONTOYA-BURGOS J.I., MULLER S., WEBER C. & PAWLOWSKI J., 1998. - Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences. *In*: Phylogeny and Classification of Neotropical Fishes (Malabarba L.R., Reis R.E., Vari R.P., Lucena Z.M.S. & C.A.S. Lucena, eds), pp. 363-374. Porto Alegre: EDIPUCRS.

MORAN P.A.P., 1950. - Notes on continuous stochastic phenomena. *Biometrika* 37: 17-23.

NÉGREL P. & LACHASSAGNE P., 2000. - Geochemistry of the Maroni River (French Guiana) during the low water stage: implications for water-rock interaction and groundwater characteristics. *J. Hydrol*., 237: 212-233.

PARADIS E., 2006. - Analysis of Phylogenetics and Evolution with R. 211 p. New York: Springer.

PARADIS E., CLAUDE J. & STRIMMER K., 2004. - APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20: 289-290.

PAVOINE S., OLLIER S., PONTIER D. & CHESSEL D., 2008. - Testing for phylogenetic signal in life history variable: Abouheif's test revisited, *Theor. Popul. Biol.*, 73: 79-91.

PLANQUETTE P., KEITH P. & LE BAIL P.Y., 1996. - Atlas des Poissons d'Eau douce de Guyane (T. 1). Collection Patrimoine Naturel, 22 : 1-429. Paris, IEGB-MNHN, INRA, CSP, Min. Environ.

PROVENZANO R.F., MACHADO-ALLISON A., CHERNOFF B., WILLINK P. & PETRY P., 2005. - *Harttia merevari*, a new species of catfish (Siluriformes: Loricariidae) from Venezuela. *Neotrop. Ichthyol.*, 3: 519-524.

R DEVELOPMENT CORE TEAM, 2009. - R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

RAPP PY-DANIEL L.H. & OLIVEIRA E.C., 2001. - Seven new species of *Harttia* from the Amazonian-Guyana region (Siluriformes: Loricariidae). *Ichthyol. Explor. Freshw.*, 12: 79-96.

REGAN C.T., 1904. - A monograph of the fishes of the family Loricariidae. *Trans. zool. Soc. Lond.*, 17: 191-350, pls 199-121.

SAITOU N. & NEI M., 1987. - The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406-425.

SARKAR D., 2010. - Lattice: lattice graphics. R package version 0.18-3. http://CRAN.R-project.org/package=lattice.

SONDAG F., GUYOT J.L., MOQUET J.S., LARAQUE A., ADÈLE G., COCHONNEAU G., DOUDOU J.C., LAGANE C. & VAUCHEL P., 2010. - Suspended sediment and dissolved load budgets for two Amazonian rivers of French Guiana: Maroni and Oyapock rivers. *Hydrol. Process*., 24: 1433-1445.

TENENHAUS M., 1998. - La Régression PLS: Théorie et Pratique. 254 p. Paris: Editions Technip.

THIOULOUSE J. & DRAY S., 2007. - Interactive multivariate data analysis in R with the ade4 and ade4TkGUI packages. *J. Stat. Soft*., 22(5): 1-14.

VALDEZ-MORENO M., IVANOVA N.V., ELÍAS-GUTIÉRREZ M., CONTRERAS-BALDERAS S. & HEBERT P.D.N, 2009. - Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. *J. Fish Biol*., 74: 377-402.

VARI R.P. & FERRARIS Jr C.J., 2009. - Fishes of the Guiana Shield. *In:* Checklist of the Freshwater Fishes of the Guiana Shield (Vari R.P., Ferraris Jr C.J., Radosavljevic A. & Funk V.A., eds). *Bull. Biol. Soc. Washington*, 17: 9-18.

VARI R.P., FERRARIS JR C.J., RADOSAVLJEVIC A. & FUNK V.A., 2009. - Checklist of the Freshwater Fishes of the Guiana Shield. *Bull. Biol. Soc. Wash.*, no. 17: i-vii, 1-95.

WARD R.D., 2009. - DNA barcode divergence among species and genera of birds and fishes. *Mol. Ecol. Res*., 9: 1077-1085.

WARD R.D., WOODWARK M. & SKININSKI D.O.F., 1994. - A comparison of genetic diversity levels in marine, freshwater and anadromous fish. *J. Fish Biol*., 44: 213-232.

WARD R.D., ZEMLAK T.S., INNES B.H., LAST P.R. & HEBERT P.D.N, 2005. - Barcoding Australia's fish species. *Phil. Trans. R. Soc. B*, 360: 1847-1857.

WARD R.D., HANNER R. & HEBERT P.D.N., 2009. - Review paper: the campaign to DNA barcode all fishes, FISH-BOL. *J. Fish Biol*., 74: 329-356.

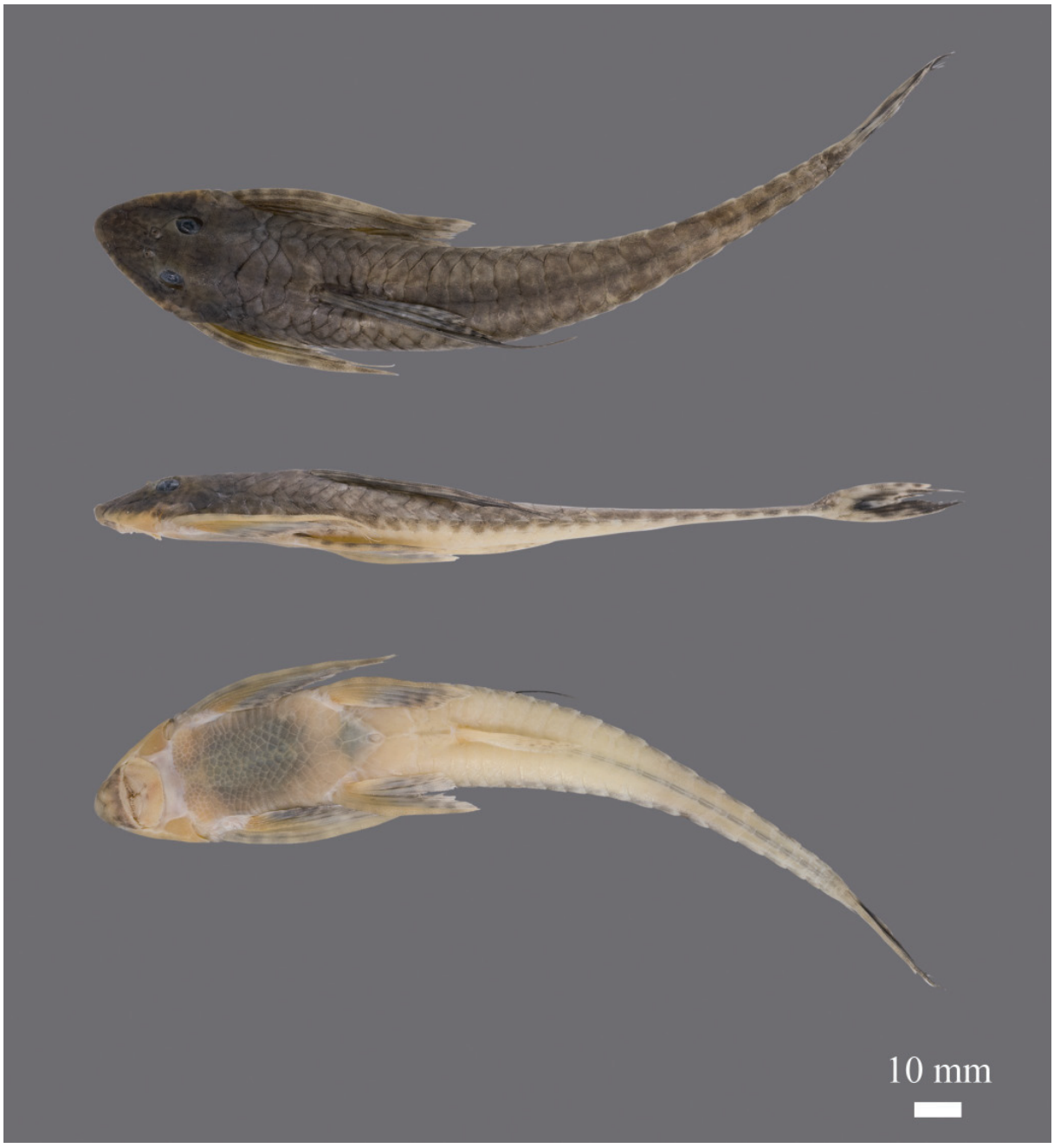
WATSON D.J. & BALON E.K., 1984. - Ecomorphological analysis of fish taxocenes in rainforest streams of northern Borneo. *J. Fish Biol*., 25: 371-384.

WOLD H., 1966. - Estimation of principal components and related models by iterative least squares. *In:* Multivariate Analysis (Krishnaiah P., ed.), pp. 391-420. New York: Academic Press.

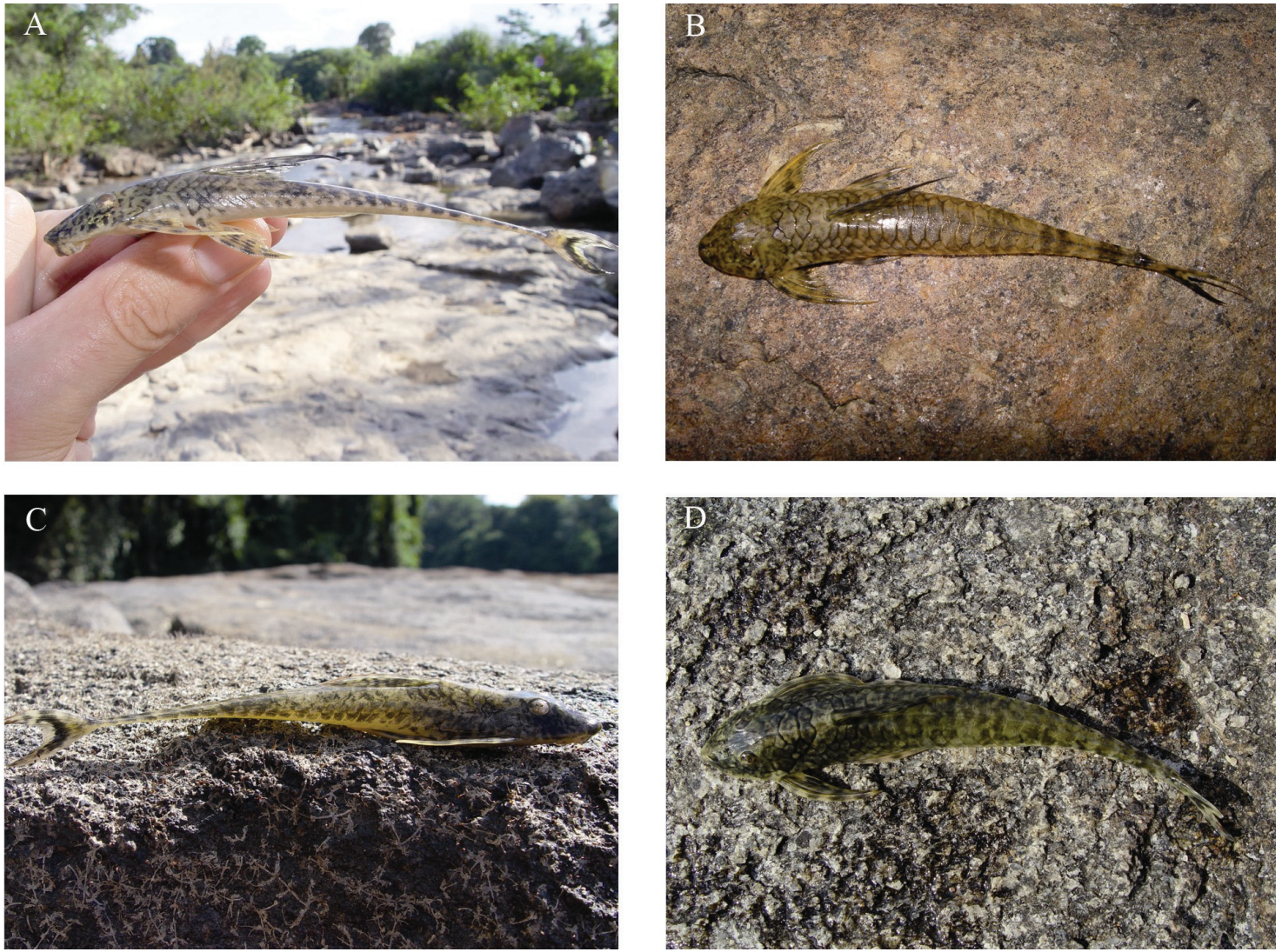
XIA X. & XIE Z., 2001. - DAMBE: Data analysis in molecular biology and evolution. *J. Hered*., 92:371-373.

XIA X., XIE Z., SALEMI M., CHEN L. & WANG Y., 2003. - An index of substitution saturation and its application. *Mol. Phylogenet. Evol.*, 26: 1-7.

Supplementary material



S1. - *Cteniloricaria platystoma*, MHNG 2704.016, 171.78 mm SL, Suriname, Sipaliwini River, Paikali rapid.



S2. - *Cteniloricaria platystoma* in life for different populations. **A**: Guyana, Essequibo River, Kurupukari Cross (R. Covain); **B**: Suriname, Corantijn River, Cow Falls (R. Covain); **C**: Suriname, Suriname River, Assigon (R. Covain); **D**: Suriname, Paloemeu River, Weyu Camp (R. Covain).

S3. - *Harttiella crassicauda*, MHNG 2674.051 (specimen MUS 221), 38.00 mm SL, Suriname, Nassau Mountains, Paramaka Creek.

S4. - *Harttiella* spp. in life. **A**: *H. crassicauda*, Suriname, Nassau Mountains, Paramaka Creek (T. Larsen); **B**: H. pilosa, French Guiana, Tortue Mountains, Orapu River drainage in Crique Grillon (R. Covain).

S5. - *Harttia guianensis*, MHNG 2643.008, 146.07 mm SL, French Guiana, Litani River, vicinity of Antecume Pata.

S6. - *Harttia* spp. in life. **A**: *H. guianensis*, Suriname, Paloemeu River, Weyu Camp (R. Covain); **B**: *H. surinamensis*, Suriname, Suriname River, Gran Rio (R. Covain); **C**: *H. fowleri*, French Guiana, Oyapock River, Alikoto Falls (R. Covain); **D**: *H. fowleri*, French Guiana, Oyapock River, Moulou Koulou (R. Covain).

S7. - *Harttia surinamensis*, MHNG 2673.033 (specimen SU05-230), 183.98 mm SL, Suriname, Suriname River, Cajana Creek.

S8. - *Harttia fowleri*, MHNG 2680.091 (specimen GF06-016), 210.47 mm SL, French Guiana, Oyapock River, Alikoto Falls.

Chapter 4

Tracking back co-dispersion events between Harttiini and *Hypostomus* (Siluriformes: Loricariidae) by comparative phylogeography: a new approach using the RLQ analysis.

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The RLQ analysis is here evaluated to detect co-dispersion events in two co-distributed groups of the Loricariidae: the Harttiini tribe, and the Hypostomus *genus. The detection of common structures in both phylogenies being potentially related to co-dispersion events, the dating provided in one phylogeny for the dispersion of* Hypostomus *species will be applied to the phylogeny of Harttiini to propose a phylogeographic hypothesis for the historical diversification of this tribe at the sub-continental scale.*

To be submited.

Abstract

Reconstructing the history of dispersion of species to understand the underlying mechanisms responsible for their current diversity and distribution remains a challenging issue. However, grasping the correct spatio-temporal frame is not always reliable in practice by lack of obvious calibration points for dating phylogenetic trees. Assuming the hypothesis that co-distributed taxa in different regions underwent co-dispersion events, we adapt the RLQ analysis, originally developed in the field of community ecology, for the comparison of phylogenies of co-distributed species. This approach has the advantage of considering the whole phylogenies without transforming them into regional trees, and uses a third table of species co-occurrences based on their spatial distributions. The RLQ analysis provides a graphical output describing the phylogenetic spatial co-structure, allowing the detection of regions of both phylogenies linked through the spatial information. We provide testing procedures aimed to detect the presence of a significant phylogenetic spatial co-structure in the data, and to highlight which part of both phylogenies are significantly linked to this co-structure. We have experienced this approach in the phylogeographic comparison of two widely distributed groups of the Neotropical catfish family Loricariidae: the Harttiini tribe and the *Hypostomus* genus. A molecular phylogeny of the Harttiini based on mitochondrial and nuclear genes has first been inferred. The resulting phylogeny indicated that the Harttiini was monophyletic and included *Harttia*, *Harttiella*, and *Cteniloricaria*. This phylogeny was then compared to a previously published phylogeny of *Hypostomus*. The RLQ analysis highlighted a strong spatial co-structure of both trees implying a common co-dispersion of species between the Amazonian and Southeastern regions. The molecular dating provided for this dispersion event in the phylogeny of *Hypostomus* was accordingly used to calibrate the tree of Harttiini. The subsequent dating estimated for the phylogeny of Harttiini meets generally those of *Hypostomus*. An explosive radiation is revealed at base of both lineages, followed by intensive diversification throughout the Miocene period. These similar patterns suggest a common temporal context in the dispersion and diversification processes of both lineages. Despite local effects, a common global factor related to the sea level fluctuations can explain such diversification.

Keywords: Neotropics, Loricariinae, Hypostominae, molecular phylogeny, biogeography, co-distributed species, molecular dating.

Introduction:

With a prediction of around 8,000 extant species (Lévêque *et al.*, 2008), the Neotropical freshwaters house the greatest icthyodiversity in the world. Several hypotheses have been proposed to explain such a tremendous diversity (review in Hubert and Renno, 2006), but despite congruencies in faunal distributions (Hubert *et al.*, 2007), the underlying mechanisms responsible of species' richness and distribution are still oddly largely misunderstood (Montoya-Burgos 2003; Chiachio *et al.*, 2008). In this context, freshwater fishes represent a group of high interest due to biological and physiological adaptations constraining their abilities to dispersion. Contrary to marine or terrestrial organisms, freshwater fishes are only able to disperse within a basin, or between adjacent basins. Major climatic and geological events shaped the modern South-American Rivers through the entire Miocene and Pleistocene (Lundberg *et al.*, 1998), providing opportunities for vicariance and/or dispersion of species through headwaters or estuaries secondary contacts (Torrico *et al.*, 2009). Given that the history of the contemporaneous rivers is tightly linked to these underlying geological events, the chronology of river connections, and accordingly species' dispersion, may be track back in time (*e.g.* Bermingham and Martin, 1998; Lovejoy and Araujo, 2000; Montoya-Burgos, 2003; Albert *et al.*, 2006; Hubert and Renno, 2006; Hubert *et al.*, 2007; Willis *et al.*, 2007; Chiachio *et al.*, 2008; Torrico *et al.*, 2009; Lovejoy *et al.*, 2010; Willis *et al.*, 2010). Nevertheless, reconstructing the history of species dispersion to explain their contemporaneous distribution may remain a challenge. Due to a lack of well documented geological archives that can be used as calibration dates, such as fossil records or orogenic events, the correct spatio-temporal framework stay often difficult, if not impossible, to grasp. A possible solution to overcome this difficulty may consist in the phylogeographic comparison of codistributed species. The comparative phylogeography represents indeed an efficient method for elucidating shared vicariant events (Edwards and Beerli, 2000). A common practice consists thereby to reconstruct phylogenies across common geographic areas, and to evaluate their topological and temporal congruencies. In this case, one can expect that if codistributed species exhibit a similar pattern in the branching order of their respective phylogenetic tree (*i.e.* reciprocal monophyly), it may be due to the fact that these species dispersed following the same processes at the same period (when the rivers connected). In other words, sister clades in different groups are expected to occupy similar geographic areas. If a dating is provided for the cladogenesis of a group, it can be accordingly apply to the other. However exploring phylogeographical patterns to assess congruence

between phylogenetic trees, and its significance (that the observed structures were not due to chance) is not always reliable in practice. Some of the major limitations in comparative phylogeography are directly dependant of the tree sources themselves. Different studies may have been conducted based on different markers, using different tree reconstruction methods assuming different assumptions, on different sample sizes, with only partial overlap of the geographic areas, making direct comparisons hazardous. Several methods have been used or proposed to assess congruencies between phylogeographic trees (*e.g.* Brooks, 1985; Page, 1994; Taberlet *et al*., 1998; Edwards and Beerli, 2000; Sullivan *et al*., 2000; Lapointe and Rissler, 2005; Ganapathy *et al*., 2006), but until recently, comparisons have been essentially made visually. Lapointe and Rissler (2005) proposed the use of the Maximum Agreement Subtrees (MAST) algorithm (Kubicka *et al*., 1995) in a permutation procedure to assess the significance of the MAST measure (congruence) between "county" trees. The maximum agreement subtree between two rooted trees is obtained by pruning the fewest number of leaves from each tree so that both trees become identical. Prior to compute the MAST score, the data are often recoded into common regional units. This step represents the Achilles' heel of this approach. Indeed, if the MAST algorithm allows the comparison of trees bearing different numbers of leaves (Dong and Kraemer, 2004; Lapointe and Rissler, 2005), the compatibility between trees is given by the leaves' labels. Since one taxon may be widespread and distributed in several areas, or to the contrary several taxa may be restricted to a single area, the recoding of phylogenies into area cladograms may force to make strong *a priori*, leading to a loss of information, or worst skewing the analysis toward the expected result.

We present here an alternative solution to assess the congruence among phylogenetic trees based on the RLQ analysis (Dolédec *et al.,* 1996), and fourth corner associated tests (Legendre *et al.*, 1997; Dray and Legendre, 2008). This approach, initially developed in community ecology, allows the detection of co-variations between two tables using a third table as link. When the two tables represent two phylogenies, and the link table contains the spatial distribution of the species, the RLQ analysis provides a graphical representation of the spatial co-structure of the two trees. The congruent parts of both phylogenetic trees under spatial constraint can thus be easily detected. Moreover, *a priori* testing provides a first general estimation of the significance of the observed spatial co-structure, and *a posteriori* tests are able to detect which parts of both phylogenies are congruent with the spatial information. A second advantage of this method is its ability to use the trees without modification, and to establish the link between both trees in a third table. Multiple links for widespread taxa or redundant taxa can therefore be easily used.

We have implemented the RLQ approach to identify common patterns of distribution between two groups of Loricariidae, the Harttiini and the *Hypostomus*, to infer co-dispersion events. The spatio-temporal framework of dispersion of *Hypostomus* has been characterized in Montoya-Burgos (2003), but no data are presently available to characterize and date the dispersion of Harttiini. To allow the comparison, this study has therefore started by the reconstruction of a molecular phylogeny of Harttiini based on mitochondrial and nuclear genes. The Harttiini is a tribe of Loricariinae among the large Neotropical catfish family Loricariidae. In Central and South America, the Siluriformes represents the most diverse order with around 1,647 described species (Reis *et al*., 2003) among which Loricariidae represents the most speciose family comprising 716 valid species and around 300 recognized as undescribed (Reis *et al*., 2003; Ferraris, 2007). Loricariids are characterized by a depressed body covered by bony plates, a unique pair of maxillary barbels, and above all, by an important modification of the mouth structure into a sucker disk. They have undergone an evolutionary radiation on a subcontinental scale that was compared to that of the Cichlidae of the Great Lakes of the Rift Valley in Africa (Schaefer and Stewart, 1993). Among Loricariidae, members of the subfamily Loricariinae are characterized by a long and depressed caudal peduncle and by the absence of an adipose fin. Phylogenetic reconstructions based both on morphological (Schaefer, 1987; Armbruster, 2004) and molecular data (Montoya-Burgos *et al*., 1998) demonstrated that Loricariinae formed the sister clade of Hypostominae. They represent a highly diversified subfamily comprising 230 species for 32 genera (Rodriguez *et al*., in press; Covain *et al*., in press) distributed in two tribes: the Harttiini and the Loricariini.

The Harttiini comprises rheophilic fishes mainly distributed in the eastern part of South America, in rivers flowing the Brazilian and Guiana Shields. Probably due to lack of obvious morphological characteristics, the systematics of Harttiini remains unclear and controversial. Isbrücker (1979), made a first tentative to classify Loricariinae on the basis of external morphological characters, but without phylogenetic inferences. He placed *Sturisoma*, *Harttia*, *Lamontichthys*, *Harttiella*, *Pterosturisoma*, *Cteniloricaria*, *Sturisomatichthys*, and *Metaloricaria* within Harttiini, and defined this tribe in having the dorsal-fin originating approximately opposite to the pelvic-fins origin, the caudal fin with 12 (rarely 11) soft rays, no orbital notch, and a poor diversity in lips and teeth structure. Montoya-Burgos *et al.* (1998) proposed the first molecular phylogeny of the family Loricariidae with emphasis on the subfamily Hypostominae. Although, their analysis included only nine representatives of the subfamily Loricariinae, they provided first evidences that Harttiini, as defined by Isbrücker

(1979), represented a paraphyletic assemblage. Isbrücker (in Isbrücker *et al*., 2001) described *Quiritixys* only based on the unusual sexual dimorphism of *Harttia leiopleura*. Rapp Py-Daniel and Oliveira (2001) described seven species of *Harttia*, and put *Cteniloricaria* in the synonymy of *Harttia*. Ferraris (2003; 2007) maintained the validity of *Cteniloricaria*, and put *Quiritixys* in the synonymy of *Harttia*. Provenzano *et al*. (2005), Covain *et al*. (2006), and Covain and Fisch-Muller (2007) maintained *Ctenilolicaria* into the synonymy of *Harttia*. Covain *et al.* (2008) proposed the first molecular phylogeny of the subfamily, and redefined the systematics of the Loricariinae, restricting Harttiini to *Harttia*. Covain and Fisch-Muller (2007) tentatively placed the monotypic genus *Harttiella* into Harttiini due to its close resemblance to *Harttia*, suggesting *Harttiella* as a dwarf form closely related to *Harttia*. At the same time, they voiced doubts concerning the synonymy of *Quiritixys* with *Harttia*, this group of species representing also dwarf forms from the Brazilian Shield potentially related to *Harttiella*. Mol and Ouboter (2004) mentioned that *H. crassicauda* was at risk of extinction or possibly already extinct because of mining activities in Nassau Mountains. Fortunately, the species has been recently collected for the second time, 56 years after its original collection. However, *H. crassicauda* remains an endangered species due to potential degradation of its habitat by both small and large scale mining, and its restricted distribution in a single creek (Mol *et al.*, 2007). *Harttiella crassicauda* has received considerable interest after Boeseman (1971) hypothesized a basal position for the species within Loricariinae. The recovery of *H. crassicauda* had, by a better grasp of its morphology and ecology, as immediate consequences the discovery of several new species of *Harttiella* in French Guiana (Covain *et al*., in press). In a recent assessment about the diversity of Harttiini within the Guianas, Covain *et al.* (in press) recognized 14 species of Harttini distributed in three genera: *Harttia* (5 species), *Cteniloricaria* (2 species), and *Harttiella* (7 species). These authors placed *C. fowleri* into *Harttia* and voiced doubts concerning the monophyly of the genus due the scattered position of *H. fowleri*, out of its Guianese counterparts. Nevertheless, no phylogenetic analysis was performed in this study. The confused systematics of Harttiini has first been clarified to assess the monophyly of *Harttia*, *Harttiella*, and *Cteniloricaria*, and the validity of *Quiritixys*. Then spatial and temporal patterns of diversification of the tribe at a continental scale have been inferred in the light of the phylogeography of *Hypostomus*.

2. Material and methods

2.1 Taxonomic sampling.

The molecular phylogeny was reconstructed on the taxonomical sampling given in Covain *et al.* (2008) with addition of 51 putative representatives of Harttiini. These later included the type species of *Harttia*, *Cteniloricaria*, *Harttiella*, and *Quiritixys*. One additional outgroup, *Pseudorinelepis genibarbis* (Valenciennes in Cuvier & Valenciennes, 1840), was added to root the tree following results of Montoya-Burgos *et al.* (1998). The list of material used for this study is provided in Table 1. The analyzed samples came from the tissue collection of the Muséum d'histoire naturelle de la Ville de Genève (MHNG), Geneva, Switzerland, the Laboratório de Biologia e Genética de Peixes (LBP) Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Botucatu Brazil, and the Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo Brazil. The sequences were deposited in GenBank.

2.2 DNA extraction, amplification and sequencing.

Tissue samples were preserved in 80% ethanol and stored at -20°C. Total genomic DNA was extracted with the DNeasy Tissue Kit (Qiagen) following the instructions of the manufacturer. The PCR amplifications of mitochondrial 12S and 16S, and the nuclear Fish Reticulon-4 (F-RTN4) genes were carried out using the Taq PCR Core Kit (Qiagen). The methodology for PCR amplifications followed Covain *et al.* (2008) for partial 12S and 16S, and Chiachio *et al.* (2008) for F-RTN4. To amplify the complete 12S gene, two additional primers were designed: Phe-L941: 5'- AAA TCA AAG CAT AAC ACT GAA GAT G 3', and Val-H2010: 5'- CCA ATT TGC ATG GAT GTC TTC TCG G 3'. The amplifications were performed in a total volume of 50 µl, containing 5 µl of 10x reaction buffer, 1 µl of dNTP mix at 10mM each, 1 µl of each primer at 10 µM, 0.2 µl of *Taq* DNA Polymerase equivalent to 1 unit of Polymerase per tube, and 1 to 4 µl of DNA. Cycles of amplification were programmed with the following profile: (1) 3 min. at 94°C (initial denaturing), (2) 35 sec. at 94°C, (3) 30 sec. at 51°C, (4) 80 sec. at 72°C, and (5) 5 min. at 72°C (final elongation). Steps 2 to 4 were repeated 35 to 39 times according to the quality and concentration of DNA.

Table 1. Taxa list, specimen and sequence data for the 71 species of Loricariinae including 52 Harttiini, and outgroup analyzed in this study. The acronyms of institutions follow Fricke and Eschmeyer (2010). Abbreviations used in the multivariate analyses are provided between square brackets.

Species	Catalog Number	Field Number	Locality	mt 12S+16S bases + GenBank No.	Ref.	F-RTN4 bases + GenBank No.	Ref.
Crossoloricaria venezuelae	INHS 35467	VZ 049	Venezuela, Rio Santa Rosa	2416 EU310444	Covain *et al.* 2008	1994 HM623647	Rodriguez *et al.* In press
Dasyloricaria tuyrensis	MHNG 2674.052	PA00–012	Panama, Rio Ipeti	2416 EU310445	Covain *et al.* 2008	2005 HM623639	Rodriguez *et al.* In press
Farlowella platoryncha	MHNG 2588.093	PE96–071	Peru, Rio Ucayali	2429 EU310446	Covain *et al.* 2008	2301 HM623649	Rodriguez *et al.* In press
Farlowella oxyrryncha	MHNG 2588.064	PE96–022	Peru, Rio Tambopata	2430 EU310443	Covain *et al.* 2008	2237 HM623650	Rodriguez *et al.* In press
Hemiodontichthys acipenserinus	MHNG 2651.012	GY04–15	Guyana, Rupununi River	2419 EU310448	Covain *et al.* 2008	2246 HM623645	Rodriguez *et al.* In press
Lamontichthys stibaros	MHNG 2677.039	MUS 208	Peru, aquarium trade, Rio Itaya[2]	2430 EU310449	Covain *et al.* 2008	2038 HM623648	Rodriguez *et al.* In press
Limatulichthys griseus	MHNG 2651.013	GY04–18	Guyana, Rupununi River	2423 EU310450	Covain *et al.* 2008	1959 HM623644	Rodriguez *et al.* In press
Loricaria clavipinna	MHNG 2640.044	PE98–002	Peru, Rio Putumayo	2424 EU310451	Covain *et al.* 2008	1964 HM623653	Rodriguez *et al.* In press
Loricaria parnahybae	MHNG 2602.067	BR98–274	Brazil, Rio Parnahyba	2421 EU310452	Covain *et al.* 2008	1985 FJ013231	Chiachio *et al.* 2008
Loricariichthys maculatus	MHNG 2621.042	SU01–56	Surinam, Sarramacca River	2425 EU310453	Covain *et al.* 2008	2221 HM623642	Rodriguez *et al.* In press
Loricariichthys microdon	MHNG 2650.054	GY04–12	Guyana, Rupununi River	2424 EU310454	Covain *et al.* 2008	1949 HM623643	Rodriguez *et al.* In press
Metaloricaria paucidens	MHNG 2677.086	GF00–083	French Guiana, Marouini River	2435 EU310455	Covain *et al.* 2008	2073 HM623637	Rodriguez *et al.* In press
Planiloricaria cryptodon	MHNG 2677.038	MUS 211	Peru, aquarium trade, Rio Itaya[2]	2415 EU310456	Covain *et al.* 2008	2006 HM623646	Rodriguez *et al.* In press
Rineloricaria platyura	MHNG 2651.009	GY04–83	Guyana, Rupununi River	2420 EU310458	Covain *et al.* 2008	2219 HM623641	Rodriguez *et al.* In press
Rineloricaria lanceolata	MHNG 2588.059	PE96–011	Peru, Rio Tambopata	2420 EU310457	Covain *et al.* 2008	2226 HM623640	Rodriguez *et al.* In press
Rineloricaria osvaldoi	UFRJ batch 6–EF4	BR 1114	Brazil, Rio Maranhão	2424 EU310459	Covain *et al.* 2008	2023 HM623652	Rodriguez *et al.* In press
Sturisoma nigrirostrum	MHNG 2588.055	PE96–001	Peru, Rio de las Piedras	2437 EU310460	Covain *et al.* 2008	2556 HM623636	Rodriguez *et al.* In press
Sturisoma monopelte	MHNG 2651.033	GY04–187	Guyana, Sawarab River	2436 EU310461	Covain *et al.* 2008	1980 HM623651	Rodriguez *et al.* In press

Sturisomatichthys citurensis	MHNG 2676.004	PA97–032	Panama, Rio Tuyra	2435 EU310462	Covain *et al.* 2008	2268 HM623635	Rodriguez *et al.* In press
Harttia guianensis [Ha 5]	MHNG 2643.016	GF00–351	French Guiana, Marouini River	2435 EU310447	Covain *et al.* 2008	2112 FJ013232	Chiachio *et al.* 2008
Harttia guianensis [Ha 4]	MHNG 2662.091	GF03-160	French Guiana, Approuague River	GBxxxxx	This study	GBxxxxx	This study
Harttia guianensis [Ha 6]	MHNG 2680.053	RV-21	French Guiana, Sinnamary River	GBxxxxx	This study	GBxxxxx	This study
Harttia fluminensis [Ha 1]	MHNG 2690.013	SU01-445	Suriname, Coppename River	GBxxxxx	This study	GBxxxxx	This study
Harttia surinamensis [Ha 2]	MHNG 2674.042	SU05-001	Suriname, Suriname River	GBxxxxx	This study	GBxxxxx	This study
Harttia tuna [Ha 3]	MHNG 2704.029	SU07-644	Brazil, Paru de Oeste River	GBxxxxx	This study	GBxxxxx	This study
Harttia fowleri [Ha 40]	MHNG 2643.022	GF99-202	French Guiana, Oyapock River	GBxxxxx	This study	GBxxxxx	This study
Harttia carvalhoi [Ha 13]	MHNG 2587.027	BR 1236	Brazil, Rio Paraíba do Sul	GBxxxxx	This study	GBxxxxx	This study
Harttia carvalhoi [Ha 14]	LBP 2115	LBP 21352	Brazil, Rio Paraíba do Sul	GBxxxxx	This study	GBxxxxx	This study
Harttia torrenticola [Ha 15]	LBP 5835	LBP 28346	Brazil, Rio São Francisco	GBxxxxx	This study	GBxxxxx	This study
Harttia gracilis [Ha 16]	LBP 6331	LBP 29819	Brazil, Rio Paraná	GBxxxxx	This study	GBxxxxx	This study
Harttia longipinna [Ha 17]	DZSJRP 2819	BR98-747	Brazil, Rio São Francisco	GBxxxxx	This study	GBxxxxx	This study
Harttia sp. Rio São Francisco [Ha 18]	LBP 5838	LBP 28352	Brazil, Rio São Francisco	GBxxxxx	This study	GBxxxxx	This study
Harttia sp. Três Marias [Ha 19]	LBP 5838	LBP 28351	Brazil, Rio São Francisco	GBxxxxx	This study	GBxxxxx	This study
Harttia sp. Serra do Cipó [Ha 20]	LBP 6528	LBP 31652	Brazil, Rio São Francisco	GBxxxxx	This study	GBxxxxx	This study
Harttia leiopleura [Ha 21]	LBP 6847	LBP 31528	Brazil, Rio São Francisco	GBxxxxx	This study	GBxxxxx	This study
Harttia leiopleura [Ha 22]	LBP 6492	LBP 31545	Brazil, Rio São Francisco	GBxxxxx	This study	GBxxxxx	This study
Harttia novalimensis [Ha 23]	LBP 5836	LBP 28348	Brazil, Rio São Francisco	GBxxxxx	This study	GBxxxxx	This study
Harttia loricariformis [Ha 24]	LBP 2121	LBP 21362	Brazil, Rio Paraíba do Sul	GBxxxxx	This study	GBxxxxx	This study
Harttia kronei [Ha 25]	MHNG 2586.058	BR 1166	Brazil, Rio Ribeira de Iguape	GBxxxxx	This study	GBxxxxx	This study
Harttia kronei [Ha 26]	LBP 2661	LBP 17427	Brazil, Rio Ribeira de Iguape	GBxxxxx	This study	GBxxxxx	This study
Harttia kronei [Ha 27]	LBP 2883	LBP 18609	Brazil, Rio Ribeira de Iguape	GBxxxxx	This study	GBxxxxx	This study
Harttia kronei [Ha 28]	LBP 1269	LBP 11215	Brazil, Rio Ribeira de Iguape	GBxxxxx	This study	GBxxxxx	This study
Harttia dissidens [Ha 29]	LBP 5859	LBP 28331	Brazil, Rio Tapajós	GBxxxxx	This study	GBxxxxx	This study
Harttia dissidens [Ha 30]	LBP 5863	LBP 28339	Brazil, Rio Tapajós	GBxxxxx	This study	GBxxxxx	This study
Harttia sp. Tapajos [Ha 31]	LBP 5857	LBP 28329	Brazil, Rio Tapajós	GBxxxxx	This study	GBxxxxx	This study
Harttia sp. 1 Xingu [Ha 33]	LBP 5845	LBP 28327	Brazil, Rio Xingu	GBxxxxx	This study	GBxxxxx	This study
Harttia sp. 2 Xingu [Ha 34]	LBP 5860	LBP 28333	Brazil, Rio Xingu	GBxxxxx	This study	GBxxxxx	This study
Harttia sp. 3 Xingu [Ha 35]	LBP 5861	LBP 28335	Brazil, Rio Xingu	GBxxxxx	This study	GBxxxxx	This study
Harttia duriventris [Ha 32]	LBP 7505	LBP 34804	Brazil, Rio Tapajós	GBxxxxx	This study	GBxxxxx	This study
Harttia punctata [Ha 37]	MHNG 2645.059	BR 995	Brazil, Rio Tocantins	GBxxxxx	This study	GBxxxxx	This study
Harttia punctata [Ha 38]	MHNG 2645.053	BR 1051	Brazil, Rio Tocantins	GBxxxxx	This study	GBxxxxx	This study
Harttia cf. *Punctata* [Ha 39]	LBP 5839	LBP 28353	Brazil, Rio Tocantins	GBxxxxx	This study	GBxxxxx	This study

Harttia sp. Tocantins [Ha 40]	LBP 5850	LBP 28367	Brazil, Rio Tocantins	GBxxxxx	This study	GBxxxxx	This study
Cteniloricaria napova [Ha 7]	MHNG 2704.030	SU07-650	Brazil, Paru de Oeste River	GBxxxxx	This study	GBxxxxx	This study
Cteniloricaria platystoma [Ha 8]	MHNG 2672.067	SU05-340	Suriname, Corantijn River	GBxxxxx	This study	GBxxxxx	This study
Cteniloricaria platystoma [Ha 9]	MHNG 2674.003	SU05-039	Suriname, Suriname River	GBxxxxx	This study	GBxxxxx	This study
Cteniloricaria platystoma [Ha 10]	MHNG 2650.082	GY04-336	Guyana, Essequibo River	GBxxxxx	This study	GBxxxxx	This study
Cteniloricaria platystoma [Ha 11]	MHNG 2700.054	GF07-265	French Guiana, Mana River	GBxxxxx	This study	GBxxxxx	This study
Cteniloricaria platystoma [Ha 12]	MHNG 2643.015	GF00-352	French Guiana, Marouini River	GBxxxxx	This study	GBxxxxx	This study
Harttiella crassicauda [Ha 41]	MHNG 2679.098	MUS 306	Suriname, Nassau Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella crassicauda [Ha 42]	MHNG 2674.051	MUS 221	Suriname, Nassau Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella crassicauda [Ha 43]	MHNG 2674.051	MUS 231	Suriname, Nassau Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella pilosa [Ha 44]	MHNG 2682.055	GF06-344	French Guiana, Tortue Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella pilosa [Ha 45]	MHNG 2682.055	GF06-343	French Guiana, Tortue Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella pilosa [Ha 46]	MHNG 2724.002	GF03-033	French Guiana, Tortue Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella longicauda [Ha 47]	MHNG 2723.094	MUS 470	French Guiana, Balenfois Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella longicauda [Ha 48]	MHNG 2723.094	MUS 463	French Guiana, Balenfois Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella longicauda [Ha 49]	MHNG 2723.094	MUS 456	French Guiana, Balenfois Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella longicauda [Ha 50]	MHNG 2699.070	GF07-026	French Guiana, Trinité Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella longicauda [Ha 51]	MHNG 2699.070	GF07-082	French Guiana, Trinité Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella longicauda [Ha 52]	MHNG 2699.070	GF07-111	French Guiana, Trinité Mountains	GBxxxxx	This study	GBxxxxx	This study
Ancistrus cirrhosus[1]	MHNG 2645.037	MUS 202	Argentina, Rio Uruguay	2420		1809	Rodriguez *et al*. In press
Pseudorinelepis genibarbis[1]	MHNG 2588.079	PE96-040	Peru, Rio Ucayali	EU310442 2434 HM592623	Covain *et al*. 2008 Rodriguez *et al*. In press	HM623638 1926 HM623634	Rodriguez *et al*. In press

[1] outgroup

[2] according to the exporter

PCR products were purified with the High Pure PCR Product Purification Kit (Roche). Sequencing reactions were performed with the Big Dye Terminator Cycle Sequencing Ready Reaction 3.1 Kit (Applied Biosystems) following instructions of the manufacturer, and were loaded on an automatic sequencer 3100-Avant Genetic Analyzer (Applied Biosystems, Perkin-Elmer).

2.3 Sequence alignment, phylogenetic reconstructions, and topological tests.

The DNA sequences were edited and assembled using BioEdit 7.0.1 (Hall, 1999), and aligned manually (for an explanation see Rodriguez *et al*., in press). Since mitochondrial DNA is presumably transmitted through maternal lineage as a single not recombining genetic unit (Meyer, 1993), a first partition corresponding to the mitochondrial genes was created. In addition, the mutational patterns in intronic and exonic regions of F-RTN4 being rather characterized by insertions/deletions in introns, and transitions/transversions in exons, two other partitions were created. Combinability between mitochondrial and nuclear markers was secondarily assessed using the Incongruence Length Difference (ILD) test (Farris *et al*., 1994) as implemented in PAUP* 4.0b10 (Swofford, 1998), and the Congruence Among Distance Matrices (CADM) test (Legendre and Lapointe, 2004) as implemented in ape 2.5 (Paradis *et al*., 2004; Paradis, 2006) in R 2.10.1 (R Development Core Team, 2009). The ILD test was conducted using a heuristic search with 1,000 replicates, TBR branch swapping, and random addition of taxa with 10 replicates. The CADM test is a generalization to several distance matrices of the Mantel test (Mantel, 1967). This test against incongruence of all distance matrices relies on the Kendall's coefficient of concordance W (Kendall and Babington Smith, 1939) among the unfolded and ranked distance matrices, and uses a Friedman's χ^2 statistic (Friedman, 1937) for its computation. An observed statistics (χ^2_{ref}) was calculated for the ordered (by rows or columns) matrices and was compared, in the upper tail, to a null hypothesis sampling distribution of randomized statistics (χ^{2*}) obtained by permuting at random all matrices, independently of one another. In case of rejection of the null hypothesis, an *a posteriori* testing procedure is available to determine witch matrices are congruent. This procedure relies on the mean of the Mantel correlations of the ranked transformed distances (Spearman's correlation r_S) between the tested matrix and all other matrices. In this case, a single matrix is permuted at a time, and repeated for all matrices in turn. It tests the null hypothesis of incongruence of the matrix subjected to the test with respect to the other matrices. A Holm (1979) correction for multiple testing is applied for all *a posteriori* tests. In

addition, pairwise Mantel correlations of the ranked distances between matrices can also be computed. Pairwise maximum likelihood (ML) (Felsenstein, 1981) distances were computed with Treefinder (Jobb *et al*., 2004) version of October 2008 for each partition using a likelihood model under which the pairwise distances are optimized. Appropriate substitution models corresponding to each potential partition were accordingly estimated with the corrected Akaike Information Criterion (Sugiura, 1978) as implemented in Treefinder. The CADM test was computed using 9,999 permutations of the three ML distances matrices. Two phylogenetic reconstruction methods allowing the analysis of partitioned data were used. First, a ML reconstruction was performed with Treefinder. Robustness of the results was estimated by resampling the data set with the nonparametric bootstrap (Efron, 1979) following Felsenstein's (1985) methodology with 1,000 pseudoreplicates. Second, a Bayesian inference analysis was conducted in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Two runs of four chains (one cold, three heated) were conducted simultaneously for $2x10^7$ generations, with the tree space sampled each 100^{th} generation. Convergence between chains occurred after $3.5x10^5$ generations (average standard deviation of split frequencies <0.01). After a visual representation of the evolution of the likelihood scores, and checking for the stationarity of all model parameters using Tracer 1.5 (Rambaut and Drummond, 2007) (*i.e.*: potential scale reduction factor (PSRF), uncorrected roughly approached 1 as runs converged (Gelman and Rubin, 1992), and Effective Sample size (ESS) of all parameters superior to 200), the $5x10^5$ first generations were discarded as burn-in. The remaining trees were used to compute the consensus tree.

Alternative topologies were tested under the null hypothesis that all phylogenetic hypotheses (trees) were not different from the best ML reconstruction using the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999), and the approximately unbiased (AU) test (Shimodaira, 2002) as implemented in Treefinder using 200,000 RELL replicates (Kishino *et al*., 1990).

2.4 Comparative phylogeography of Harttiini and Hypostomus.

The area of dispersion of Harttiini and *Hypostomus* being largely over-lapping, and the Loricariinae forming the sister subfamily of Hypostominae, we can reasonably expect that both groups dispersed following a common process that can be assessed by a close exploration of tree topologies (*i.e*. if sister clades of both subfamilies occupy similar geographic areas). For this, we performed a RLQ analysis of both phylogenies constrained by

the distribution of the different species. Prior to the analysis, the two phylogenies were restricted to the single Harttiini for the first tree, and to the species of *Hypostomus* sharing common distributions with Harttiini for the second tree (Appendix S1). These two trees being reconstructed using different markers, models of evolution, and programs, their branch lengths were not directly comparable. All branch lengths were consequently set to one, and a patristic distance matrix was computed for each tree using ape 2.5 in R 2.10.1. To perform the RLQ analysis of these two distances matrices, a binary coding table of co-distributed species (1 if species are co-distributed, 0 otherwise) was firstly created and submitted to a Correspondence Analysis (CA). This analysis looks for scores of rows and columns of maximal correlation, and allows a re-ordination of the table according to the co-occurrences. Five geographic areas adapted from Montoya-Burgos (2003), Chiachio *et al.* (2008), and Torrico *et al.* (2009) were used to construct the co-occurrence table. These five areas correspond to the current co-distribution of Harttiini and *Hypostomus*, namely: the East coastal rivers of Brazil, the Upper Paraná basin, the São Francisco basin, The Amazon basin, and the coastal rivers of Guianas. Then the distances matrices were rendered Euclidian using Cailliez's (1983) method. Principal Coordinate Analyses (PCoA) (Gower, 1966) were performed on these corrected distance matrices using the CA weights to reveal their structuring. Scores of the species on the principal coordinates were used as descriptors of the phylogenies in the RLQ analysis. The RLQ analysis aims to investigate the relationships between the two tables R (phylogeny of Harttiini) and Q (phylogeny of *Hypostomus*), using a link table L (species co-occurrences), and to extract the joint structure between them. Here table R was a 52 x 52 table containing row scores of the PCoA of Harttiini, table Q a 34 x 34 table containing row scores of the PCoA of *Hypostomus*, and table L a 52 x 34 cross table of co-occurrences. The mathematical model of RLQ is described in Dolédec *et al*. (1996) with adaptations in Dray *et al*. (2002), and Dray and Legendre (2008). The RLQ analysis consists in an eigenvalue decomposition and provide sets of scores for the two phylogenies of maximal covariance. Thus the RLQ analysis looks for combinations of the principal coordinates of both distance matrices that maximize the spatial covariance (*i.e.* the phylogenetic spatial co-structure). To assess the significance of the RLQ results, a Monte-Carlo permutation test was computed on the total coinertia of the analysis. Two models of permutation were used. First entire rows of table L were permuted to destroy the link between L and R but preserve L linked to Q, and second, entire columns of L were permuted to destroy the link between L and Q but preserve the link between L and R. 99,999 random permutations were used to allow adjustments for multiple testing, and the results of both models were

assembled (see Dray and Legendre 2008 for details). This procedure tests the general spatial relationship between the two tables. Then an *a posteriori* testing procedure was computed using these same models to test the link between the RLQ axes and the principal coordinates describing the topologies. These permutation tests rely on the fourthcorner statistics (Legendre *et al*., 1997; Dray and Legendre, 2008) and allow the detection of the trees' structures significantly linked to the compromise highlighted by the RLQ analysis. The RLQ analysis and associated tests were performed using the ade4 1.4-14 package (Dray and Dufour, 2007) in R.

2.5 Inferences of spatial and temporal patterns of diversification in Harttiini.

In a second time, we dated our phylogenetic tree using secondary dating events. Since no geological events are presently accurately dated within the geographical range of distribution of Harttiini (eastern part of South-America), we used dates inferred from the previous study conducted on Hypostominae by Montoya-Burgos (2003). The trees of this previous study were dated using the geological splitting event between *Hypostomus hondae* from the Maracaibo Lake in Venezuela, and *H. plecostomoides* from the Rio Orinoco estimated around 8 Ma. Due to a constant molecular clock, dating of the nodes was accordingly inferred throughout the *Hypostomus* phylogenies.

The assumption of constant molecular clock was assessed for the Harttiini subtree using the distances based test of Xia (2009) under the GTR model using DAMBE 4.5.56 (Xia, 2001; Xia and Xie, 2001), and for each node of the tree using the mean path lengths method (MPL) of Britton *et al.* (2002) as implemented in ape in R. Prior to the computation of the MPL tests, the tree's branch lengths were converted in mean numbers of substitutions. Since the tests of molecular clock may reject the null hypothesis of global molecular clock and equal rates of substitutions in the subtrees, the chronogram was reconstructed using two methods that account for different rates of substitutions along branches. First: the Penalized Likelihood (PL) method (Sanderson, 2002) that correct for the Non Parametric Rate Smoothing (NPRS) method (Sanderson, 1997) was performed on the Harttiini subtree using ape in R. The methodology proposed by Paradis (2006) was followed for the estimation of the smoothing parameter λ. This parameter controls for a trade-off between a parametric formulation where each branch has its own rate, and a nonparametric term where changes in rates are minimized between contiguous branches. If λ is small then the parametric component dominates and rates vary as much as possible among branches (local rates),

whereas for increasing values of λ, the variations in rates are smoother to tend to be uniform (clock-like model). The ideal value of λ was estimated by cross validation for increasing values ranging from 0.1 to 10^{12}. Second: we used a Bayesian tree calibration method allowing relaxed molecular clock models. Node ages and substitution rates were estimated using an uncorrelated lognormal relaxed clock in BEAST 1.5.4 (Drummond *et al*., 2006; Drummond and Rambaut, 2007). The GTR + G model was applied on the three partitions using a Yule tree prior and the Harttiini subtree as fixed topology. Twenty million generations were used with parameters sampling each 1,000th generation for the Markov Chain Monte Carlo (MCMC) exploration of parameters' space. A normal distribution was applied for the tmrca prior. Other parameters were set to default. The convergence of the chain was assessed by inspection of the trace plots and ESS using Tracer 1.5. Since all parameters converged (ESS > 200), the default 10% parameters and trees were discarded as burn-in, and summarized using TreeAnnotator 1.5.4. The chronogram was edited using FigTree 1.3.1. In addition, to confirm results of both calibration methods, we used the Local Rate Minimum Deformation (LRMD) method (Jobb *et al*., 2004) that tries to keep the real rates as similar as possible to ideal local rates. It reflects the assumption that rates are similar between neighbouring edges (autocorrelated model). To compute confidence limits of rates and divergence times, we performed a bootstrap analysis of the best ML tree as fixed topology, using 1,000 pseudoreplicates.

To reconstruct the ancestral range of Harttiini, we performed a dispersal-vicariance analysis (Ronquist, 1997) that accounts for phylogenetic uncertainties in ancestral reconstructions using S-DIVA (Yu *et al*., 2010). S-DIVA relies on DIVA 1.2 (Ronquist, 2001), but uses a sample of trees (*e.g.* collection of Bayesian trees) rather than a single fully resolved tree to reconstruct ancestral areas following the Bayes-DIVA method (Nylander et al., 2008), and estimates confidence in reconstructions following Harris and Xiang (2009). DIVA estimates the possible ancestral distribution of species by parsimony optimization of the number of dispersal and local extinction events to explain the current distribution of species using a three dimensional cost matrix. The program does not necessitate any assumption about the ancestral distribution of species, implying that ancestral species can be distributed in several areas at a time. DIVA assumes no cost to vicariance events relative to dispersal and extinction that are assigned a cost of one. We used the five geographic areas previously defined to describe the current distribution of Harttiini and the trees file obtained from BEAST 1.5.4.

3. Results

3.1 Phylogenetic analyses

We sequenced the almost complete 12S and 16S mitochondrial genes, and the partial nuclear gene F-RTN4 for 52 representatives of putative Harttiini. Other sequences for twenty representatives of Loricariinae representing 14 genera, *Ancistrus cirrhosus* and *Pseudorinelepis genibarbis* were obtained from GenBank using the accession numbers given in Covain *et al.* (2008), Chiachio *et al.* (2008), and Rodriguez *et al.* (in press). The sequence alignment included 6,931 positions from which 984 corresponded to the 12S rRNA gene, 73 to the tRNA Val gene, 1,479 to the 16S rRNA gene, 895 to the exonic regions of the F-RTN4 gene, and 3,500 to the intronic regions of the F-RTN4 gene. No significant conflicting phylogenetic signal was detected in the data set, as the ILD test failed to reject the null hypothesis of congruence between data partitions (ILD: $p_{(X>Xobs)} = 0.115$), and the global CADM test rejected the null hypothesis of incongruence between matrices (CADM: W = 0.9193, $\chi^2_{ref} = 7245.0886$, $p_{(\chi 2ref \geq \chi 2^*)} = 0.0001$). The CADM *a posteriori* tests did not detect any conflicting matrix in the data ($\bar{r}_S$ mitochondrion = 0.8669287, $p_{(\bar{r}_S\ ref \geq \bar{r}_S\ ^*)} = 0.0003$; $\bar{r}_S$ exons = 0.8808559, $p_{(\bar{r}_S\ ref \geq \bar{r}_S\ ^*)} = 0.0003$; $\bar{r}_S$ introns = 0.8891141, $p_{(\bar{r}_S\ ref \geq \bar{r}_S\ ^*)} = 0.0003$). The sequences were consequently concatenated, and three partitions corresponding to mitochondrial genes, exonic parts of F-RTN4, and intronic parts of F-RTN4 were used to reconstruct the tree. The models GTR + G (Tavaré, 1986) for mitochondrial genes and intronic regions of F-RTN4, and TN + I + G (Tamura and Nei, 1993) for exonic regions of F-RTN4 fitted our data the best as indicated by Treefinder. The GTR + G model was used for each of the three partitions for the Bayesian inference, with each partition assigned its own among-sites heterogeneity rate.

Bayesian and ML phylogenetic reconstructions lead to equivalent tree topologies, both comparable to the one obtained by Covain *et al.* (2008), and Rodriguez *et al.* (in press). The best ML tree (-lnL = 39708.37) (Fig. 1) split the Loricariinae into two lineages: the Harttiini (clade 1) including the genus *Harttia, Cteniloricaria*, *Harttiella*, and the type species of *Quiritixys*, and the Loricariini (clade 2) including all other Loricariinae. The phylogenetic relationships within Loricariini were fully congruent with Covain *et al*. (2008), and Rodriguez *et al*. (in press), and are not redescribed here. Within Harttiini, the first diverging group included the species of *Harttia* inhabiting coastal rivers of the Guiana Shield, with the exception of *H. tuna* that formed the sister group of Surinamese representatives (*H.*

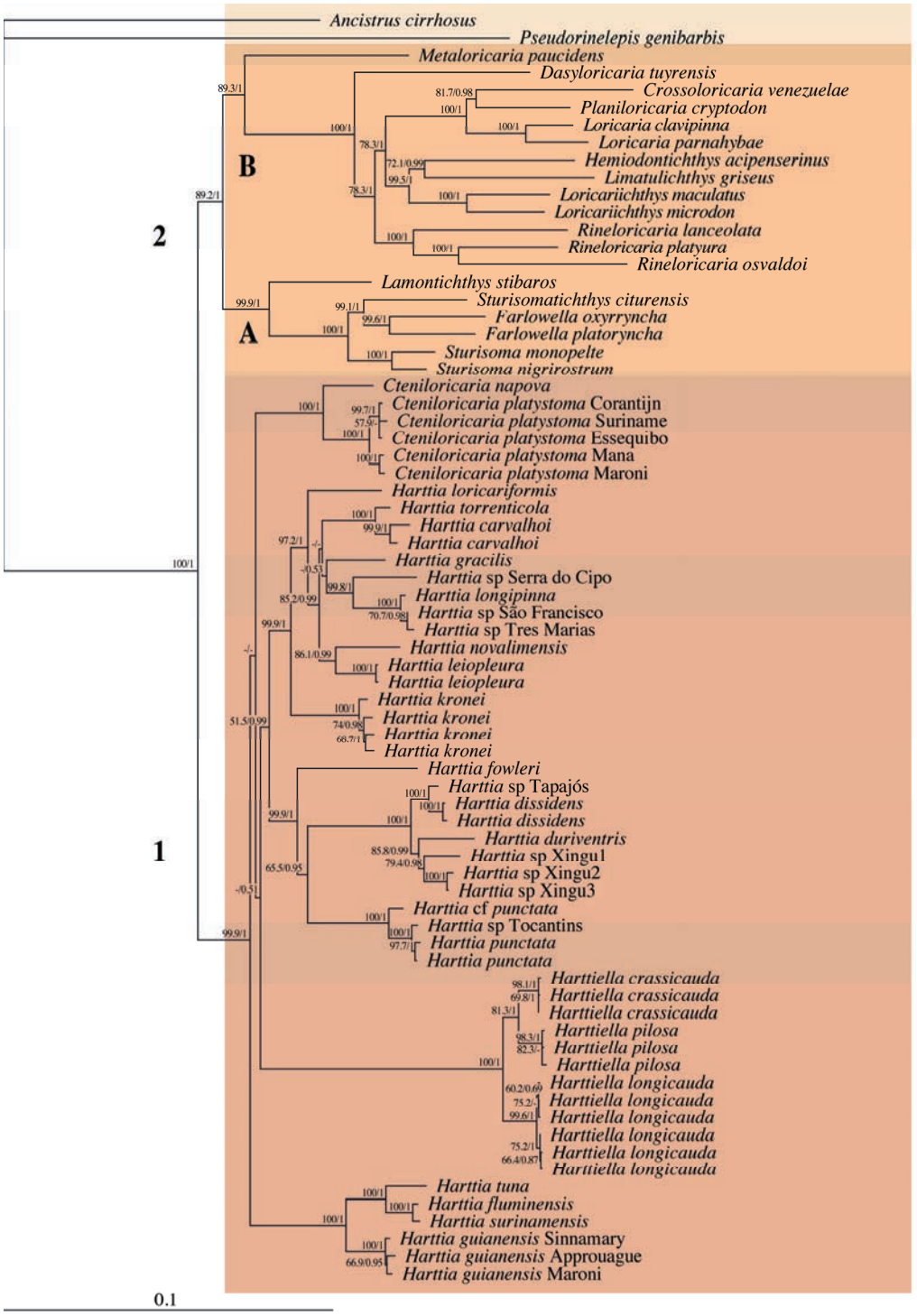


Fig. 1. Maximum likelihood tree of the Loricariinae including 52 Harttiini inferred from the analysis of partial mt 12S and 16S, and F-RTN4 nuclear gene sequences (-lnL = 39708.37). The best fit substitution models used were GTR + G for mitochondrial genes and intronic regions of F-RTN4, and TN + I + G for exonic regions of F-RTN4. The GTR + G model was used for each of the three partitions for the Bayesian inference. Both reconstructions lead to equivalent tree topologies. Numbers above branches indicate bootstrap supports above 50 for the ML analysis followed by posterior probabilities above 0.5 for the Bayesian inference. Sign (-) indicates values below 50 % bootstrap and 0.5 posterior probabilities leading to polytomies in the ML bootstrap and Bayesian inference majority rule consensus trees (consensus level = 50, and 0.5 respectively). 1: Harttiini, 2: Loricariini, A: Farlowellina, B: Loricariina. Scale indicates the number of substitution per site as expected by the model.

surinamensis and *H. fluminensis*). *H. tuna* inhabits a tributary of the Paru do Oeste River that flows toward the Amazon. These three species formed in turn the sister group of all populations of *H. guianensis* from French Guiana (Sinnamary, Approuague, and Maroni Rivers). Internal relationships in this clade were highly supported by bootstrap values and posterior probabilities, whereas deeper relationships suffered from a significant lack of statistical support in both ML and Bayesian analyses. The second clade was constituted by all *Cteniloricaria* but *C. fowleri*. *Cteniloricaria napova* formed the sister group of all Guianese representatives that included all populations of the type species *C. platystoma*. *Cteniloricaria napova* also inhabits the Paru do Oeste River that flows toward the Amazon. The populations of *C. platystoma* from French Guiana (Maroni and Mana Rivers) formed the sister group of the populations from Suriname (Suriname and Corantijn Rivers) and Guayana (Esssequibo River). Internal relationships were also strongly supported, contrary to the base of the clade in both analyses. The third clade was constituted of representatives of *Harttiella*, including the type species *H. crassicauda*. This clade formed the sister group of Amazonian and Southeastern representatives of *Harttia*. Internal relationships between *Harttiella* representatives were strongly supported, with *H. crassicauda* from Suriname forming the sister group of *H. pilosa* (Orapu River) in French Guiana, both in turn forming the sister group of the species *H. longicauda* (Approuague and Mana Rivers) in French Guiana. Deeper relationships were also poorly supported in both reconstructions. The fourth diverging group was split into two clades, one made of all *Harttia* representatives from South-East Brazil, and one made of all *Harttia* representatives from the Amazon basin plus *C. fowleri*. This species inhabits the Oyapock River, a coastal river that forms the boarder between French Guiana and Brazil, and connected at base of the Amazonian clade, a strongly supported position. The sister group of *C. fowleri* was constituted on one side by *Harttia* representatives from Tapajós and Xingu Rivers (*H. dissidens*, *H. duriventris*…), and on the other side by *Harttia* representatives from Tocantins River (*H. punctata*…). Internal relationships within the Amazonian clade were highly supported by both bootstrap values and posterior probabilities. In the Southeastern clade, representatives of *H. kronei* from the Ribeira de Iguape River, a coastal river of the Brazilian Shield, formed the sister group of all remaining *Harttia* species. The second diverging species was *H. loricariformis* (type species of *Harttia*) from the Paraíba do Sul River, also a coastal river of South East Brazil, that formed the sister group of two groups. The first one was constituted of *H. novalimensis*, and *H. leiopleura* (type species of *Quiritixys*) from the São Francisco basin, in a sister position to a second clade that included all remaining species of *Harttia*. This clade split into two groups, on one hand *H. torrenticola*

from the São Francisco basin as sister species of *H. carvalhoi* from the Paraíba do Sul River, and on the other hand *H. gracilis* from the Upper Paraná basin forming the sister group of the remaining *Harttia* representatives from the São Francisco basin (*H. longipinna*…). Within the Southeastern clade, internal relationships were generally strongly supported, except for the three subclades forming the sister group of *H. loricariformis* that suffered from a lack of statistical support. The bootstrap majority rule consensus tree (consensus level = 50) over 1,000 pseudoreplicates, and the Bayesian majority rule consensus tree (consensus level = 0.50) were fully congruent with the exception of the position of *H. gracilis* that was better supported in the Bayesian reconstruction. Both trees showed the same polytomies in the deepest part of the trees, with no resolution of the positions of the clades containing the Guianese representatives of *Harttia* and *Cteniloricaria*, in regards to the other Harttiini, as well as between the sister groups of *H. loricariformis*.

The only strongly supported relationships in both analyses being the sister relationship between the Amazonian clade, and the Southeastern clade, alternative hypotheses were evaluated for deeper relationships involving the three Guianese lineages (Table 2). All tested topologies lead to equivalent alternatives hypotheses since none of the tests succeeded in rejecting the null hypothesis. Consequently, the best ML tree was considered as the best estimates of the true phylogeny (greater likelihood), and used as reference tree in subsequent analyses.

Table 2. Alternative phylogenetic relationships evaluated using the Shimodaira and Hasegawa (SH) and Approximately Unbiased (AU) testing procedures. C: *Cteniloricaria* clade; Ht: *Harttiella* clade; HG: *Harttia* Guianese clade; HA: *Harttia* Amazonian clade; HSE: *Harttia* South-eastern clade.

Hypothesis	ln*L*	Δ ln*L*	SH	AU
H0: (HG,(C,(Ht,(HA,HSE))))	-39708.37	-	-	-
H1: (C,(HG,(Ht,(HA,HSE))))	-39708.67	-0.30	0.708	0.576
H2: (HG,(Ht,(C,(HA,HSE))))	-39710.19	-1.82	0.595	0.430
H3: (Ht,(HG,(C,(HA,HSE))))	-39711.09	-2.72	0.406	0.380
H4: (Ht,(C,(HG,(HA,HSE))))	-39711.96	-3.59	0.389	0.274
H5: (C,(Ht,(HG,(HA,HSE))))	-39711.00	-2.63	0.600	0.320

3.2 Spatial co-structure analysis of Harttiini and Hypostomus' *phylogenies.*

In order to highlight the spatial co-structure of both phylogenies, the PCoA scores describing the topologies were submitted to the RLQ analysis. Prior to the analysis, the link table L was submitted to a CA to provide a new ordination of the table according to the co-occurrence of species within the five communities corresponding to the five geographic areas.

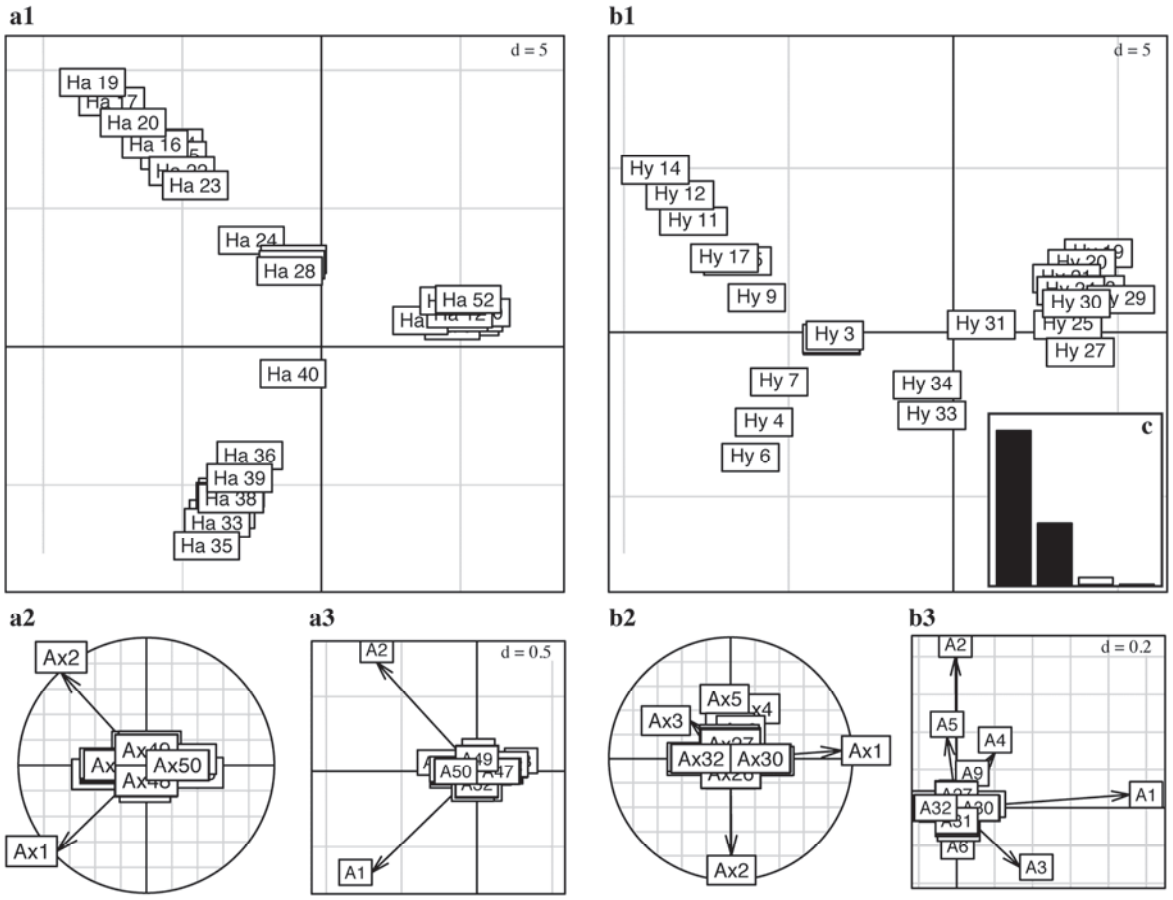


Fig. 2. RLQ analysis of the phylogenies of Harttini and *Hypostomus* constrained by the spatial co-distribution of the species. Projection of data coordinates of preliminary analyses (PCoA of Harttiini and *Hypostomus*) onto co-inertia axes 1-2 of the RLQ analysis. a: analysis of the Harttiini (table R); a1: projection of the normalized individuals' scores in the co-inertia plan (labeled as in table 1); a2: projection of inertia axes of the simple analysis onto co-inertia axes of RLQ analysis (inertia axes of PCoA of Harttiini); a3: coordinates of variables in the co-inertia plan of the RLQ analysis (principal coordinates of PCoA of Harttiini); b: analysis of *Hypostomus* (table Q); b1: projection of the normalized individuals' scores in the co-inertia plan (labeled as in Appendix S1); b2: projection of inertia axes of the simple analysis onto co-inertia axes of RLQ analysis (inertia axes of PCoA of *Hypostomus*); b3: coordinates of variables in the co-inertia plan of the RLQ analysis (principal coordinates of PCoA of *Hypostomus*); c: eigenvalues of RLQ analysis.

Then PCoA were computed using the CA rows and columns' weights for the Harttiini and *Hypostomus* phylogenies respectively. A first general assessment of the relationships between both phylogenies under spatial constraint was performed using a monte carlo testing procedure based on the total spatial coinertia (sum of eigenvalues) of the RLQ analysis, and showed a significant link between both data sets ($p = 0.02012$). The first plan of RLQ accounted for 96.74 % of the total spatial co-structure (69.07 % for axis 1 and 27.67 % for axis 2) (Fig. 2c). RLQ analysis characteristics are provided in Table 3. Covariance associated

Table 3. Main characteristics of the RLQ analysis. covariance: covariance (maximized by the analysis) between linear combinations of variables of R and Q (principal coordinates of Harttiini and *Hypostomus* phylogenies) using the link table L (species co-distribution); inertiaR: maximum inertia projected onto the axes of the simple analysis of Harttiini (eigenvalues of the PCoA); coinertiaR: maximum inertia of the simple analysis of Harttiini projected on the axes of the RLQ analysis; inertiaQ: maximum inertia projected onto the axes of the simple analysis of *Hypostomus* (eigenvalues of the PCoA); coinertiaQ: maximum inertia of the simple analysis of *Hypostomus* projected on the axes of the RLQ analysis; corr: correlation between both systems of coordinates (R and Q) onto RLQ axes.

	covariance	inertiaR	coinertiaR	inertiaQ	coinertiaQ	corr
Axis 1	13.48	24.6	20.99	26.3	22.71	0.62
Axis 2	8.53	19.45	21.51	5.45	4.96	0.83

to the first axis was twice greater than the one associated to the second axis.. The inertia projected onto RLQ axes was very close to the one projected onto inertia axes of the initial analyses: 96.47 % (42.5/44.05) of the Harttiini data structure and 87.14 % (27.67/31.75) of the *Hypostomus* data structure were recovered by axes 1 and 2 of the spatial co-structure analysis. Correlations between both data sets and RLQ axes were also high (0.62 on the first RLQ axis and 0.83 on the second one). Axis 1 of the RLQ analysis defined the continental scale of distribution of both lineages and split representatives from the Brazilian Shield plus Amazon from representatives of the Guiana Shield. Axis 2 defined the regional distributions of both lineages and ordered the species according to their current distribution within the five areas. The projections of Harttiini and *Hypostomus* data coordinates onto RLQ axes are given in Fig. 2. Comparison of both sets of coordinates, (Fig. 2, a1 and b1) allowed highlighting the most congruent regions between both phylogenies. These mainly concerned the grouping of species from the São Francisco system in negative values of axis 1 and positive values on axis 2 for both lineages, as well as the grouping of species from the Amazon in the negative values of axes 1 and 2. The grouping of species from the Guiana Shield was also consistent for both representations with all species grouping in positive values on axes 1 and 2, but with a poorer splitting in *Hypostomus* corresponding to a mix of Guianese, Amazonian, and Southeastern species. The variables involved the most in the RLQ compromise (Fig.2, a3 and b3) corresponded to the principal coordinates (PCO) 1 (scores of -0.70 on axis 1 and -0.67 on axis 2) and 2 (scores of -0.66 on axis 1 and 0.73 on axis 2) for Harttiini, and PCO1 (scores of 0.91 on axis 1 and 0.07 on axis 2), and 2 (scores of -0.007 on axis 1 and 0.80 on axis 2) for *Hypostomus*. Concerning individuals' scores on PCO1 (Fig. 3a), the highest corresponded to a splitting between Amazonian (positive values), and Guianese (negative values) lineages in Harttiini, and to a splitting between the clade D2 (positive values) comprising representatives of Amazonian, Guianese, and Southeastern lineages, and the clades D3 and D4 (negative

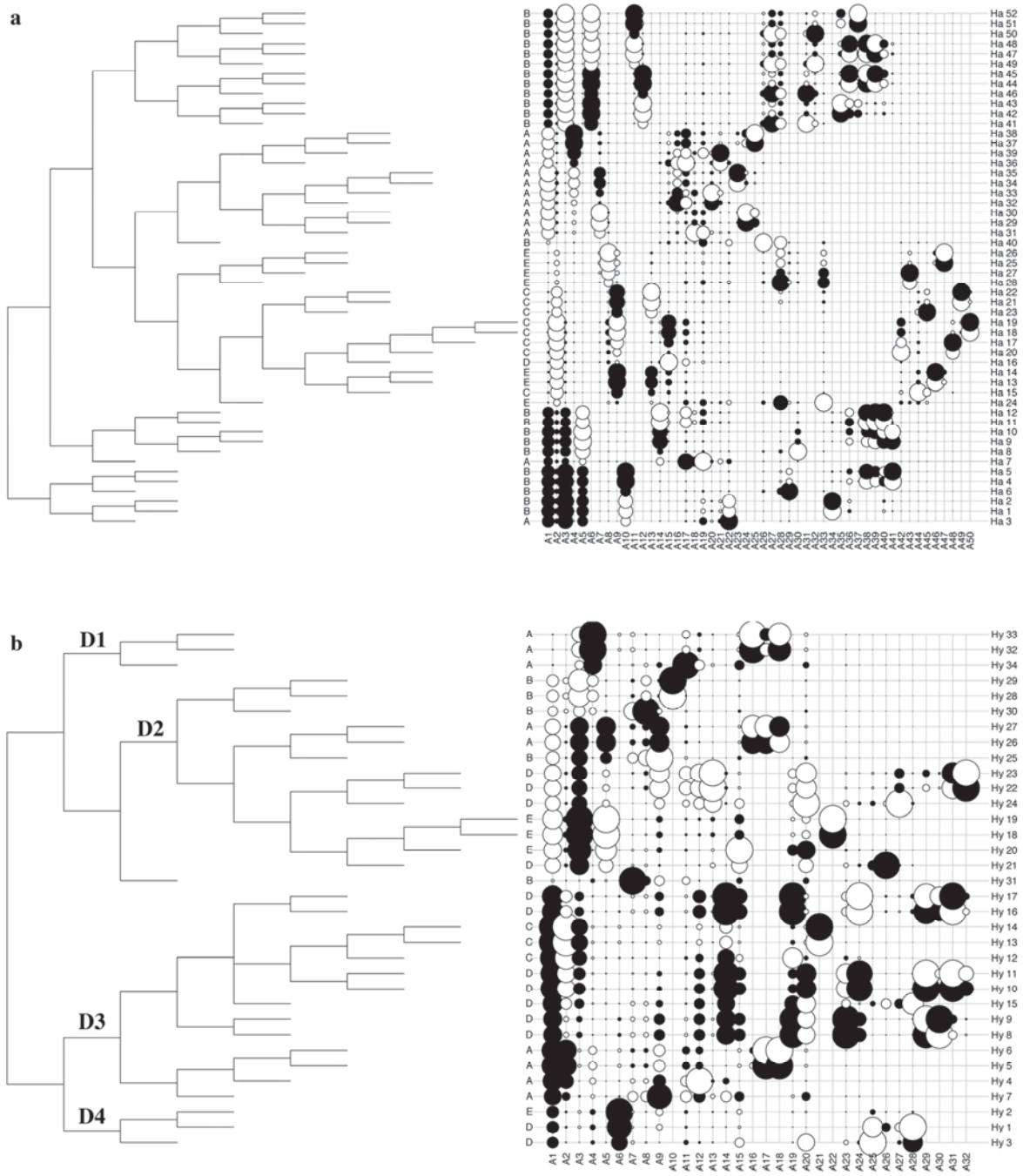


Fig. 3. Description of the phylogenies of Harttiini and *Hypostomus* by the principal coordinates of their respective PCoA. a: phylogenetic tree of Harttiini in relation to individuals' scores of its PCoA (species labeled as in table 1); b: phylogenetic tree of *Hypostomus* in relation to individuals' scores of its PCoA (species and clades labeled as in Appendix S1). Size of circles proportional to scores, positive scores in white and negative scores in black. Letters refer to the biogeographic regions as provided in figure 6.

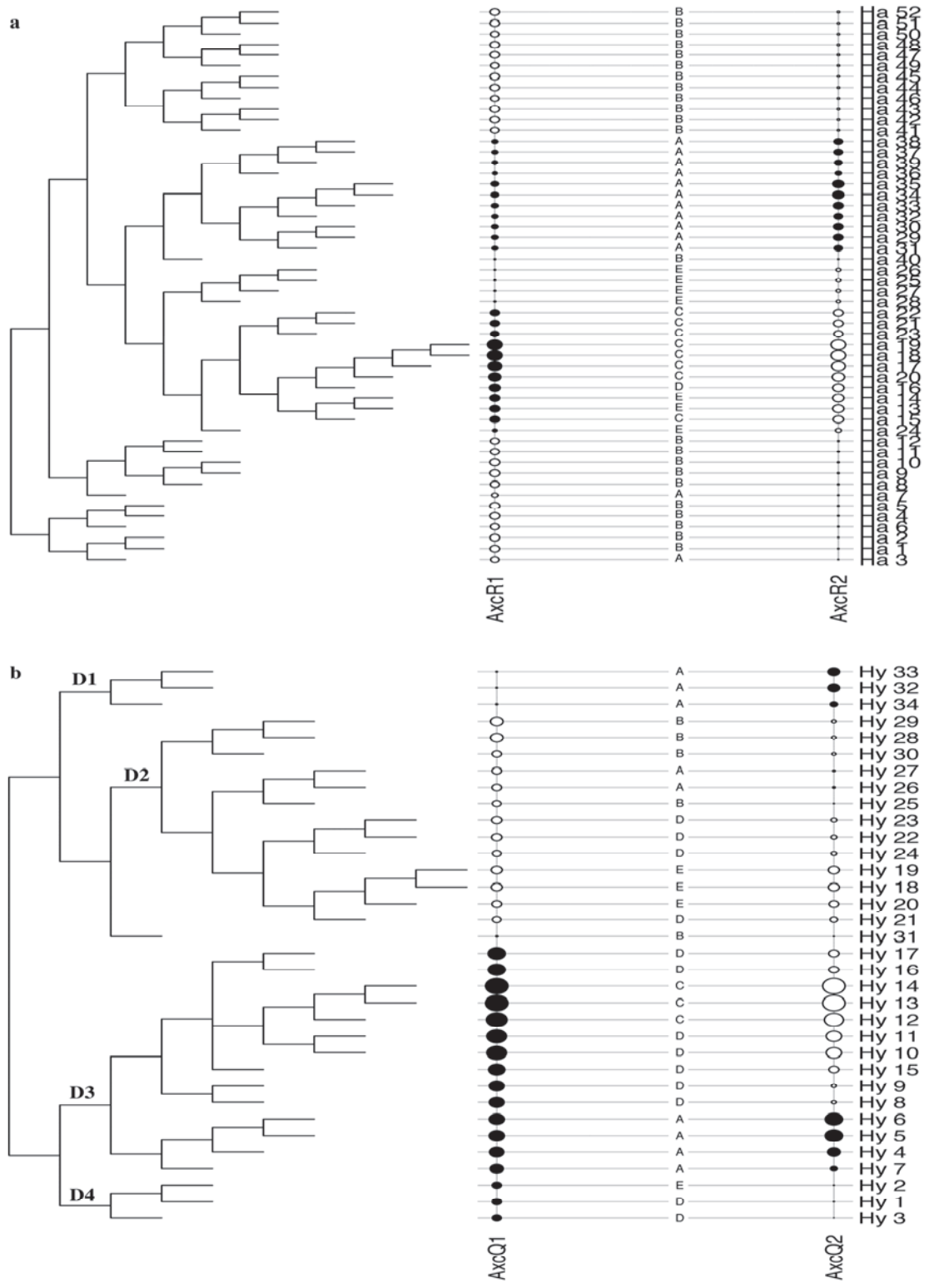


Fig. 4. Results of the RLQ analysis. Linear combinations of PCOs of both phylogenies maximizing the spatial covariance. a: phylogenetic tree of Harttiini (R) in relation to the scores of combinations of its principal coordinates in the RLQ analysis (species labeled as in table 1), axes 1 and 2; . b: phylogenetic tree of *Hypostomus* (Q) in relation to the scores of combinations of its principal coordinates in the RLQ analysis (species and clades labeled as in Appendix S1), axes 1 and 2. Size of circles proportional to scores, positive scores in white and negative scores in black. Letters refer to the biogeographic regions as provided in figure 6.

values) comprising Southeastern and Amazonian representatives in *Hypostomus* (Fig. 3b). PCO2 split Southeastern representatives (including East coastal rivers of Brazil, the Upper Paraná basin, and the São Francisco basin) in positive values from Guianese lineages of *Harttia* in negative values, whereas representatives of *Hypostomus* from the East coastal rivers of Brazil, the Upper Paraná basin, and the São Francisco basin in positive values were split from Amazonian representatives in negative values. In Harttiini, linear combinations of PCOs maximizing the phylogenetic spatial co-structure resulting from the RLQ analysis recovered the splitting between Guianese lineages (positive values) from Amazonian and Southeastern lineages (negative values) on the first axis (Fig. 4a). On the second axis, lineages from the Amazon (negative values) were split from the East coastal rivers of Brazil, the Upper Paraná basin, and the São Francisco basin (positive values). Concerning *Hypostomus*, these concerned the splitting between the D3 (positive) and D1 plus D2 (negative) clades on axis 1 of the RLQ, and the splitting between the Amazon (negative) and Upper Paraná and São Francisco basins (positive) of clade D3 on axis 2 (Fig. 4b). The *a posteriori* fourth corner testing procedure based on the PCOs and RLQ axes identified a strong and significant link between PCO2 of both phylogenies, and the second axis of the RLQ (Fig. 5). The phylogenetic spatial co-structure highlighted corresponded therefore to the common splitting event between the Amazon and the Southeastern drainages (East coastal rivers of Brazil, Upper Paraná basin, and São Francisco basin).

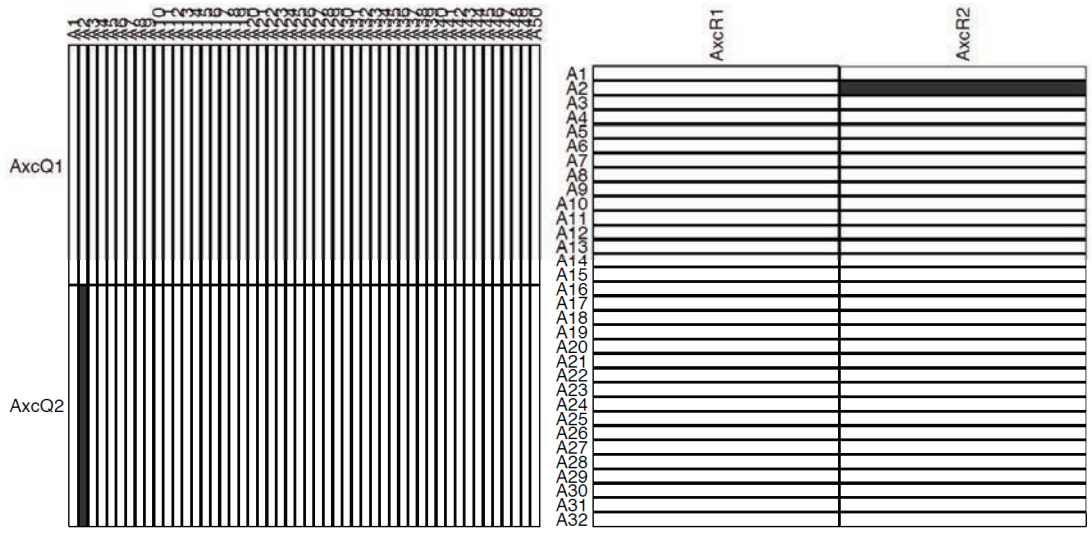


Fig. 5. Synthetic representation of the a *posteriori* fourthcorner testing procedure of the possible existing link between the RLQ axes and the principal coordinates describing the topologies of Harttiini and *Hypostomus*. Two models were used under 99,999 random permutations. A1 to A50, and A1 to A32: principal coordinates of the PCOs of the phylogenies of Harttiini (R) and *Hypostomus* (Q) respectively. AxcQ1 and R1, and AxcQ2 and R2: RLQ axes 1 and 2.

3.3 Spatial and temporal patterns of diversification.

The global test of constant molecular clock was significantly rejected ($AICu_{clock} > AICu_{non\ clock}$; $2\Delta\ln L_{RSS} > 2\Delta\ln L_{RSS0.01}$), and the MPL tests of equal rates of substitution for each node provided a mix of non significant and significant rejections (p-values ranging from 0 to 0.927) with more numerous rejections toward the deepest nodes implying local clocks. Relaxed molecular clock methods were accordingly applied. According to previous results, we used the calibration dates provided in Montoya-Burgos (2003) for the dispersal event illustrated in his clade D3 (Appendix S1). This node described the splitting event between Amazonian lineages (Tocantins River) of *Hypostomus* on one hand and lineages from Upper Paraná-São Francisco basins on the other hand. The estimated dates provided were -10.2 and -10.1 Ma for D-loop and ITS markers respectively. The cross validation procedure for the evaluation of the smoothing parameter λ required for the PL method, provided scores that reached a minimum for an estimate of $1x10^{-1}$ and a second (higher) for $1x10^{9}$. The use of the smallest value of λ as smoothing parameter provided an estimation for the root of the Harttiini located around -20 Ma with estimated substitution rates (in expected number of substitution per site and per Ma) ranging between$1x10^{-8}$ and $6.20x10^{-6}$ (mean: $9.01x10^{-7}$, SD: $1.20x10^{-6}$). The use of the higher value of λ estimated the root of Harttiini around -11.72 Ma. with rates varying between $6.39x10^{-7}$ and $6.51x10^{-7}$ (mean: $6.44x10^{-7}$, SD: $4.34x10^{-9}$). The Bayesian calibration of the tree estimated the root of Harttiini around -11.99 Ma., and the LRMD calibration around -11.63 Ma. Since the results converged toward comparable solutions and that most of MPL estimations were included in between Bayesian and LRMD estimations, the MPL calibration was performed with the higher value of the smoothing parameter.

The Dispersal-vicariance analysis resulted in the exact solution of 2 equally optimal reconstructions, and required seven dispersal events to explain the current distribution of Harttiini. The spatio-temporal pattern of diversification of Harttiini at the continental scale is provided in Fig. 6. The diversification of Harttiini initiated around -11.99 to -11.63 Ma. from ancestors occurring in the coastal rivers of the Guianas (B = 100%, P = 1) by the splitting of the *H. surinamensis* clade from all other Harttiini. Within this clade, the diversification of the different species occurred within the Guianas from a Guianese ancestor (B = 100%, P = 1) between -6.01 and -4.32 Ma., with nevertheless a dispersion event recorded toward the Amazon (AB = 100%, P = 1) between -6.01 to -4.32 and -2.27 to -1.87 Ma.; the species *H. tuna* representing an Amazonian vicariance of the Guianese *H. fluminensis* and *H. surinamensis*. The different populations constituting *H. guianensis* diversified within the

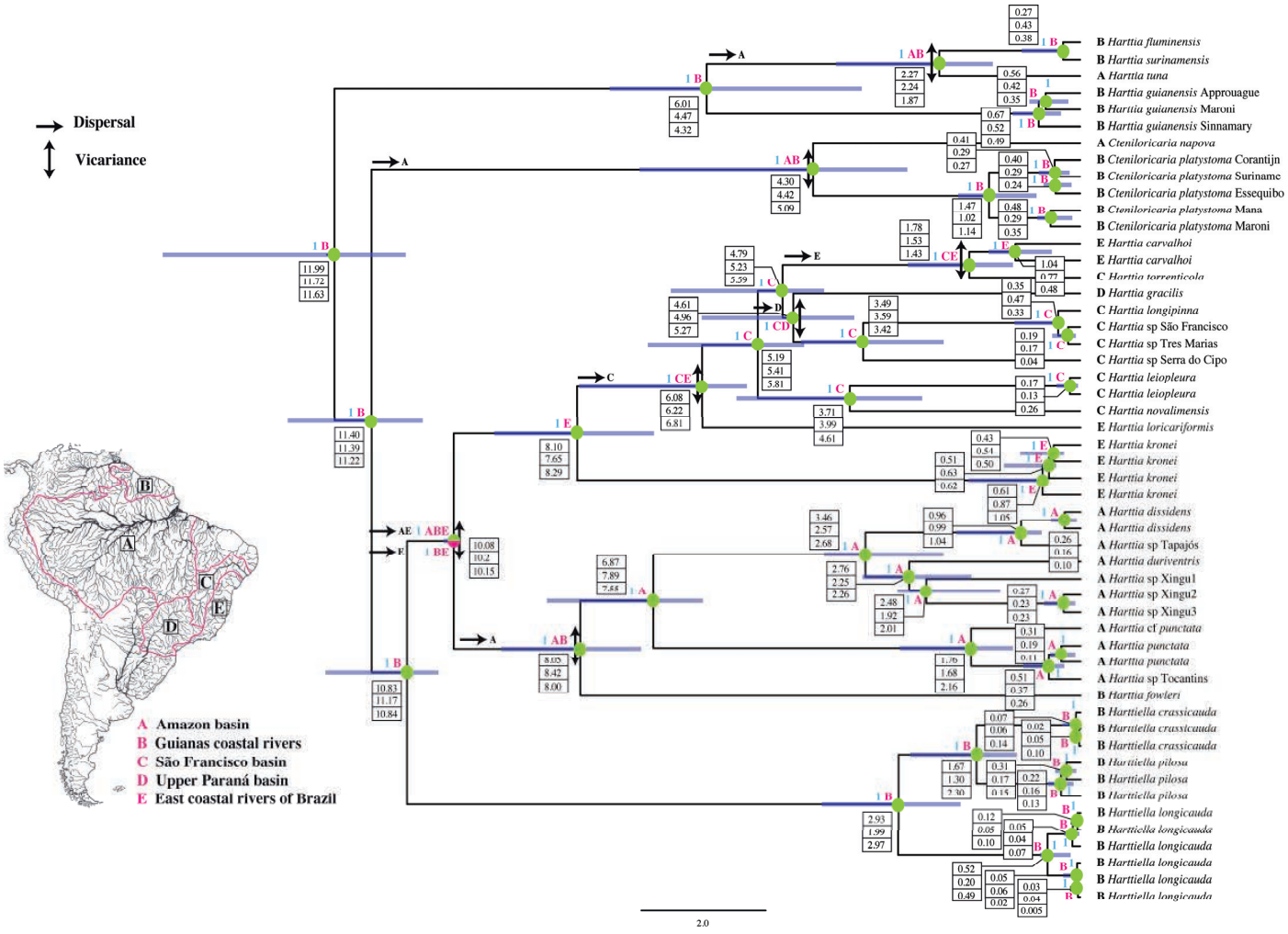


Fig. 6. Historical biogeography of Harttiini. Bayesian maximum clade credibility chronogram computed under the GTR + G model on the three data partitions, and dispersal-vicariance analysis of Harttiini using the five geographic areas provided on the map of South America as terminals. Bars indicate confidence intervals for the aging of the nodes. Boxes indicate the inferred dates for the nodes: up: Bayesian estimations, middle: PL estimations, and down: LRMD estimations. Pie charts show probabilities of alternative reconstructed ancestral ranges. Numbers above branches indicate support value for the ancestral range reconstruction. Letters above branches refer to the reconstructed biogeographic areas.

different rivers of French Guiana since -0.67 to -0.49 Ma. The second diverging clade including the *Cteniloricaria* representatives dispersed mainly within the Guianas from Guianese ancestors (B = 100%, P = 1) located around -11.40 to -10.84 Ma., with a dispersion event toward the Amazon (AB = 100%, P = 1) between -11.40 to -10.84 and -5.09 to –4.30 Ma. for the ancestor of *C. napova* and *C. platystoma*. The latter dispersed within the Guianas since -1.47 to -1.02 Ma. The third clade corresponding to *Harttiella* representatives diversified within the Guianas from a Guianese ancestor (B = 100%, P = 1) located around -11.17 to -10.83 Ma., the diversification of the different species initiating between -2.97 and -1.99 Ma. The fourth diverging group including at base our calibration point showed an ambiguous yet strongly supported dispersal-vicariance reconstruction with two equiprobable states (BE = 50%, ABE = 50%, P = 1). From a Guianese ancestor (B = 100%, P = 1), the species dispersed or toward the East costal rivers of Brazil (state BE) or toward the Amazon and East coastal rivers of Brazil (state ABE). This last hypothesis appeared nevertheless more likely since the Amazonian region splits the Guiana and Brazilian Shields, rendering unlikely direct pathway from Guianese costal rivers to Brazilian coastal rivers. From that ancestral area the species split by vicariance with on one side the Amazonian and remaining Guianese representatives (AB = 100%, P = 1) and on the other side the Southeastern representatives (E = 100%, P = 1). The ancestor of the last Guianese representative (*C. fowleri*) split from the Amazonian representatives around -8.42 to -8 Ma. The Amazonian species of *Harttia* diversified within the Amazon basin since -7.89 to -6.87 Ma. with the splitting between representatives from Tocantins drainage and representatives from Tapajós and Xingu drainages. Diversification within the Tocantins occurred since -2.16 to -1.68 Ma, whereas splitting between Tapajós and Xingu Rivers representatives took place between -3.46 and -2.57 Ma. Diversification of species within these drainages occurred around -1.04 to -0.96 Ma. and around -2.26 to -2.76 Ma. for Tapajos and Xingu Rivers respectively. Within the last group comprising all Southeastern representatives, diversification occurred around -8.29 to -7.65 Ma. from ancestors originating from coastal rivers of Brazil (E = 100%, P = 1). From this ancestral distribution the ancestral species dispersed toward the Rio São Francisco (CE = 100%, P = 1) between -8.29 to -7.65 and -6.81 to -6.08 Ma. The ancestors of *H. loricariformis* split by vicariance from other remaining Harttiini. From an ancestral distribution located in the Rio São Francisco dated around -5.81 to -5.19 Ma. ago a first group of species including *H. leiopleura* and *H. novalimensis* diversified within this drainage since -4.61 to -3.71 Ma. From the Rio São Francisco ancestral area, the ancestral species went on their diversification within this drainage until -5.59 to -4.79 Ma. ago. Then the ancestral species dispersed toward

two distinct regions, with on one hand dispersion toward the coastal rivers of South East Brazil (CE = 100%, P = 1) between -5.59 to -4.79 and -1.78 to -1.43 Ma., and on the other hand a dispersion toward the Upper Paraná drainage (CD = 100%, P = 1) between -5.59 to -4.79 and -5.27 to -4.61 Ma. ago. The diversification of both lineages occurred by vicariance between Upper Paraná and Rio São Francisco on one hand and between Rio São Francisco and the coastal rivers of Brazil on the other hand from ancestors located around -5.27 to -4.61 Ma. and -1.78 to -1.43 Ma. respectively. A second diversification occurred within the Rio São Francisco basin since -3.58 to -3.42 Ma.

Discussion

In this work we were interested in exploring the phylogeography of the Harttiini, a tribe among the highly specialized Neotropical catfish subfamily Loricariinae, and in deciphering their history of dispersion at the subcontinental scale. Unfortunately a direct dating of the dispersion events was not made possible since no external geological events open to explain the current distribution of species was accurately recorded within the range of distribution of the different species. To circumvent this trouble, we used a new approach to detect co-structures in phylogenies under the spatial constraint of co-distribution of species. Initially devoted to the ecological study of the joint structure between three tables, such as species traits and environmental variables through the constraint of environment's species composition, the RLQ analysis (Dolédec *et al.,* 1996) has proven to be an efficient and relevant tool as exploratory and ordination method. The strength of the RLQ relies on the link table L providing the hypothesis constraining the analysis. The co-structures revealed are thus directly interpretable in the light of the emitted hypothesis, all other apparently visible co-structure being potentially related to unrevealed factors. Moreover, philosophically tightly related, the fourthcorner testing procedure introduced by Legendre *et al.* (1997) has also been efficiently adapted to the study of coevolution between hosts and their parasites (Legendre *et al*., 2002). The ParaFit method (Legendre *et al.* 2002) tests the significance of a global hypothesis of coevolution between parasites and their host using the phylogenetic trees of both parasites and hosts beforehand described by their respective PCOs, and an host-parasite binary coding association matrix as link. The method also allows to test the significance of individual host-parasite association link. Recently, Dray and Legendre (2008) adapted the fourthcorner method to allow the combination of different models in the global testing procedures. Dray (in prep.) developed the possibility to test the individual link between each

variables of R and Q (here the PCOs of each phylogeny) and the axes of the RLQ analysis (the compromise established between the phylogenies and the co-distribution of species).

Theoretically well adapted to our problematic the use of the RLQ analysis has also the advantage over existing methods to treat the full phylogenies at once (beforehand converted into distances matrices), and to use a third table as spatial link table. This allows the use of a wide range of tree sources (cladograms, phylograms, phenograms, supertrees, consensus trees…) and does not necessitate any modification such as converting phylogenies into area cladograms. The RLQ analysis offers a graphical output which allows a detailed analysis of the contribution of each phylogeny to the overall trend, within the frame of the spatial distribution of the species. Co-structures between both data sets are highlighted on the factorial map of individuals (Fig. 2, a1 and b1) by the relative position of both systems of coordinates (phylogenies of Harttiini and *Hypostomus*) onto RLQ axes. In our case, a consistent spatial co-structure pattern is clearly highlighted by the RLQ along the second axis of both systems of coordinates. The factorial maps of variables (Fig. 2, a3 and b3) reveal the contribution of each variable (here the principal coordinates) to the phylogenetic spatial co-structure, and identify the groups defined by these variables. The fact to use PCOs as descriptors of the phylogenies could not be without consequences, poorly balanced topologies resulting in poor descriptors for example. Moreover, first PCOs often characterize deepest nodes implying more distant relationships. These nodes display more variations onto axes, and consequently possess a greater weight in the analysis. Nevertheless, in the situation illustrated here, even though the first PCOs displayed the greater variance, the RLQ analysis perfectly identified the second PCOs to be significantly linked to the spatial co-structure as shown by the greater correlation recorded between both PCO2 and the second axis of the RLQ (Table 3). A second advantage to use PCOs is that they allow treating with poorly resolved or poorly supported phylogenies by breaking of the tree representation. The graph of eigenvalues (Fig. 2c) identifies the axes explaining the major part of the congruent information between data sets under spatial constraint. Thus, the RLQ provides an ordination of the species according to their phylogenetic position in both trees, constrained by their current co-distribution. A last major advantage of the RLQ analysis is that the significance of the observed structure can be subjected to fourthcorner *a priori* tests evaluating the strength of the revealed global phylogenetic spatial co-structure, and *a posteriori* tests allowing detecting which parts of both phylogenies are significantly linked to the highlighted structures.

In order to study the phylogeography of Harttiini catfishes, we first inferred the phylogeny of the subfamily using 12S and 16S mitochondrial genes, as well as the F-RTN4

nuclear gene. The results show that Harttiini form a monophyletic group comprising all *Harttia*, *Cteniloricaria*, and *Harttiella* representatives, and forms the sister tribe of the Loricariini. This corroborates the findings of Montoya-Burgos *et al.* (1998) and Covain *et al.* (2008) who recovered this topology with a more restricted Loricariinae sampling.

The phylogenetic reconstruction confirms the validity of *Cteniloricaria* and *Harttiella* as revised by Covain et al. (in press), both genera being monophyletic. *Cteniloricaria* contained initially three valid species (Isbrücker, 1979): *C. platystoma* (type species), *C. maculata*, and *C. fowleri*. Covain *et al*. (loc. cit.) demonstrated that *C. maculata* was a junior synonym of *C. platystoma*, and described in the same work *C. napova* from the Paru de Oeste River, a tributary of the Trombetas River flowing toward the Amazon. They also noted that *C. fowleri* possessed strong morphological and genetic divergences to other *Cteniloricaria* and placed temporarily the species within *Harttia*. Our phylogenetic reconstruction placed *C. fowleri* at base of the Amazonian clade of *Harttia*, a position strongly supported by bootstrap values and posterior probabilities. This position excludes it definitely from *Cteniloricaria* and it is here assigned to *Harttia*. The authors also voiced doubts concerning the monophyly of *Harttia* due to the obtained scattered position of *H. fowleri* in regards to other *Harttia* and to *Cteniloricaria*. Their analysis was based on DNA barcodes for an assessment of the global diversity of the Harttiini within the Guianas, and was mainly aimed as identification purposes using a phenetic approach. Based on robust phylogenetic methods, this result is confirmed in the present study, but the non significance of the constrained monophyly of *Harttia* with Guianese representatives forming the sister group of Amazonian + Southeastern groups, and with *Harttiella* or *Cteniloricaria* as sister taxa (Table 2, H4 and H5) does not allow envisaging the placement of Guianese *Harttia* in a distinct genus. In the same way, Boeseman (1971) hypothesized a rather basal position concerning *Harttiella*, as sister group (excluding Farlowellina) of *Cteniloricaria* (*Parasturisoma* in Boeseman) and *Harttia*. The different alternative topologies evaluated here do not allow to reject this hypothesis. Even though a more nested position was found by the best ML reconstruction, the AU and SH tests failed to reject Boeseman's hypothesis (Table 2, H3 and H4). Finally, the nested position of *H. leiopleura* (type species of *Quiritixys*) within the Southeastern clade, as well as the position of *H. loricariformis* (type species of *Harttia*) as sister species of all Southeastern *Harttia* but *H. kronei*, does not allow the recognition of *Quiritixys* as a valid genus. The unusual sexual dimorphism expressed in *H. leiopleura* and the small size, comparable to that of a *Harttiella*, of the two known representative of the group (*H. leiopleura* and *H. novalimensis*), should

therefore be interpreted as local adaptations of *Harttia*, or to a morphological stasis, *Hartttiella* forming the sister group of the Amazonian-Southeastern representatives.

Despite evident sampling bias between Harttiini and *Hypostomus* data sets (*e. g*. Harttiini sampling rich in Guianese and poor in Upper Paraná representatives versus an opposite sampling in *Hypostomus*) making direct comparison difficult, the RLQ analysis highlighted a significant spatial co-structure of both phylogenies. If the hypothesis of co-dispersion events is correct, the inferred subsequent dating events should be comparable between both sub-trees corresponding to Amazonian and Southeastern representatives. Montoya-Burgos (2003) reported a second cladogenetic event within his clade D3 corresponding to the isolation of the Southeastern *Hypostomus* species of the Rio São Francisco from the Upper Paraná species estimated around -6.4 to -5.7 Ma. Even though a single representative of *Harttia* from Upper Paraná is present in our data set (*H. gracilis*), the splitting of this species from representatives from the Rio São Francisco (*H. longipinna*, *H*. sp. Tres Marias, and *H*. sp. São Francisco) is estimated around -5.3 to -4.6 Ma. This estimation appears slightly inferior, but the confidence interval computed by the Bayesian reconstruction for this node includes the *Hypostomus* dating. This result can thus reasonably be regarded as a confirmation of the co-dispersion hypothesis. Montoya-Burgos (2003) hypothesized the boundary displacement between the Upper Paraná-São Francisco basins during the Tertiary (-65 to -1.8 Ma.; Beurlen, 1970) to explain the colonization of the Rio São Francisco. A second exchange within the Southeastern clade of Harttiini may be corroborated by our study. A small variation is indeed recorded on both second PCOs that are significantly linked to the second axis of the RLQ analysis. It concerns the faunal exchange between the coastal rivers of South East Brazil, particularly the Rio Paraíba do Sul, and the Upper Paraná drainage. The dispersion events reconstructed from the node leading on one side to *H. carvalhoi* and *H. torrenticola* (Paraíba do Sul and São Francisco respectively), and to *H. gracilis* (Upper Paraná) and *H. longipinna*, *H*. sp. Tres Marias, and *H*. sp. São Francisco (São Francisco) on the other side is estimated to have initiated around -5.6 to -4.7 Ma. These estimations meet the -4.2 Ma. provided by Montoya-Burgos (2003) for the faunal exchange between Paraíba do Sul (*H. affinis*, *H. punctatus*) and Ribeira de Iguape (*H*. sp. 1161) on one hand, and Upper Paraná (*H. ancistroides*) on the other hand within his clade D2. The disconnection between the coastal Paraíba do Sul and the Upper Paraná in the middle Miocene (16-10 Ma. ago) has been put forward to explain this distribution. Montoya-Burgos (2003) moderated however this difference between both dating by possible more recent headwater captures. The multiple exchanges between the Rio São Francisco, Upper Paraná,

and coastal rivers of South East Brazil revealed in the present study corroborate this hypothesis and imply a highly complex pattern of multiple headwaters captures between these regions during the past 8 million years. The frequency of shared species between these three areas was estimated around 14% (Ribeiro, 2006). The fact that diversification in these regions was not followed by cladogenetic events suggests relatively recent dispersions. These regions are indeed prone to tectonic activity and deformations favoring stream captures resulting of direct tectonic stress or differential erosion (Ribeiro, 2006). The youngest tectonic activity concerning headwaters of Ribeira de Iguape, Iguaçu and Paranapanema rivers on one hand, and upper Rio Tietê on the other hand, both sharing a mixed fish fauna, was estimated to less than 1.6 Ma. (Ribeiro, 2006). To the contrary, a potential false co-dispersion of species concerns a sub-group nested within the clade D2 of *Hypostomus* (*sensu* Montoya-Burgos, 2003), and refers to *H. plecostomus* from Oyapock River in French Guiana, as sister group of *H*. sp. 49 + 36 from the Amazon. This branching pattern is strikingly similar to that of *H. fowleri* from the Oyapock River as sister group of Amazonian species of *Harttia*. The splitting between *H. plecostomus* and *H*. sp. 49 and *H*. sp. 36 was estimated around -5.5 Ma by Montoya-Burgos (2003), whereas splitting of *H. fowleri* from other Amazonian species is estimated around -8.42 to -8 Ma. This estimation appears superior and the dating for *Hypostomus* is out of the confidence interval for this node, what could imply different dispersion processes (so no direct co-dispersion). Indeed, *H. plecostomus* and its sister species are inhabitants of the lower part of rivers, in quiet and muddy waters, whereas *Harttia* are rheophilic species inhabiting the upper part of rivers in clear and swift current. Due to these ecological constraints, *H. plecostomus* and relatives are able to disperse through coalescing river mouths in low sea level periods, contrary to *Harttia* representatives that are more likely able to disperse through headwater captures. Moreover, this branching pattern does not display variation on the second PCO of *Hypostomus*, implying a different tree's structure. These apparent similarities between branching patterns are only observed by chance and may lead to false interpretations. Trees' topologies should thus be first submitted to robust inferences such as RLQ in order to detect significantly congruent patterns, as it is the case here. Visual comparisons of trees should be consequently avoided as much as possible. Only *Hypostomus watwata* displays small variations on PCO2. This Guianese species forms the base of clade D2 as sister group of the previous group on one hand, and of a group of *Hypostomus* from coastal rivers of Southeastern Brazil and Paraná River on the other hand. The origin of clade D2 has been estimated around -11.4 to -10.5 Ma. (Montoya-Burgos, 2003). This estimation meets the date at base of clade D3 of *Hypostomus* used as calibration

date for the phylogeny of Harttiini, and the origin of clade D4 of *Hypostomus* estimated around -11.8 Ma. Excluding the node used to calibrate the phylogeny of Harttiini, the origin of the Guianese *Harttia*, *Cteniloricaria*, and *Harttiella* lineages are estimated around -11.99 to -11.63 Ma. for *Harttia*, -11.4 to -10.84 Ma. for *Cteniloricaria*, and -11.17 to -10.83 Ma. for *Harttiella*. These estimations perfectly meet those provided for *Hypostomus*, suggesting a common temporal context of diversification even though only one spatial co-structure is revealed. The sudden diversification of Harttiini and *Hypostomus* reveals an explosive radiation pattern at base of both lineages, each clade appearing at the same period. These concomitant cladogenetic events suggest a global common factor explaining the origin of the different lineages. The sea level fluctuations are often put forward to explain habitat fragmentations or river mouth connections. A major marine regression favoring river mouth connections is indeed reported at the beginning of the Tortonian period of Upper Miocene around -11 and -10 Ma. (Haq *et al*., 1987) that may explain the origin of the different clades of Harttiini and *Hypostomus*. However, species diversification within each clade occurred quite early in both phylogenies, more or less around -5.5 Ma., suggesting, despite local effects, a second global common factor responsible for fish diversification. An important marine transgression favoring habitat fragmentations is also reported for the Zanclan period of Lower Pliocene around -5 Ma. (Haq *et al*., 1987) that may explain such species diversification. Corroborating these results, a recent study conducted on Serrasalmidae by Hubert *et al*. (2007) revealed a rapid diversification of the species initiating during the Late Miocene and ending at the Pliocene-Pleistocene transition. These authors estimated the splitting between two genera of piranha (*Serrasalmus* and *Pygocentrus*) around -8.73±1.79 Ma. with a rapid diversification of *Serrasalmus* achieved between -8 and -5.66±0.8 Ma. The genus *Serrasalmus* is widely distributed within Orinoco, Amazon, Paraná, and São Francisco Rivers, and may accordingly represent another guild of co-distributed species. Nevertheless, their current pattern of distribution appears highly complex, and their phylogenetic relationships, and spatio-temporal context of diversification should be first scrutinized (*e.g*. using the RLQ approach) prior to reach any conclusion.

Acknowledgments

We are grateful to Philippe Gaucher, CNRS Guyane; Régis Vigouroux and Philippe Cerdan, Hydreco Guyane; Claude Weber, Patrick de Rham and Alain Merguin, MHNG; Maël Dewynter, ONF Guyane; Frédéric Melki, Biotope France; Kenneth Wan Tong You,

Paramaribo; Paul Ouboter, NZCS; Calvin Bernard, CSBD; the North Rupununi District Development Board; and the Iwokrama organization for the collection of tissue samples and/or their field and logistic assistance; the G. and A. Claraz Foundation for their financial support for the missions in Suriname in 2001, 2005, 2007 and 2008, and in French Guiana in 2006; the Académie Suisse des Sciences Naturelles (ScNat) for their financial support for the missions in Guyana 2004 and French Guiana 2006; The Constantin Topali Found for their financial contribution to the acquisition of field material for the mission Suriname 2007, and lab material in 2009; André Huser, Sallmann-Fehr AG for the gift of gill nets for the mission in Suriname in 2005 and 2007; the Guyana Environmental Protection Agency, and Ministry of Amerindian affairs; the French Guiana Diren, and Préfecture; and the Surinamese Ministry of Agriculture, Animal Husbandry and Fisheries for the different necessary authorizations and collecting permits. Part of this project was supported by the Fond National Suisse de la Recherche Scientifique (JIMB 3100A0–104005). The figures were finalized by Florence Marteau, MHNG. Preliminary results of this study were presented in 4ème rencontres de l'Ichtyologie en France (RIF), Paris, 03-2009; the 6ème symposium national de morphométrie et évolution des formes (SMEF), Montpellier, 06-2009; and the 13th Evolutionary Biology Meeting at Marseilles, 09-2009 with the financial support of the Société Académique de Genève. John A. Hollier, MHNG, provided useful comments and suggestions on the earliest version of the manuscript; Jan Pawlowski, University of Geneva, is acknowledged for the laboratory facilities; José Fahrni, University of Geneva, for the sequencing process; Lionel Cavin, MHNG, Pierre Legendre and François-Joseph Lapointe, University of Montreal, Emmanuel Paradis, IRD Montpellier, Christoph Heibl, University of Munich, and Eileen Kraemer, University of Georgia for fruitful discussions and help concerning different methods. This work represents part of the PhD dissertation of the first author, who would particularly acknowledge Denis Duboule, University of Geneva, Sonia Fisch-Muller, MHNG, and Stéphane Dray, University of Lyon for their supervision.

References

Albert, J.S., Lovejoy, N.R., and Crampton, W.G.R., 2006. Miocene tectonism and the separation of cis- and trans-Andean river basins: evidence from Neotropical fishes. *Journal of South American Earth Sciences* **21:** 14-27.

Armbruster, J. W., 2004. Phylogenetic relationship of the suckermouth armoured catfishes (Loricariidae) with emphasis on the Hypostominae and the Ancistrinae. *Zoological Journal of the Linnean Society* **141:** 1-80.

Bermingham, E., and Martin, A.P., 1998. Comparative mtDNA phylogeography of Neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology* **7**: 499-518.

Beurlen, K., 1970. Geologie von Brasilien. Gebrüder Borntraeger, Berlin/Stuttgart, 444 pp.

Boeseman, M., 1971. The "comb-toothed" Loricariinae of Surinam, with reflections on the phylogenetic tendencies within the family Loricariidae (Siluriformes, Siluroidei). *Zoologische Verhandelingen (Leiden)* **116:** 1-56.

Britton, T., Oxelman, B., Vinnersten, A., and Bremer, K., 2002. Phylogenetic dating with confidence intervals using mean path lengths. *Molecular Phylogenetics and Evolution* **24:** 58–65.

Brooks, D.R., 1985. Historical ecology: a new approach to studying the evolution of ecological associations. *Annals of the Missouri Botanical Garden* **72**, 660-680.

Cailliez, F., 1983. The analytical solution of the additive constant problem. *Psychometrika* **48**: 305–310.

Chiachio, M.C., Oliveira, C., and Montoya-Burgos, J.I., 2008. Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). *Molecular Phylogenetics and Evolution* **49:** 606-617.

Covain, R., Le Bail, P.-Y., Sagnes, P., and Fisch-Muller, S., 2006. Les espèces du genre *Harttia* (Siluriformes : Loricariidae) en Guyane Française : morphologie, taxinomie et distribution. *Cybium* **30**: 3-18.

Covain, R., and Fisch-Muller, S., 2007. The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: Loricariidae): a practical key and synopsis. *Zootaxa* **1462**: 1-40.

Covain, R., Dray, S., Fisch-Muller, S., and Montoya-Burgos, J.I., 2008. Assessing phylogenetic dependence of morphological traits using co-inertia prior to investigate character evolution in Loricariinae catfishes. *Molecular Phylogenetics and Evolution* **46**: 986-1002.

Covain, R., Fisch-Muller, S., Montoya-Burgos J.I., Mol J., Le Bail, P-Y., and Dray, S. (in press). The Harttiini (Siluriformes, Loricariidae) from the Guianas: a multi-table approach to assess their diversity, evolution, and distribution. *Cybium*.

Doledec, S., Chessel, D., ter Braak, C.J.F., and Champely, S., 1996. Matching species traits to environmental variables: a new three-table ordination method. *Environmental and Ecological Statistics* **3**: 143–166.

Dong, S., and Kraemer, E., 2004. Calculation, visualization, and manipulation of MASTs (Maximun Agreement Subtrees). In: *Proceedings of the IEEE Computational Systems Bioinformatics Conference*, pp. 1–10.

Dray, S., Pettorelli, N., and Chessel, D., 2002. Matching data sets from two different spatial samplings. *Journal of Vegetation Science* **13**: 867–874.

Dray, S., and Dufour, A-B., 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* **22(4)**: 1-20.

Dray, S., and Legendre, P., 2008. Testing the species traits-environment relationships: the fourth-corner problem revisited. *Ecology* **89**: 3400–3412.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., and Rambaut, A., 2006. Phylogenetics and dating with confidence. *PLoS Biology* **4**, e88.

Drummond A.J., and Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**:214.

Edwards, S.V., and Beerli, P., 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* **54**, 1839-1854.

Efron, B., 1979. Bootstrap methods: Another look at the jackknife. *Annals of Statistics* **7**: 1–26.

Farris, J.S., Källersjö, M., Kluge, A.G. and Bult, C., 1994. Testing significance of incongruence. *Cladistics* **10**: 315–319.

Felsenstein, J., 1981. Evolutionary trees from DNA-sequences- a maximum-likelihood approach. *Journal of Molecular Evolution* **17**: 368–376.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Ferraris, Jr. C.J., 2003. Subfamily Loricariinae (Armored catfishes). In: *Check List of the Freshwater Fishes of South and Central America (CLOFFSCA)* (Reis R.E., Kullander S.O. and C. J. Ferraris, eds.), pp. 330-350. Porto Alegre: EDIPUCRS.

Ferraris, Jr. C.J., 2007. Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. *Zootaxa* **1418**: 1-628.

Fricke, R., and Eschmeyer, W.N., 2010. A guide to Fish Collections in the Catalog of Fishes database. On-line version of 15 January 2010. http://research.calacademy.org/redirect?url=http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp.

Friedman, M., 1937. The use of ranks to avoid the assumption of normality implicit in the analysis of variance. *Journal of the American Statistical Association* **32**: 675-701.

Ganapathy, G., Goodson, B., Jansen R., Le H-S., Ramachandran, V., and Warnow, T., 2006. Pattern identification in biogeography. *IEEE/ACM Transactions of Computational Biology and Bioinformatics* **3**: 334-346.

Gelman, A., and Rubin, D.B., 1992. Inference from Iterative Simulation Using Multiple Sequences. *Statistical Science* **7(4)**:457-511.

Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* **53**: 325-338.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.

Haq, B.U., Hardenbol, J. and Vail, P.R., 1987. Chronology of fluctuating sea levels since the Triassic (250 million year ago to present). *Science* **235 (4793)**: 1156-1167.

Harris, A.J., and Xiang, Q-Y., 2009. Estimating ancestral distributions of lineages with uncertain sister groups: a statistical approach to Dispersal-Vicariance Analysis and a case using Aesculus L (Sapindaceae) including fossils. *Journal of Systematics and Evolution* **47**: 349–368.

Holm S., 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6**: 65–70.

Hubert, N., and Renno, J-F., 2006. Historical biogeography of South American freshwater fishes. *Journal of Biogeography* **33**: 1414-1436.

Hubert, N., Duponchelle, F., Nuñez, J., Garcia-Davila, C., Paugy, D., and Renno, J-F., 2007. Phylogeography of the piranha genera *Serrasalmus* and *Pygocentrus*: implications for the diversification of the Neotropical ichthyofauna. *Molecular Ecology* **16**: 2115-2136.

Huelsenbeck, J. P., and Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**:754-755.

Isbrücker, I.J.H., 1979. Description préliminaire de nouveaux taxa de la famille des Loricariidae, poissons-chats cuirassés néotropicaux, avec un catalogue critique de la sous-famille nominale (Pisces, Siluriformes). *Revue Française d'Aquariologie* **5**: 86–116.

Isbrücker, I. J. H., Seidel, I., Michels, J.P., Schraml E. and Werner, A., 2001. Diagnose vierzehn neuer Gatungen der Familie Loricariidae Rafinesque, 1815 (Teleostei, Ostariophysi). In*:* R. Stawikowski (Ed.), *Harnischwelse 2*, Die Aquarien und Terrarien Zeitschrift, Sonderheft, Eugen Ulmer, Stuttgart, Germany, p. 17-24.

Jobb, G., von Haeseler, A. and Strimmer, K., 2004. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology* **4**: 18.

Kendall, M. G., and Babington Smith, B., 1939. The problem of m rankings. *Annals of Mathematical Statistics* **10**: 275-287.

Kishino, H., T., Miyata, and Hasegawa, M., 1990. Maximum likelihood inference of protein phylogeny and the origin of chloroplasts. *Journal of Molecular Evolution* **31**:151–160.

Kubicka, E., Kubicki, G. and McMorris, F.R., 1995. An algorithm to find agreement subtrees. *Journal of Classification* **12**: 91-99.

Lapointe, F-J., and Rissler, L.J., 2005. Congruence, consensus, and the comparative phylogeography of codistributed species in California. *American Naturalist* **166**: 290-299.

Legendre, P., Galzin, R., and Harmelin-Vivien, M.L., 1997. Relating behavior to habitat: solutions to the fourth-corner problem. *Ecology* **78**: 547–562.

Legendre, P., Desdevises, Y., and Bazin, E., 2002. A statistical test for host-parasite coevolution. *Systematic Biology* **51(2)**: 217–234.

Legendre, P., and Lapointe, F-J., 2004. Assessing congruence among distance matrices: single malt Scotch whiskies revisited. *Australian and New Zealand Journal of Statistics* **46**: 615-629.

Lévêque, C., Oberdorff, T., Paugy, D., Stiassny, M.L.J., and Tedesco, P.A., 2008. Global diversity of fish (Pisces) in freshwater. *Hydrobiologia* **595**: 547-567.

Lovejoy, N.R., and Araújo, L.G., 2000. Molecular systematics, biogeography and population structure of Neotropical freshwater needlefishes of the genus *Potamorrhaphis*. *Molecular Ecology* **9**: 259-268.

Lovejoy, N.R., Lester, K., Crampton, W.G.R., Marques, F.P.L. and Albert, J.S., 2010. Phylogeny, biogeography, and electric signal evolution of Neotropical knifefishes of the genus *Gymnotus* (Osteichthyes: Gymnotidae). *Molecular Phylogenetics and Evolution* **54**: 278-290.

Lundberg, J.G., Marshall, L.G., Guerrero, J., Horton, B., Malabarba, M.C.S.L. and Wesselingh, F., 1998. The stage for Neotropical fish diversification: a history of tropical South American Rivers. *In*: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena Z.M.S. and Lucena, C.A.S. (Eds.) *Phylogeny and Classification of Neotropical Fishes*. Edipucrs Porto Alegre, Porto Alegre, Brazil, pp. 13–48.

Mantel N., 1967. - The detection of desease clustering and a generalized regression approach. *Cancer Research* **27**: 209-220.

Meyer, A., 1993. Evolution of mitochondrial DNA in fishes. In: *Biochemistry and Molecular Biology of Fishes, Volume 2: Molecular Biology Frontiers.* (Hochachka, P.W. and Mommsen, P., eds), pp 1-38. Amsterdam, the Netherlands: Elsevier Science.

Mol, J.H., and Ouboter, P.E., 2004. Downstream effects of erosion from small-scale gold mining on the instream habitat and fish community of a small neotropical rainforest stream. *Conservation Biology* **18**: 201-214.

Mol, J.H., Wan Tong You, K., Vrede, I., Flynn, A., Ouboter, P., and Van der Lugt, F., 2007. Fishes of Lely and Nassau Mountains, Suriname. *In* A Rapid Biological Assessment of the Lely and Nassau Plateaus, Suriname (with additional information on the Brownsberg

Plateau) (Alonso L.E. & J.H. Mol, eds.), pp. 107-118. RAP Bulletin of Biological Assessment 43. Conservation International, Arlington, USA.

Montoya-Burgos, J.I., 2003. Historical biogeography of the catfish genus *Hypostomus* (Siluriformes: Loricariidae), with implications on the diversification of Neotropical ichthyofauna. *Molecular Ecology* **12**: 1855-1867.

Montoya-Burgos, J.I., Muller, S., Weber, C., and Pawlowski, J., 1998. Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences. *In*: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena Z.M.S. and Lucena, C.A.S. (Eds.) *Phylogeny and Classification of Neotropical Fishes*. Edipucrs Porto Alegre, Porto Alegre, Brazil, pp. 363–374.

Nylander, J.A.A., Olsson, U., Alström, P., and Sanmartín, I., 2008. Accounting for phylogenetic uncertainty in biogeography: a Bayesian approach to Dispersal vicariance Analysis of the thrushes (Aves: *Turdus*). *Systematic Biology* **57**: 257–268.

Page, R.D.M., 1994. Maps between trees and cladistic analysis of historical associations among genes, areas and organisms. *Systematic Zoology* **43**: 58-77.

Paradis, E., 2006. Analysis of Phylogenetics and Evolution with R. Springer, New York, 211 pp.

Paradis, E., Claude, J., and Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289-290.

Provenzano, R. F., Machado-Allison, A., Chernoff, B., Willink, P. and Petry, P., 2005. *Harttia merevari*, a new species of catfish (Siluriformes: Loricariidae) from Venezuela. *Neotropical Ichthyology* **3**: 519-524.

R Development Core Team, 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rambaut, A, and Drummond, A.J., 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer

Rapp Py-Daniel, L.H., and Oliveira, E.C., 2001. Seven new species of *Harttia* from the Amazonian-Guyana region (Siluriformes: Loricariidae). *Ichthyological Exploration of Freshwaters* **12**: 79-96.

Reis, R. E., Kullander, S. O., and Ferraris, C.J. Jr. (Eds.), 2003. *Check list of the freshwater fishes of South and Central America*, Edipucrs Porto Alegre, Porto Alegre, Brazil, 742 pp.

Ribeiro, A.C., 2006. Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil: an example of faunal evolution associated with a divergent continental margin. *Neotropical Ichthyology* **4**: 225-246.

Rodriguez, M.S., Ortega, H., and Covain, R., (in press). Intergeneric phylogenetic relationships in Loricariinae catfishes (Siluriformes: Locariidae), with description of *Fonchiiloricaria nanodon*: a new genus and species from Peru. *Journal of Fish Biology*.

Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* **46**: 195–203.

Ronquist, F., 2001. DIVA version 1.2. Computer program for MacOS and Win32. Evolutionary Biology Centre, Uppsala University. Available at http://www.ebc.uu.se/systzoo/research/diva/diva.html.

Ronquist, F., and Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**:1572-1574.

Sanderson, M.J., 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution* **14(12)**:1218-1231.

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* **19**: 101–109.

Schaefer, S.A., and Stewart, D.J., 1993. Systematics of the *Panaque dentex* species group (Siluriformes: Loricariidae), wood-eating armored catfishes from tropical South America. *Ichthyological Exploration of Freshwaters* **4**: 309-342.

Schaefer, S.A., 1987. Osteology of *Hypostomus plecostomus* (Linnaeus) with a phylogenetic analysis of the loricariid subfamilies (Pisces: Siluroidei). *Contributions in Science, Natural History Museum of Los Angeles County* **394**: 1-31.

Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. *Systematic Biology* **51(3)**: 492–508.

Shimodaira, H., and Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**: 1114–1116.

Sugiura, N., 1978. Further analysis of the data by Akaike's information criterion and the finite corrections. *Communications in Statististics, Theory and Methods* **M A7**: 13-26.

Sullivan, J., Aranello, E., and Rogers, D.S., 2000. Comparative phylogeography of Mesoamerican highland rodents: concerted versus independent response to past climatic fluctuations. *American Naturalist* **155**: 755-768.

Swofford, D.L., 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.

Taberlet, P., Fumagalli, L., Wust-Saucy, A-G., and Cosson, J-F., 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* **7**: 453-464.

Tamura, K., and Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**: 512-526.

Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. In: *Some mathematical questions in biology-DNA sequence analysis* (Miura, R.M., ed), pp 57–86. Providence, RI: American Mathematics Society.

Torrico, J.P., Hubert, N., Desmarais, E., Duponchelle, F., Nuñez Rodriguez, J., Montoya-Burgos, J.I., Garcia Davila, C., Carvajal-Vallejos, F.M., Grajales, A.A., Bonhomme, F., and Renno, J-F., 2009. Molecular phylogeny of the genus *Pseudoplatystoma* (Bleeker,

1862): Biogeographic and evolutionary implications. *Molecular Phylogenetics and Evolution* **51**: 588-594.

Willis, S.C., Nunes, M., Montaña, C.G., Farias, I.P., and Lovejoy, N.R., 2007. Systematics, biogeography, and evolution on the neotropical peacock basses *Cichla* (Perciformes: Cichlidae). *Molecular Phylogenetics and Evolution* **44**: 291-307.

Willis, S.C., Nunes, M., Montaña, C.G., Farias, I.P., Ortí, G., and Lovejoy, N.R., 2010. The Casiquiare River acts as a corridor between the Amazonas and Orinoco river basins: biogeographic analysis of the genus *Cichla*. *Molecular Ecology* **19**: 1014-1030.

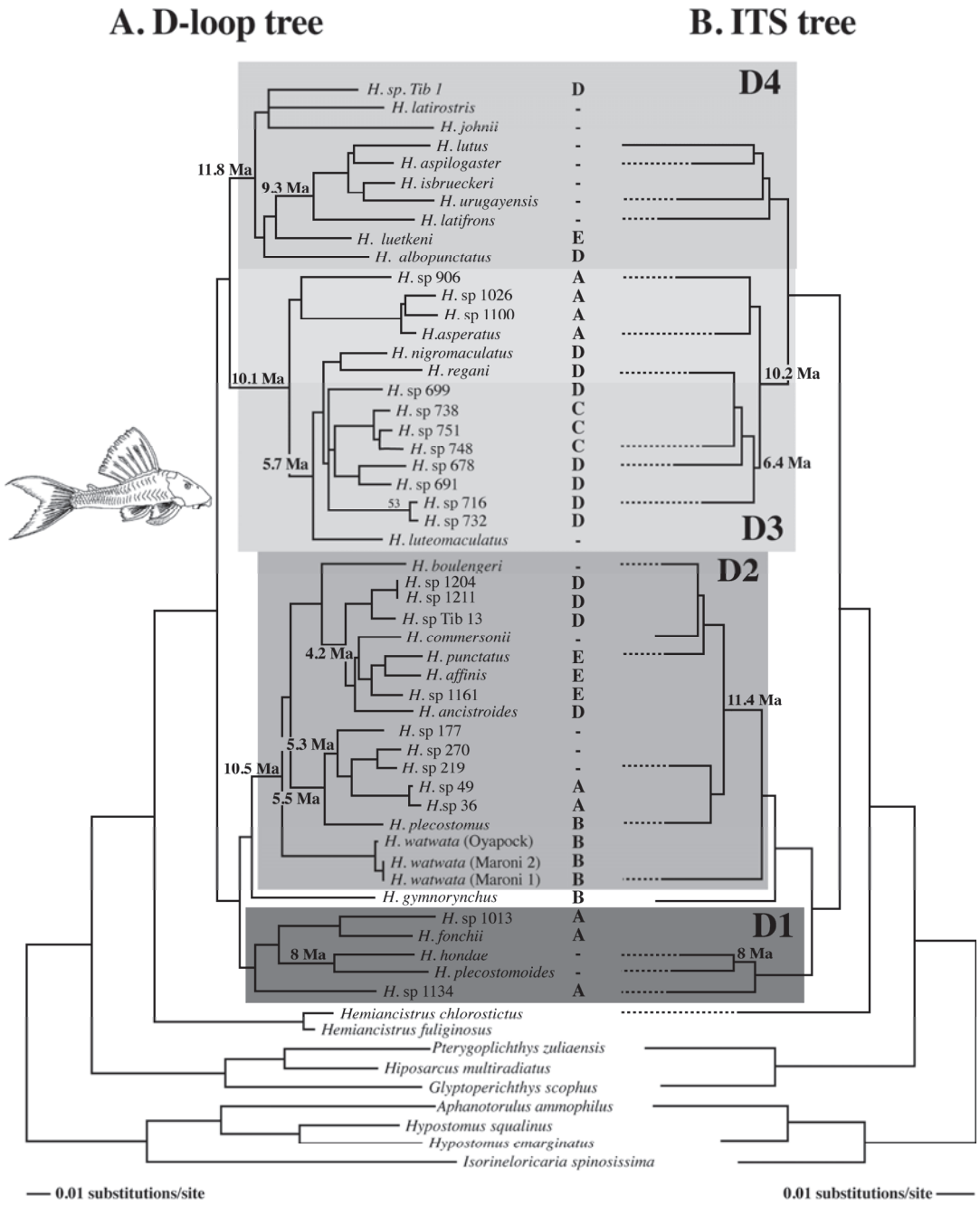
Xia, X., 2001. Data analysis in molecular biology and evolution. Kluwer Academic Publishers, Boston.

Xia, X., 2009. Information-theoretic indices and an approximate significance test for testing the molecular clock hypothesis with genetic distances. *Molecular Phylogenetics and Evolution* **52(3)**: 665-676

Xia, X., and Xie, Z., 2001. DAMBE: Software package for data analysis in molecular biology and evolution. *Journal of Heredity* **92**: 371-373.

Yu, Y, Harris, A.J., and He, X.J., 2010. S-DIVA (Statistical Dispersal-Vicariance Analysis): a tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution* **56(2)**: 848-850.

Supplementary material



S1. Maximum likelihood trees adapted from Mntoya-Burgos (2003) based on the D-loop (left) and ITS (right) sequences of *Hypostomus* species. Clades named as in Montoya-Burgos (2003). Numbers at nodes refer to age estimates. Letters refer to the biogeographic areas as provided in Fig. 6 for the species sharing a common distribution with Harttiini. Abbreviations used in the multivariate analyses are as followed: *H. albopunctatus* **[Hy 1]**, *H. luetkeni* **[Hy 2]**, *H*. sp. Tib 1 **[Hy 3]**, *H. asperatus* **[Hy 4]**, *H*. sp. 1026 **[Hy 5]**, *H*. sp. 1100 **[Hy 6]**, *H*. sp. 906 **[Hy 7]**, *H. nigromaculatus* **[Hy 8]**, *H. regani* **[Hy 9]**, *H*. sp. 678 **[Hy 10]**, *H*. sp. 691 **[Hy 11]**, *H*. sp. 738 **[Hy 12]**, *H*. sp. 748 **[Hy 13]**, *H*. sp. 751 **[Hy 14]**, *H*. sp. 699 **[Hy 15]**, *H*. sp. 716 **[Hy 16]**, *H*. sp. 732 **[Hy 17]**, *H. affinis* **[Hy 18]**, *H. punctatus* **[Hy 19]**, *H*. sp. 1161 **[Hy 20]**, *H. ancistroides* **[Hy 21]**, *H*. sp. 1204 **[Hy 22]**, *H.* sp. 1211 **[Hy 23]**, *H*. sp. Tib 13 **[Hy 24]**, *H. plecostomus* **[Hy 25]**, *H*. sp. 36 **[Hy 26]**, *H*. sp. 49 **[Hy 27]**, *H. watwata* Maroni 1 **[Hy 28]**, H. *watwata* Maroni 2 **[Hy 29]**, *H. watwata* Oyapock **[Hy 30]**, *H. gymnorhynchus* **[Hy 31]**, *H. fonchii* **[Hy 32]**, *H*. sp. 1013 **[Hy 33]**, *H*. sp. 1134 **[Hy 34]**.

Chapter 5

Assessing phylogenetic dependence of biological traits to investigate character evolution in Loricariinae catfishes, episode II. The orthograms strike back.

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An exhaustive phylogeny is reconstructed (350 OTUs), the orthograms are generalized and the MSPA is evaluated on an extended data set mixing quantitative (discrete and continuous), qualitative (binary, multistate, and ordinal), intraphenotypic (morphology, ethology) or extraphenotypic (environmental parameters) to detect co-evolution among multiple traits along the phylogeny, and thus revealing variables involved in the main evolutionary innovations of the Loricariinae. In addition evolutionary patterns for these innovations are revealed and a dating for the appearance of these structures is proposed.

To be submited.

Abstract

The non independence of biological traits among species due to the effect of the phylogeny is nowadays a concept widely admitted in comparative biology. Several methods have been proposed to detect phylogenetic autocorrelation in biological data, but until present, each method relied on the statistical nature of the data under study. Here we proposed a unifying tool to detect phylogenetic dependence in both quantitative and qualitative data, as well as in a multivariate dataset. This method extends existing methods (orthogram and multi scale pattern analysis, MSPA) and allows to describe the evolutionary patterns of multiple biological traits along a phylogeny beforehand described by a set of orthogonal vectors. We used this analysis in deciphering the evolution of biological traits in a highly specialized group of Neotropical catfishes: the Loricariinae. Prior to the analyses, an exhaustive molecular phylogeny of this group based on mitochondrial and nuclear genes was inferred, and the systematics of the subfamily was revised. The multivariate orthogram computed on the dataset containing intraphenotypic (morphological and ethological) and extraphenotipic (ecological) information decribed by quantitative (continuous and discrete), and qualitative (binary, multimodal, and ordinal) variables, revealed that the data were strongly phylogenetically autocorrelated and implied the deepest nodes in the explanation of the observed patterns. Several univariate orthograms mostly related to mouth characteristics also displayed such a similar pattern of phylogenetic dependence. The MSPA perfectly confirmed these results and revealed strong associations among all traits related to the mouth characteristics and the deepest nodes of the phylogeny, confirming thus the co-evolution of all these characters due to similar selective pressure. Unexpectedly, the co-evolution in mouth characteristics was not related to ecological habits, but was shaped by reproductive necessities responsible for a third evolutionary adaptation in Loricariinae. All these innovations appeared during the tertiary, a period characterized by the orogenesis of the Andes and progressive establishment of the modern Amazon and Orinoco.

Keywords: Siluriformes, Loricariidae, molecular phylogeny, multivariate analyses, Multi-Scale Pattern Analysis, co-evolution, evolutionary constraints, molecular dating.

1. Introduction

The phylogenetic dependence of biological traits due to their heritability from common ancestors (Harvey and Pagel, 1991) is nowadays a concept widely accepted in comparative biology. The evolution of traits (*e.g.* morphology, ecology, behavior) may be indeed plastic and stochastic or, to the contrary tightly linked to the evolutionary history of a group implying non independence among observations. This idea of non independence of traits had numerous implications in different field of evolutionary biology [*e.g.* univariate ancestral state reconstruction (Schluter *et al*., 1997; Pagel, 1999a; Huelsenbeck *et al*., 2003; Pagel *et al*., 2004); multivariate ancestral shape reconstruction (Wiley *et al*., 2005); molecular dating assuming autocorrelation of rates (Gillespie, 1986; Sanderson, 1997, 2002; Thorne and Kishino, 2002); or reconstruction of missing data (Bruggeman *et al*., 2009)]. The study of the evolution of traits along a phylogeny requires thus the prerequisite of testing for phylogenetic dependence. Several methods have been developed to detect or correct for phylogenetic dependence in comparative data (*e.g.* Felsenstein, 1985a; Cheverud *et al*., 1985; Gittleman and Kot, 1990; Harvey and Pagel, 1991; Lynch, 1991; Diniz-Filho *et al*., 1998; Pagel, 1999b; Abouheif, 1999; Paradis and Claude, 2002; Blomberg *et al*., 2003; Ollier *et al*., 2006; Felsenstein, 2008; Pavoine *et al.*, 2008; for reviews see Rholf, 2001; Blomberg *et al*. 2003; Freckleton, 2009). A popular method was introduced by Abouheif (1999) who modified two previously existing tests to detect phylogenetic autocorrelation for quantitative and qualitative data respectively: the Test For Serial Independence (TFSI) (von Neumann *et al*., 1941), and the RUNS test (Sokal and Rholf, 1995). Very intuitive, these tests only rely on the topological structure of the tree allowing the use of a wide range of tree sources (*e.g.* cladograms, phylograms, consensus trees, supertrees). Each character under study must be however individually tested according to its quantitative or qualitative nature. Therefore, this procedure becomes fastidious or even intractable for very large phylogenies, complex topologies, and when the number of traits under study is important. Pavoine *et al.* (2008) demonstrated that the TFSI test following Abouheif's procedure, designed to detect self similarities among adjacent observations in quantitative traits, was strictly equivalent to a Moran's I (Moran, 1950) test of spatial autocorrelation using a particular proximity matrix. However, the RUNS test, as modified by Abouheif, remains to date the only mean to deal with qualitative data. Alternatively, Ollier *et al.* (2006) developed a relevant approach to detect and characterize phylogenetic dependence, and at the same time highlight different patterns of evolution along a phylogenetic tree. However, this method also suffers from the impossibility to deal with qualitative data. Moreover, all methods developed until now are univariate, and only consider

one variable at a time against a phylogenetic tree. No method is presently able to test a multivariate table as a whole or to explore patterns of co-evolution among multiple traits. A first attempt to solve this trouble was proposed by Covain *et al.* (2008) who proposed the use of the co-inertia analysis (CIA) (Dolédec and Chessel, 1994) to extract the joint structure between a phylogeny (previously converted into a distance matrix) and a table of biological traits (quantitative and/or qualitative). In this case, CIA highlights the traits that possess the maximum covariation with the phylogeny as well as phylogenetic associations among traits. However, the method relies on the representation of a phylogenetic distance matrix (*e.g.* pairwise corrected or uncorrected genetic distances, patristic distances optimized or not using an evolutionary model) using principal coordinates (Gower, 1966) that are not always the best descriptors for a phylogeny (*e.g.* when the tree possesses strong imbalance). In another context, Pavoine *et al.* (2010) proposed the use of the quadratic entropy index (Rao, 1982) to measure the trait diversity among species, and decomposed this index along a phylogeny to characterize its phylogenetic pattern among communities. Even though traits may be numerous and of different statistical natures, the computation of this index required the conversion of the table of traits into a distances matrix, providing therefore a global estimation of trait diversity, and rendering comparisons between traits impossible in a multivariate frame.

To fill this gap, we extend the orthogram method developed by Ollier *et al*. (2006) to deal with categorical variables and also multivariate data including tables mixing qualitative and quantitative data, and provided therefore a new global test of phylogenetic autocorrelation. These new tools give thus a clear prominence to the phylogenetic dependence of a table at different levels (global or local) using the same statistical frame. This unifying structure, making each test directly comparable, subsequently allowed the development of a new multivariate method for the exploration of patterns of co-evolution among traits along a phylogeny. This new approach adapts the multi-scale pattern analysis (MSPA) technique developed for the analysis of spatial data (Jombart *et al*., 2009) into a phylogenetic context. The method corrects for the possible artifact introduced by the use of principal coordinates in CIA by using any orthonormal basis representing the phylogeny (Ollier *et al*., 2006). The MSPA also possesses all advantages of classical multivariate analysis: reliability, robustness, reduction of dimensionality and easiness of interpretation by the use of graphics, or possibility to reveal several structures at a time.

The new tools developed herein were experienced on a real multivariate data set comprising quantitative, qualitative, intra-phenotypic and extra-phenotypic variables to

explore the patterns of evolution of traits in a group of highly derived catfishes, the Loricariinae. This work has therefore started by the reconstruction of an exhaustive and robust molecular phylogeny of this group based on mitochondrial and nuclear genes. The Loricariinae represents a highly diversified subfamily among the large Neotropical catfish family Loricariidae, or armored catfish. Loricariids have undergone an evolutionary radiation at a subcontinental scale, from Costa Rica to Argentina, which has been compared to that of the Cichlidae of the Great Lakes of the Rift Valley in Africa (Schaefer and Stewart, 1993). The species flock Loricariidae represents indeed the most speciose family of the Siluriformes in the world with 716 valid species and an estimated 300 undescribed species distributed in 96 genera (Reis *et al*., 2003; Ferraris, 2007). Extremely variable colour patterns and body shapes among loricariid taxa reflect their high degree of ecological specialization, and because of their highly specialized morphology loricariids have been recognized as a monophyletic assemblage in the earliest classifications of the Siluriformes (de Pinna 1998). The Loricariidae are characterized by a depressed body covered by bony plates, a single pair of maxillary barbels, and above all, by the modification of the mouth into a sucker disk. Within the Loricariidae, members of the subfamily Loricariinae are united by a long and depressed caudal peduncle and by the absence of an adipose fin. They live stuck to the substrate and show accordingly marked variations in body shape due to the various habitats colonized, from lotic to lentic systems, on inorganic or organic substrates (*e.g.* members of *Farlowella* resemble a thin stick of wood and blend remarkably among submerged wood and leafs; alternatively members of *Pseudohemiodon* are large and flattened and bury themselves in sandy substrates). Some groups have numerous teeth, pedunculated, and organized in a comblike manner, while other groups have few teeth or even no teeth on the premaxillae. These latter are often strongly differentiated, and can be bicuspid straight and thick, spoon-shaped, reduced in size or very long. An important diversity in lip's characteristics, which can be strongly papillose, filamentous or smooth, also characterizes this subfamily (Isbrücker, 1979; Covain and Fisch-Muller, 2007). Reproductive strategies are also diverse in Loricariinae. Members of Harttiini and Farlowellina are indeed known to be open brooders (*i.e*. eggs are laid on an exposed surface and guarded by the male), and Loricariina members display numerous alternative strategies: members of the *Pseudohemiodon-Loricaria* groups are abdomino-lip brooders (*i.e*. eggs are laid in a single layered mass, and are maintained to the surface of the lower lip and abdomen of the male); members of the *Loricariichthys* group are lip brooders (*i.e.* eggs are laid in a mass and held by the male in the fold made by its enlarged lips); and others such as *Rineloricaria* representatives are cavity brooders (*i.e.* eggs

are laid attached to one another in single layer masses on the cavity floor, and are brooded by the male) (Covain and Fisch-Muller, 2007). Evers and Siedel (2005) also reported the use of a vegetal support such as a dead leaf by members of *Limatulichthys*. In this case, the eggs are laid in a mass and attached to the surface of the support. The eggs and support are then held by the male in the fold made by its lips. Sexual dimorphism displays accordingly substantial variations related to breeding strategies. If most of the time in species that lay eggs on surfaces, sexual dimorphism is expressed through the hypertrophy of odontodes along the sides of head, on pectoral spines (and sometimes fins), or on the predorsal area (or even on the entire body) of males, in lip and abdomino-lip brooder species the sexual dimorphism is often expressed through differences in lip surface (smoother in males), tooth shape (tooth cusps rounded in males), or lip enlargement. There are currently 230 valid species of Loricariinae, distributed in 32 genera (for a review see Covain and Fisch-Muller, 2007; also Ghazzi, 2008; Ingenito *et al*., 2008; Fichberg and Chamon, 2008; Rapp Py-Daniel and Fichberg, 2008; Rodriguez and Miquelarena, 2008; Rodriguez and Reis 2008; Rodriguez *et al*., 2008; Thomas and Rapp Py-Daniel, 2008; de Carvalho Paixão and Toledo-Piza, 2009; Thomas and Sabaj Pérez, 2010; Rodriguez *et al*., in press; Covain *et al*., in press).

The evolutionary history of Loricariinae has been only recently explored by Covain *et al*. (2008), who proposed the first molecular phylogeny of the subfamily and assessed the phylogenetic dependence of the morphological traits classically used as diagnostic features. Although their analysis included only 20 representatives of the Loricariinae, they redefined its systematics with the restriction of the tribe Harttiini to *Harttia*, and the placement of all remaining genera of the study within the tribe Loricariini. Within the latter, they redefined the subtribes Loricariina and Farlowellina, and confirmed the natural groupings of members of the *Loricariichthys* and *Loricaria-pseudohemiodon* groups within Loricariina, but rejected the monophyly of the *Rineloricaria* group (*sensu* Covain and Fisch-Muller, 2007). Covain *et al*. (2008) furthermore demonstrated that the characteristics of the mouth (including tooth) and of the caudal fin were strongly positively autocorrelated with the phylogeny and that they were sufficient to define naturally tribal and subtribal ranks, as well as several of the morphological groups proposed in Covain and Fisch-Muller (2007). Moreover, a recent study conducted on the tribe Harttiini within the Guianas, revealed phylogenetic autocorrelation in morphometric, ecological, and distributional data suggesting that the evolution of shape was linked to adaptations to a particular type of habitats and potentially to dispersion abilities (Covain *et al*., in press). The data set proposed by Covain and Fisch-Muller (2007) was extended to ecomorphological, ecological and ethological data to evaluate the new tests developed here.

Then the co-evolutionary patterns among multivariate traits were explored using the MSPA to decipher the main evolutionary trends in Loricariinae. A dating of these main innovations was proposed to evaluate if major paleogeological events that shaped South-America through the Miocene and Pleistocene could explain the appearance and diversification of such traits or behaviour, especially the large diversity of reproductive strategies, unique among Loricariidae.

2. Material and methods

2.1 Taxonomic sampling.

The molecular phylogeny was reconstructed using the taxonomical sampling given in Covain *et al.* (2008) with the addition of 330 species of the Loricariinae and 18 outgroup species. The outgroup was chosen in other subfamilies of the Loricariidae. The list of material used for this study is provided in Table 1. The analyzed samples came from the tissue collection of the Muséum d'histoire naturelle de la Ville de Genève (MHNG); Academy of Natural Sciences, Philadelphia (ANSP); Smithsonian Tropical Research Institute (STRI), Panama; Laboratório de Biologia de Peixes, Departamento de Morfologia, Universidade Estadual Paulista, Campus de Botucatu (LBP); Auburn University, Montgomery (AUM); and Museu de Ciências e Tecnologia of the Pontifícia Universidade Católica do Rio Grande do Sul (MCP), Porto Alegre. The sequences were deposited in GenBank.

2.2 DNA extraction, amplification and sequencing.

Tissue samples were preserved in 80% ethanol and stored at -20°C. Total genomic DNA was extracted with the DNeasy Tissue Kit (Qiagen) following the instructions of the manufacturer. The PCR amplifications of mitochondrial 12S and 16S, and the nuclear Fish Reticulon-4 (F-RTN4) genes were carried out using the Taq PCR Core Kit (Qiagen). The methodology for PCR amplifications followed Chiachio *et al.* (2008) for F-RTN4. To amplify the almost complete 12S, $tRNA_{val}$ and 16S mitochondrial genes in a single 2,500 bp long fragment, a Nested PCR protocol was used. The external round of PCR was performed using the pair of primers Phe-L941: 5'- AAA TCA AAG CAT AAC ACT GAA GAT G 3', and H3059 (Alves-Gomes *et al*., 1995). The external amplifications were performed in a total volume of 50 µl, containing 5 µl of 10x reaction buffer, 1 µl of dNTP mix at 10mM each, 1 µl of each primer at 10 µM, 0.2 µl of *Taq* DNA Polymerase equivalent to 1 unit of Polymerase per tube, and 1 to 4 µl of DNA. Cycles of amplification were programmed with the following

Table 1. Taxa list, specimen and sequence data for the 350 Loricariinae and 18 outgroup representatives analyzed in this study. The acronyms of institutions follow Fricke and Eschmeyer (2010).

Species	Catalog Number	Field Number	Locality	mt 12S+16S bases		F-RTN4 bases	
				+ GenBank No.	Ref.	+ GenBank No.	Ref.
Harttia guianensis	MHNG 2643.016	GF00–351	French Guiana, Marouini River	2435 EU310447	Covain *et al.* 2008	2112 FJ013232	Chiachio *et al.* 2008
Loricaria parnahybae	MHNG 2602.067	BR98–274	Brazil, Rio Parnahyba	2421 EU310452	Covain *et al.* 2008	1985 FJ013231	Chiachio *et al.* 2008
Crossoloricaria venezuelae	INHS 35467	VZ 049	Venezuela, Rio Santa Rosa	2416 EU310444	Covain *et al.* 2008	1994 HM623647	Rodriguez *et al.* In press
Dasyloricaria tuyrensis	MHNG 2674.052	PA00–012	Panama, Rio Ipeti	2416 EU310445	Covain *et al.* 2008	2005 HM623639	Rodriguez *et al.* In press
Farlowella aff. *oxyrryncha**	MHNG 2588.064	PE96–022	Peru, Rio Tambopata	2430 EU310443	Covain *et al.* 2008	2237 HM623650	Rodriguez *et al.* In press
Farlowella platorynchus	MHNG 2588.093	PE96–071	Peru, Rio Ucayali	2429 EU310446	Covain *et al.* 2008	2301 HM623649	Rodriguez *et al.* In press
Hemiodontichthys acipenserinus	MHNG 2651.012	GY04–15	Guyana, Rupununi River	2419 EU310448	Covain *et al.* 2008	2246 HM623645	Rodriguez *et al.* In press
Lamontichthys stibaros	MHNG 2677.039	MUS 208	Peru, aquarium trade, Rio Itaya[2]	2430 EU310449	Covain *et al.* 2008	2038 HM623648	Rodriguez *et al.* In press
*Limatulichthys punctatus**	MHNG 2651.013	GY04–18	Guyana, Rupununi River	2423 EU310450	Covain *et al.* 2008	1959 HM623644	Rodriguez *et al.* In press
Loricaria clavipinna	MHNG 2640.044	PE98–002	Peru, Rio Putumayo	2424 EU310451	Covain *et al.* 2008	1964 HM623653	Rodriguez *et al.* In press
Loricariichthys maculatus	MHNG 2621.042	SU01–56	Surinam, Sarramacca River	2425 EU310453	Covain *et al.* 2008	2221 HM623642	Rodriguez *et al.* In press
Loricariichthys microdon	MHNG 2650.054	GY04–12	Guyana, Rupununi River	2424 EU310454	Covain *et al.* 2008	1949 HM623643	Rodriguez *et al.* In press
Metaloricaria paucidens	MHNG 2677.086	GF00–083	French Guiana, Marouini River	2435 EU310455	Covain *et al.* 2008	2073 HM623637	Rodriguez *et al.* In press
Planiloricaria cryptodon	MHNG 2677.038	MUS 211	Peru, aquarium trade, Rio Itaya[2]	2415 EU310456	Covain *et al.* 2008	2006 HM623646	Rodriguez *et al.* In press
Rineloricaria lanceolata	MHNG 2588.059	PE96–011	Peru, Rio Tambopata	2420 EU310457	Covain *et al.* 2008	2226 HM623640	Rodriguez *et al.* In press
*Rineloricaria osvaldoi**	UFRJ 6–EF4	BR 1114	Brazil, Rio Maranhão	2424 EU310459	Covain *et al.* 2008	2023 HM623652	Rodriguez *et al.* In press
Rineloricaria platyura	MHNG 2651.009	GY04–83	Guyana, Rupununi River	2420 EU310458	Covain *et al.* 2008	2219 HM623641	Rodriguez *et al.* In press
Sturisoma monopelte	MHNG 2651.033	GY04–187	Guyana, Sawarab River	2436 EU310461	Covain *et al.* 2008	1980 HM623651	Rodriguez *et al.* In press
*Sturisoma robustum**	MHNG 2588.055	PE96–001	Peru, Rio de las Piedras	2437 EU310460	Covain *et al.* 2008	2556 HM623636	Rodriguez *et al.* In press
Sturisomatichthys citurensis	MHNG 2676.004	PA97–032	Panama, Rio Tuyra	2435 EU310462	Covain *et al.* 2008	2268 HM623635	Rodriguez *et al.* In press
Fonchiiloricaria nanodon	MHNG 2710.048	PE08-199	Peru, Rio Monzon	2429 HM592626	Rodriguez *et al.* In press	2015 HM623656	Rodriguez *et al.* In press
Fonchiiloricaria nanodon	MHNG 2710.060	PE08-336	Peru, Rio Aucayacu	2429 HM592627	Rodriguez *et al.* In press	2015 HM623657	Rodriguez *et al.* In press
Spatuloricaria aff. *caquetae**	MHNG 2710.050	PE08-230	Peru, Rio Huallaga	2418 HM592624	Rodriguez *et al.* In press	1981 HM623654	Rodriguez *et al.* In press
Spatuloricaria sp. Nanay	MHNG 2677.071	PE05-014	Peru, aquarium trade, Rio Nanay[2]	2419 HM592625	Rodriguez *et al.* In press	1979 HM623655	Rodriguez *et al.* In press
Cteniloricaria napova	MHNG 2704.030	SU07-650	Brazil, Paru de Oeste River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Cteniloricaria platystoma	MHNG 2672.067	SU05-340	Suriname, Corantijn River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Cteniloricaria platystoma	MHNG 2674.003	SU05-039	Suriname, Suriname River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Cteniloricaria platystoma	MHNG 2650.082	GY04-336	Guyana, Essequibo River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Cteniloricaria platystoma	MHNG 2700.054	GF07-265	French Guiana, Mana River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Cteniloricaria platystoma	MHNG 2643.015	GF00-352	French Guiana, Marouini River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.

Harttia aff. *punctata**	LBP 5839	LBP 28353	Brazil, Rio Tocantins	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia carvalhoi	MHNG 2587.027	BR 1236	Brazil, Rio Paraíba do Sul	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia carvalhoi	LBP 2115	LBP 21352	Brazil, Rio Paraíba do Sul	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia dissidens	LBP 5859	LBP 28331	Brazil, Rio Tapajós	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia dissidens	LBP 5863	LBP 28339	Brazil, Rio Tapajós	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia duriventris	LBP 7505	LBP 34804	Brazil, Rio Tapajós	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia fluminensis	MHNG 2690.013	SU01-445	Suriname, Coppename River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia fowleri	MHNG 2643.022	GF99-202	French Guiana, Oyapock River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia gracilis	LBP 6331	LBP 29819	Brazil, Rio Paraná	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia guianensis	MHNG 2662.091	GF03-160	French Guiana, Approuague River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia guianensis	MHNG 2680.053	RV-21	French Guiana, Sinnamary River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia kronei	MHNG 2586.058	BR 1166	Brazil, Rio Ribeira de Iguape	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia kronei	LBP 2661	LBP 17427	Brazil, Rio Ribeira de Iguape	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia kronei	LBP 2883	LBP 18609	Brazil, Rio Ribeira de Iguape	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia kronei	LBP 1269	LBP 11215	Brazil, Rio Ribeira de Iguape	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia leiopleura	LBP 6847	LBP 31528	Brazil, Rio São Francisco	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia leiopleura	LBP 6492	LBP 31545	Brazil, Rio São Francisco	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia longipinna	DZSJRP 2819	BR98-747	Brazil, Rio São Francisco	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia loricariformis	LBP 2121	LBP 21362	Brazil, Rio Paraíba do Sul	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia novalimensis	LBP 5836	LBP 28348	Brazil, Rio São Francisco	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia punctata	MHNG 2645.059	BR 995	Brazil, Rio Tocantins	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia punctata	MHNG 2645.053	BR 1051	Brazil, Rio Tocantins	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia sp. 1 Xingu	LBP 5845	LBP 28327	Brazil, Rio Xingu	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia sp. 2 Xingu	LBP 5860	LBP 28333	Brazil, Rio Xingu	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia sp. 3 Xingu	LBP 5861	LBP 28335	Brazil, Rio Xingu	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia sp. Rio São Francisco	LBP 5838	LBP 28352	Brazil, Rio São Francisco	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia sp. Serra do Cipó	LBP 6528	LBP 31652	Brazil, Rio São Francisco	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia sp. Tapajos	LBP 5857	LBP 28329	Brazil, Rio Tapajós	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia sp. Tocantins	LBP 5850	LBP 28367	Brazil, Rio Tocantins	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia sp. Três Marias	LBP 5838	LBP 28351	Brazil, Rio São Francisco	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia surinamensis	MHNG 2674.042	SU05-001	Suriname, Suriname River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia torrenticola	LBP 5835	LBP 28346	Brazil, Rio São Francisco	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttia tuna	MHNG 2704.029	SU07-644	Brazil, Paru de Oeste River	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella crassicauda	MHNG 2679.098	MUS 306	Suriname, Nassau Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella crassicauda	MHNG 2674.051	MUS 221	Suriname, Nassau Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella crassicauda	MHNG 2674.051	MUS 231	Suriname, Nassau Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.

Harttiella longicauda	MHNG 2723.094	MUS 470	French Guiana, Balenfois Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella longicauda	MHNG 2723.094	MUS 463	French Guiana, Balenfois Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella longicauda	MHNG 2723.094	MUS 456	French Guiana, Balenfois Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella longicauda	MHNG 2699.070	GF07-026	French Guiana, Trinité Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella longicauda	MHNG 2699.070	GF07-082	French Guiana, Trinité Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella longicauda	MHNG 2699.070	GF07-111	French Guiana, Trinité Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella pilosa	MHNG 2682.055	GF06-344	French Guiana, Tortue Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella pilosa	MHNG 2682.055	GF06-343	French Guiana, Tortue Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Harttiella pilosa	MHNG 2724.002	GF03-033	French Guiana, Tortue Mountains	GBxxxxx	Covain *et al.* in prep.	GBxxxxx	Covain *et al.* in prep.
Apistoloricaria ommation	ANSP 182331	P6265	Peru, Rio Amazonas	GBxxxxx	This study	GBxxxxx	This study
Apistoloricaria ommation	MHNG	MUS 437	Peru, aquarium trade, Rio Amazonas[2]	GBxxxxx	This study	GBxxxxx	This study
Aposturisoma myriodon	MHNG	PE08-004	Peru, Rio Huacamayo	GBxxxxx	This study	GBxxxxx	This study
Aposturisoma myriodon	MHNG	PE08-131	Peru, Rio Huyhuantal	GBxxxxx	This study	GBxxxxx	This study
Brochiloricaria macrodon	LBP	LBP 24033	Brazil, Rio Paraguay	GBxxxxx	This study	GBxxxxx	This study
Brochiloricaria sp. Uruguay	MCP 28414	MCP 28414	Brazil, Rio Ibicui-Mirim	GBxxxxx	This study	GBxxxxx	This study
Crossoloricaria aff. bahuaja	MHNG	PE08-714	Peru, Rio Cushabatai	GBxxxxx	This study	GBxxxxx	This study
Crossoloricaria bahuaja	ANSP 180793	P4078	Peru, Rio Madre de Dios	GBxxxxx	This study	GBxxxxx	This study
Crossoloricaria cephalaspis	Stri	Stri-1449	Colombia, Rio San Juan	GBxxxxx	This study	GBxxxxx	This study
Crossoloricaria cephalaspis	Stri	Stri-1577	Colombia, Rio Atrato	GBxxxxx	This study	GBxxxxx	This study
Crossoloricaria rhami	MHNG	PE08-120	Peru, Rio Aguaytia	GBxxxxx	This study	GBxxxxx	This study
Crossoloricaria variegata	Stri	Stri-6781	Panama, Rio Tuira	GBxxxxx	This study	GBxxxxx	This study
Dasyloricaria latiura	Stri	Stri-1559	Panama, Rio Atrato	GBxxxxx	This study	GBxxxxx	This study
Dasyloricaria tuyrensis	Stri	Stri-4140	Panama, Rio Tuira	GBxxxxx	This study	GBxxxxx	This study
F. schreitmuelleri	MHNG 2601.087	BR98-106	Brazil, Rio Guamá	GBxxxxx	This study	GBxxxxx	This study
Farlowella acus	Stri	MER95T-22	Venezuela, Valencia Lake	GBxxxxx	This study	GBxxxxx	This study
Farlowella aff. *rugosa*	ANSP 179 768	T2200	Guyana, Simoni River	GBxxxxx	This study	GBxxxxx	This study
Farlowella amazona	MHNG 2601.065	BR98-052	Brazil, Rio Acara	GBxxxxx	This study	GBxxxxx	This study
Farlowella curtirostra	Stri	MER95T-13	Venezuela, Rio Motatan	GBxxxxx	This study	GBxxxxx	This study
Farlowella hahni	MHNG	PR-29	Argentina, Santa Fé	GBxxxxx	This study	GBxxxxx	This study
Farlowella knerii	MHNG	PE08-259	Peru, Rio Aspuzana	GBxxxxx	This study	GBxxxxx	This study
Farlowella mariaelenae	Stri	VZ-59	Venezuela, Rio Caipe	GBxxxxx	This study	GBxxxxx	This study
Farlowella martini	Stri	VZ-126	Venezuela, Rio Aroa	GBxxxxx	This study	GBxxxxx	This study
Farlowella nattereri	MHNG 2650.099	GY04-291	Guyana, Kurupukari cross	GBxxxxx	This study	GBxxxxx	This study
Farlowella nattereri	MHNG 2654.067	GY04-306	Guyana, Kurupukari cross	GBxxxxx	This study	GBxxxxx	This study
Farlowella oxyrryncha	MHNG	PE08-051	Peru, Rio Huacamayo	GBxxxxx	This study	GBxxxxx	This study

Farlowella oxyrryncha	MHNG 2613.035	CA 21	Peru, Rio Ucayali	GBxxxxx	This study	GBxxxxx	This study
Farlowella oxyrryncha	MHNG 2601.095	BR98-118	Brazil, Rio Guamá	GBxxxxx	This study	GBxxxxx	This study
Farlowella oxyrryncha	LBP 16200	LBP 16200	Brazil, Rio Araguiaia	GBxxxxx	This study	GBxxxxx	This study
Farlowella oxyrryncha	MHNG	PE08-698	Peru, Rio Neshua	GBxxxxx	This study	GBxxxxx	This study
Farlowella oxyrryncha	MHNG	PE08-823	Peru, Rio Cushabatai	GBxxxxx	This study	GBxxxxx	This study
Farlowella oxyrryncha	LBP	LBP 22907	Brazil, Rio Jurua	GBxxxxx	This study	GBxxxxx	This study
Farlowella paraguayensis	Stri	Stri-2205	Paraguay, Arroyo Curuguati	GBxxxxx	This study	GBxxxxx	This study
Farlowella paraguayensis	LBP 26396	LBP 26396	Brazil, Rio Paraná	GBxxxxx	This study	GBxxxxx	This study
Farlowella platorynchus	MHNG 2650.096	GY04-290	Guyana, Kurupukari cross	GBxxxxx	This study	GBxxxxx	This study
Farlowella platorynchus	MHNG 2602.021	BR98-163	Brazil, Rio Peritoro	GBxxxxx	This study	GBxxxxx	This study
Farlowella platorynchus	MHNG	PE08-906	Peru, Rio Ucayali	GBxxxxx	This study	GBxxxxx	This study
Farlowella reticulata	MHNG 2683.081	GF06-637	French Guiana, Maroni River	GBxxxxx	This study	GBxxxxx	This study
Farlowella reticulata	MHNG 2683.070	GF06-588	French Guiana, Mana River	GBxxxxx	This study	GBxxxxx	This study
Farlowella reticulata	MHNG 2681.060	GF06-118	French Guiana, Oyapock River	GBxxxxx	This study	GBxxxxx	This study
Farlowella smithi	ANSP 180541	P4099	Peru, Rio Manuripe	GBxxxxx	This study	GBxxxxx	This study
Farlowella taphorni	Stri	VZ-89	Venezuela, Rio Mayupa	GBxxxxx	This study	GBxxxxx	This study
Farlowella vittata	Stri	VZ-63	Venezuela, Rio Caipe	GBxxxxx	This study	GBxxxxx	This study
Harttiella intermedia	MHNG 2713.087	MUS 650	French Guiana, Trinité Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella intermedia	MHNG 2713.087	MUS 651	French Guiana, Trinité Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella intermedia	MHNG 2713.087	MUS 652	French Guiana, Trinité Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella lucifer	MHNG 2721.088	GF10-034	French Guiana, Lucifer Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella lucifer	MHNG 2721.088	GF10-043	French Guiana, Lucifer Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella lucifer	MHNG 2721.088	GF10-037	French Guiana, Lucifer Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella lucifer	MHNG 2721.091	GF10-051	French Guiana, Lucifer Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella lucifer	MHNG 2721.091	GF10-053	French Guiana, Lucifer Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella lucifer	MHNG 2721.091	GF10-055	French Guiana, Lucifer Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella lucifer	MHNG 2712.085	MUS 592	French Guiana, Crique Limonade	GBxxxxx	This study	GBxxxxx	This study
Harttiella lucifer	MHNG 2712.085	MUS 593	French Guiana, Crique Limonade	GBxxxxx	This study	GBxxxxx	This study
Harttiella lucifer	MHNG 2712.085	MUS 594	French Guiana, Crique Limonade	GBxxxxx	This study	GBxxxxx	This study
Harttiella parva	MHNG 2723.093	MUS 606	French Guiana, Atachi Bakka Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella parva	MHNG 2723.093	MUS 607	French Guiana, Atachi Bakka Mountains	GBxxxxx	This study	GBxxxxx	This study
Harttiella parva	MHNG 2723.093	MUS 611	French Guiana, Atachi Bakka Mountains	GBxxxxx	This study	GBxxxxx	This study
Hemiodontichthys acipenserinus	MHNG 2588.057	PE96-005	Peru, Madre de Dios	GBxxxxx	This study	GBxxxxx	This study
Hemiodontichthys acipenserinus	MHNG 2602.007	BR98-138	Brazil, Rio Guamá	GBxxxxx	This study	GBxxxxx	This study

Hemiodontichthys acipenserinus	MCP 28819	MCP 28819	Brazil, Rio Purus	GBxxxxx	This study	GBxxxxx	This study
Hemiodontichthys acipenserinus	LBP	LBP 26640	Brazil, Rio Jari	GBxxxxx	This study	GBxxxxx	This study
Ixinandria steinbachi	-	IXS2	Argentina, Salta	GBxxxxx	This study	GBxxxxx	This study
Lamontichthys filamentosus	MHNG	MUS	Peru, aquarium trade	GBxxxxx	This study	GBxxxxx	This study
Lamontichthys filamentosus	LBP	LBP 4038	Brazil, Rio Branco	GBxxxxx	This study	GBxxxxx	This study
Lamontichthys llanero	MHNG	MUS 356	Colombia, aquarium trade	GBxxxxx	This study	GBxxxxx	This study
Lamontichthys stibaros	MHNG	PE08-224	Peru, Rio Huallaga	GBxxxxx	This study	GBxxxxx	This study
Limatulichthys punctatus	ANSP 182707	P6232	Peru, Rio Itaya	GBxxxxx	This study	GBxxxxx	This study
Limatulichthys punctatus	MHNG	BR98-140	Brazil, Rio Guamá	GBxxxxx	This study	GBxxxxx	This study
Limatulichthys punctatus	AUM 42223	V5319	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Limatulichthys punctatus	LBP	LBP 23618	Brazil, Rio Jurua	GBxxxxx	This study	GBxxxxx	This study
Limatulichthys punctatus	MHNG	PE08-112	Peru, Rio Agaytia	GBxxxxx	This study	GBxxxxx	This study
Limatulichthys punctatus	LBP 26769	LBP 26769	Brazil, Rio Jari	GBxxxxx	This study	GBxxxxx	This study
Limatulichthys punctatus	LBP 26472	LBP 26472	Brazil, Rio Jari	GBxxxxx	This study	GBxxxxx	This study
Lorcaria sp. Guyana	MHNG	GY04-110	Guyana, Pirara River	GBxxxxx	This study	GBxxxxx	This study
Lorcaria sp. Guyana	MHNG	GY04-191	Guyana, Sawarab bridge	GBxxxxx	This study	GBxxxxx	This study
Loricaria aff. *nickeriensis*	MHNG	GF06-044	French Guiana, Oyapock River	GBxxxxx	This study	GBxxxxx	This study
Loricaria aff. *panahybae*	LBP 11690	LBP 11690	Brazil, Rio Araguaia	GBxxxxx	This study	GBxxxxx	This study
Loricaria apeltogaster	-	-	Argentina, Entre Rios	GBxxxxx	This study	GBxxxxx	This study
Loricaria cataphracta	MHNG	GF98-044	French Guiana, Kourou River	GBxxxxx	This study	GBxxxxx	This study
Loricaria cataphracta	MHNG	GF06-570	French Guiana, Mana River	GBxxxxx	This study	GBxxxxx	This study
Loricaria cataphracta	MHNG	SU08-042	Suriname, Suriname River	GBxxxxx	This study	GBxxxxx	This study
Loricaria cataphracta	MHNG	SU08-943	Suriname, Commewijne River	GBxxxxx	This study	GBxxxxx	This study
Loricaria cf. *lata*	LBP 16148	LBP 16148	Brazil, Rio Araguaia	GBxxxxx	This study	GBxxxxx	This study
Loricaria nickeriensis	MHNG	SU05-334	Suriname, Corantijn River	GBxxxxx	This study	GBxxxxx	This study
Loricaria prolixa	LBP 34925	LBP 34925	Brazil, Rio Paraná	GBxxxxx	This study	GBxxxxx	This study
Loricaria prolixa	LBP 34924	LBP 34924	Brazil, Rio Paraná	GBxxxxx	This study	GBxxxxx	This study
Loricaria simillima	MHNG	PE05-030	Peru, aquarium trade, Rio Amazonas[2]	GBxxxxx	This study	GBxxxxx	This study
Loricaria sp. Araguaia	LBP	LBP 11506	Brazil, Rio Araguaia	GBxxxxx	This study	GBxxxxx	This study
Loricaria sp. Branco	LBP 4032	LBP 4032	Brazil, Rio Branco	GBxxxxx	This study	GBxxxxx	This study
Loricaria sp. Branco	LBP	LBP 4101	Brazil, Rio Branco	GBxxxxx	This study	GBxxxxx	This study
Loricaria sp. Mato Grosso	MCP 36566	MCP 36566	Paraguay, Mato Grosso	GBxxxxx	This study	GBxxxxx	This study
Loricaria sp. Orinoco	AUM 42224	V5315	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Loricaria sp. Paraguay	MHNG	PY9093	Paraguay, Rio Paraguay	GBxxxxx	This study	GBxxxxx	This study
Loricaria sp. Rupununi	MHNG	GY04-129	Guyana, Rupununi River	GBxxxxx	This study	GBxxxxx	This study

Loricaria tucumanensis	-	AG06-018	Argentina, Ita-Ibate	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys anus	MCP 28415	MCP 28415	Brazil, Rio Grande do Sul	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys anus	MCP 28317	MCP 28317	Brazil, Rio Grande do Sul	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys anus	LBP 7309	LBP 7309	Brazil, Rio Guiaba	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys castaneus	MHNG	BR 162	Brazil, surroundings of Rio de Janeiro	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys castaneus	LBP 35548	LBP 35548	Brazil	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys castaneus	LBP 35549	LBP 35549	Brazil	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys cf. *ucayalensis*	ANSP 182668	P6046	Peru, Rio Nanay	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys derbyi	MHNG	BR98-250	Brazil, Rio Parnahyba	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys derbyi	LBP 27214	LBP 27214	Brazil, Rio Parnahyba	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys labialis	MHNG	PY9094	Paraguay, Rio Paraguay	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys melanocheilus	MCP 28915	MCP 28915	Brazil, Rio Ibicui-Mirim	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys platymetopon	MHNG	PY9098	Paraguay, Rio Paraguay	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys sp. Amazonas	Stri	Stri-531	Peru, Rio Amazonas	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys sp. Jari	LBP	LBP 26622	Brazil, Rio Jari	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys sp. Jari	LBP 27135	LBP 27135	Brazil, Rio Jari	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys sp. Orinoco	AUM 42225	V5310	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Loricariichthys sp. Rio Baia	LBP	LBP 19263	Brazil, Rio Baia	GBxxxxx	This study	GBxxxxx	This study
Metaloricaria nijsseni	MHNG	SU05-012	Suriname, Suriname River	GBxxxxx	This study	GBxxxxx	This study
Metaloricaria nijsseni	MHNG	SU05-359	Suriname, Corantijn River	GBxxxxx	This study	GBxxxxx	This study
Metaloricaria nijsseni	MHNG	SU01-459	Suriname, Coppename River	GBxxxxx	This study	GBxxxxx	This study
Metaloricaria paucidens	MHNG 2681.042	GF06-108	French Guiana, Oyapock River	GBxxxxx	This study	GBxxxxx	This study
Paraloricaria agastor	-	AG06-017	Argentina, Ita-Ibate	GBxxxxx	This study	GBxxxxx	This study
Paraloricaria agastor	-	AG06-019	Argentina, Puerto Abra	GBxxxxx	This study	GBxxxxx	This study
Paraloricaria vetula	-	YC-008	Argentina, Entre Rios	GBxxxxx	This study	GBxxxxx	This study
Pseudohemiodon aff. *apithanos*	MHNG	PE05-009	Peru, aquarium trade, Rio Amazonas[2]	GBxxxxx	This study	GBxxxxx	This study
Pseudohemiodon aff. *apithanos*	ANSP 178115	P1759	Peru, aquarium trade, Rio Itaya[2]	GBxxxxx	This study	GBxxxxx	This study
Pseudohemiodon aff. *apithanos*	MHNG	PE08-852	Peru, Rio Cushabatai	GBxxxxx	This study	GBxxxxx	This study
Pseudohemiodon apithanos	MHNG	PE05-020	Peru, aquarium trade, Rio Itaya[2]	GBxxxxx	This study	GBxxxxx	This study
Pseudohemiodon apithanos	MHNG	PE05-026	Peru, aquarium trade, Rio Nanay[2]	GBxxxxx	This study	GBxxxxx	This study
Pseudohemiodon laminus	MHNG	PE05-035	Peru, aquarium trade, Rio Amazonas[2]	GBxxxxx	This study	GBxxxxx	This study
Pseudohemiodon laticeps	LBP	LBP 24034	Brazil, Rio Paraguay	GBxxxxx	This study	GBxxxxx	This study
Pseudohemiodon laticeps	-	-	Argentina, Corrientes	GBxxxxx	This study	GBxxxxx	This study
Pseudohemiodon sp.	MHNG	PE05-034	Peru, aquarium trade, Rio Amazonas[2]	GBxxxxx	This study	GBxxxxx	This study

Pseudoloricaria laeviuscula	AUM 44646	G5231	Guyana, Takutu River	GBxxxxx	This study	GBxxxxx	This study
Pseudoloricaria laeviuscula	AUM 44646	G5232	Guyana, Takutu River	GBxxxxx	This study	GBxxxxx	This study
Pseudoloricaria laeviuscula	AUM 44646	G5233	Guyana, Takutu River	GBxxxxx	This study	GBxxxxx	This study
Pseudoloricaria laeviuscula	INPA 28991	MUS 517	Brazil, Rio Madeira	GBxxxxx	This study	GBxxxxx	This study
Pterosturisoma microps	MHNG	PE05-016	Peru, aquarium trade	GBxxxxx	This study	GBxxxxx	This study
Rhadinoloricaria aff. *macromystax*	ANSP 182349	T2364	Guyana, Rupununi River	GBxxxxx	This study	GBxxxxx	This study
Rhadinoloricaria sp. Orinoco	ANSP 185044	T4029	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rhadinoloricaria sp. Orinoco	AUM 42094	V5507	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rhadinoloricaria sp. Orinoco	AUM 42094	V5508	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aequalicuspis	MCP 29282	MCP 29282	Brazil, Arroio Molha Coco	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *cadeae*	LBP 7359	LBP 7359	Brazil, Eldorado do Sul	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *cadeae*	LBP 25580	LBP 25580	Brazil, Rio Guiaba	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *cadeae*	LBP 10585	LBP 10585	Brazil, Rio Iguaçu	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *fallax*	LBP 23512	LBP 23512	Brazil, Rio Japim	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *latirostris*	MHNG	MUS 491	Brazil, aquarium trade	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *phoxocephala*	MCP 28832	MCP 28832	Brazil, Rio Purus	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *phoxocephala*	MCP 28832	495	Brazil, Rio Purus	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *phoxocephala*	MCP 28832	496	Brazil, Rio Purus	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *phoxocephala*	LBP	LBP 23617	Brazil, Rio Jurua	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *phoxocephala*	MHNG	PI 720	Peru, Rio Momon	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *phoxocephala*	ANSP 182368	T2101	Guyana, Essequibo River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *stewarti*	MHNG 2663.003	GF03-196	French Guiana, Approuague River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *stewarti*	MHNG 2681.019	GF06-077	French Guiana, Oyapock River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *stewarti*	MHNG 2682.091	GF06-428	French Guiana, Maroni River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *stewarti*	MHNG 2683.049	GF06-538	French Guiana, Mana River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *stewarti*	MHNG 2617.015	MUS	French Guiana, Sinnamary River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *stewarti*	MHNG	SUJM-064	Suriname, Suriname River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *stewarti*	MHNG	SU08-945	Suriname, Commewijne River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria aff. *strigilata*	LBP 19534	LBP 19534	Brazil, Corrego da batata	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria altipinnis	Stri	Stri-3589	Panama, Rio Chucunaque	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria altipinnis	MHNG	PA97-045	Panama, Rio Chucunaque	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria cadeae	MCP 21217	MCP 21217	Brazil, Rio Grande do Sul	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria catamarcensis	-	RC	Argentina, Salta	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria catamarcensis	MHNG 2680.033	MUS	Argentina, Rio Sali	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria cf. *kronei*	LBP 11175	LBP 11175	Brazil, Rio Ribeira do Iguape	GBxxxxx	This study	GBxxxxx	This study

Rineloricaria cf. *latirostris*	LBP 8534	LBP 8534	Brazil, Rio Marumbi	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria eigenmanni	MHNG	MUS 494	Colombia, aquarium trade	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria fallax	MHNG 2651.054	GY04-146	Guyana, Takutu River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria fallax	MHNG 2672.015	SU05-412	Suriname, Corantijn River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria fallax	MHNG 2650.072	GY04-009	Guyana, Rupununi River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria fallax	MHNG 2651.034	GY04-396	Guyana, Berbice River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria fallax	MHNG 2650.067	GY04-384	Guyana, Demerara River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria fallax	LBP 24075	LBP 24075	Brazil, Boa Vista	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria fomosa	ANSP 185291	V5405	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria fomosa	AUM 43885	V5531	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria heteroptera	AUM 43886	V5530	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria heteroptera	AUM 43886	V5534	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria heteroptera	AUM 43886	V5529	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria heteroptera	AUM 43928	V5562	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria hoehnei	MHNG	PR-018	Paraguay, Paraguay River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria jaraguensis	LBP 8268	LBP 8268	Brazil, Jaragua do Sul, Rio Itapocu	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria lanceolata	MHNG 2613.029	CA-01	Peru, Rio Ucayali	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria lanceolata	MHNG 2651.029	GY04-172	Guyana, Takutu River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria lanceolata	MHNG 2651.059	GY04-252	Guyana, Mauishparu River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria lanceolata	MHNG	MUS	Brazil, Purus River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria lanceolata	Stri	Stri-2422	Argentine Rio Corrientes	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria lanceolata	LBP	LBP 11505	Brazil, Rio Araguaia	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria lanceolata	MHNG	PE08-053	Peru, Rio Huacamayo	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria longicauda	MCP 38347	MCP 38347	Brazil, Rio Grande do Sul	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria melini	LBP 24247	LBP 24247	Brazil, Rio Negro, Barcelos	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria melini	MHNG	MUS	Brazil, aquarium trade	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria microlepidogaster	MCP 21263	MCP 21263	Brazil, Rio Grande do Sul	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria misionera	MHNG 2680.034	MUS	Argentina, Rio Cuna-Piru	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria morrowi	MHNG	PI719	Peru, Rio Momon	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria parva	MHNG	PR-009	Argentina, Santa Fé	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria parva	LBP 3656	LBP 3656	Brazil, Rio Paraguay	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria platyura	LBP 12864	LBP 12864	Brazil, Rio Amazonas	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria platyura	MHNG	BR98-088	Brazil, Rio Guamá	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria platyura	MHNG	GF03-193	French Guiana, Approuague River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria platyura	MHNG	MUS 334	Brazil, Rio Purus	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria platyura	MHNG	GY04-197	Guyana, Takutu River	GBxxxxx	This study	GBxxxxx	This study

Rineloricaria platyura	MHNG	GF99-009	French Guiana, Kaw	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria platyura	MHNG	BR98-049	Brazil, Rio Acara	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria platyura	MCP 28832	497	Brazil, Rio Purus	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria quadrensis	MCP 21195	MCP 21195	Brazil, Lagoa Fortaleza	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sneiderni	Stri	Stri-1399	Colombia, Rio Baudo	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Agua Santa	MHNG 2587.054	BR1253	Brazil, Rio Paraíba do Sul	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Ao Itai	MHNG 2587.015	BR1215	Brazil, Rio Paraíba do Sul	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Araguaia	LBP	LBP 11818	Brazil, Rio Araguaia	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Betari	MHNG 2586.083	BR1190	Brazil, Rio Betari	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Betari	MHNG 2586.072	BR1184	Brazil, Rio Betari	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Carombe	LBP 17410	LBP 17410	Brazil, Rio Carombé	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Corantijn	MHNG 2671.085	SU05-450	Suriname, Corantijn River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Corantijn	MHNG	SU07-017	Suriname, Sipaliwini River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Corantijn	MHNG	SU01-417	Suriname, Nickerie River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Corrego Seco	MHNG 2586.065	BR1176	Brazil, Rio Ribeira do Iguape	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Guama 4	MHNG 2601.091	BR98-112	Brazil, Rio Guamá	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Guama 5	MHNG 2601.044	BR98-010	Brazil, Rio Gurupi	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Guama 5	MHNG	BR98-008	Brazil, Rio Gurupi	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Guama 5	MHNG	BR98-167	Brazil, Rio Piria	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Guama 6	MHNG 2601.063	BR98-047	Brazil, Rio Acara	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Huacamayo	MHNG 2613.032	CA-33	Peru, Rio Pisqui	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Huacamayo	MHNG	PE08-057	Peru, Rio Huacamayo	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Macacu	MHNG 2587.082	BR1273	Brazil, Rio da Toca	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Managua	LBP	LBP 21378	Brazil, Riacho Sitio do Meio	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Maroni 2	MHNG	SU08-441	Suriname, Paloemeu River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Maroni 2	MHNG	SU08-442	Suriname, Paloemeu River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Martinso	MHNG 2586.089	BR1196	Brazil, Rio Martinso	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Orinoco	AUM 44067	V5435	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Orinoco	AUM 44067	V5437	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Panama	MHNG	PA00-014	Panama, Rio Ipeti	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Paraiba do Sul	MHNG	BR 156	Brazil, Rio Paraíba do Sul	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Parguaza	LBP 15846	LBP 15846	Venezuela, Rio Parguaza	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Piedade	MHNG 2586.055	BR1163	Brazil, Rio Piedade	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Previsto	MHNG	PE08-186	Peru, Rio Previsto	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Puerto Ayacucho	MHNG	MUS 489	Venezuela, aquarium trade	GBxxxxx	This study	GBxxxxx	This study

Rineloricaria sp. Ribeira	MHNG 2586.088	BR1195	Brazil, Rio Ribeira do Iguape	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Rio da Toca	MHNG 2587.078	BR1269	Brazil, Rio Macacu	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Sao Joao	LBP 7954	LBP 7954	Brazil, Rio São João	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Sao Joao 2	MHNG 2586.052	BR1155	Brazil, Rio Araraguara	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Sao Joao 2	MHNG 2586.052	BR1156	Brazil, Rio Araraguara	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Tibagi	LBP 11000	LBP 11000	Brazil, Rio Tibagi	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Ucayali	MHNG	PE08-905	Peru, Rio Ucayali	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Ucayali 2	LBP	LBP 20081	Peru, Rio Huancabamba	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Uruguay	MCP 21616	MCP 21616	Brazil, Rio Grande do Sul	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria sp. Von Humbolt	MHNG	PE08-697	Peru, bosque Von Humbolt	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria stewarti	MHNG 2651.057	GY04-257	Guyana, Mauishparu River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria stewarti	MHNG 2651.027	GY04-183	Guyana, Takutu River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria stewarti	MHNG 2671.015	SU05-592	Suriname, Coppename River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria stewarti	MHNG 2671.084	SU05-457	Suriname, Corantijn River	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria strigilata	MCP 23751	MCP 23751	Brazil, Rio Grande do Sul	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria teffeana	-	-	SR, aquarium specimen	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria uracantha	Stri	Stri-1662	Panama, Rio Mandinga	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria uracantha	LBP	LBP 18551	Panama, Santa Rita Arriba	GBxxxxx	This study	GBxxxxx	This study
Rineloricaria wolfei	ANSP 182695	P6236	Peru, Rio Itaya	GBxxxxx	This study	GBxxxxx	This study
Spatuloricaria cf. *evansii*	LBP 16145	LBP 16145	Brazil, Rio Araguaia	GBxxxxx	This study	GBxxxxx	This study
Spatuloricaria puganensis	ANSP 182372	P4743	Peru, Rio Yanatili	GBxxxxx	This study	GBxxxxx	This study
Spatuloricaria puganensis	ANSP 180789	P4747	Peru, Rio Urubamba	GBxxxxx	This study	GBxxxxx	This study
Spatuloricaria sp. Araguaia	LBP	LBP 11507	Brazil,Rio Araguaia	GBxxxxx	This study	GBxxxxx	This study
Spatuloricaria sp. Ireng	ANSP 180 486	T2361	Guyana, Ireng River	GBxxxxx	This study	GBxxxxx	This study
Spatuloricaria sp. Magdalena 1	MHNG	MUS 353	Colombia, aquarium trade	GBxxxxx	This study	GBxxxxx	This study
Spatuloricaria sp. Magdalena 2	IAvHP	6635	Colombia, Rio Magdalena, Honda	GBxxxxx	This study	GBxxxxx	This study
Spatuloricaria sp. Magdalena 2	IAvHP	6637	Colombia, Rio Magdalena, Honda	GBxxxxx	This study	GBxxxxx	This study
Spatuloricaria sp. Magdalena 2	IAvHP	6638	Colombia, Rio Magdalena, Honda	GBxxxxx	This study	GBxxxxx	This study
Spatuloricaria sp. Orinoco	ANSP 185303	P4006	Venezuela, Rio Orinoco	GBxxxxx	This study	GBxxxxx	This study
Sturisoma aureum	MHNG	MUS 286	Colombia, aquarium trade	GBxxxxx	This study	GBxxxxx	This study
Sturisoma aureum	MHNG	MUS 357	Colombia, aquarium trade	GBxxxxx	This study	GBxxxxx	This study
Sturisoma cf. *guentheri*	ANSP 182587	P6330	Peru, Rio Nanay	GBxxxxx	This study	GBxxxxx	This study
Sturisoma dariense	MHNG	PA97-019	Panama, Darien	GBxxxxx	This study	GBxxxxx	This study
Sturisoma festivum	Stri	MER95T-20	Venezuela, Maracaibo Lake	GBxxxxx	This study	GBxxxxx	This study
Sturisoma frenatum	Stri	Stri-872	Colombia, Rio San Juan	GBxxxxx	This study	GBxxxxx	This study
Sturisoma nigrirostrum	ANSP 178322	P1593	Peru, Rio Amazonas	GBxxxxx	This study	GBxxxxx	This study

Sturisoma panamense	MHNG	PA00-013	Panama, Rio Ipeti	GBxxxxx	This study	GBxxxxx	This study
Sturisoma robustum	MHNG	PY9091	Paraguay, Rio Paraguay	GBxxxxx	This study	GBxxxxx	This study
Sturisoma sp. Rio Branco	LBP	LBP 4044	Brazil, Rio Branco	GBxxxxx	This study	GBxxxxx	This study
Sturisomatichthys leightoni	MHNG	MUS 327	Colombia, aquarium specimen	GBxxxxx	This study	GBxxxxx	This study
Ancistrus cirrhosus[1]	MHNG 2645.037	MUS 202	Argentina, Rio Uruguay	2420 EU310442	Covain *et al*. 2008	1809 HM623638	Rodriguez *et al*. In press
Pseudorinelepis genibarbis[1]	MHNG 2588.079	PE96-040	Peru, Rio Ucayali	2434 HM592623	Rodriguez *et al*. In press	1926 HM623634	Rodriguez *et al*. In press
Neoplecostomus microps[1]	MHNG	BR 1283	Brazil,	GBxxxxx	Montoya-Burgos *et al.* In prep.	GBxxxxx	Montoya-Burgos *et al.* In prep.
Hemiancistrus medians[1]	MHNG	SU08-173	Suriname, Tapanahony River	GBxxxxx	Fisch-Muller *et al.* In press	GBxxxxx	Fisch-Muller *et al.* In press
Guyanancistrus brevispinis[1]	MHNG	GF00-103	French Guiana, Maroni River	GBxxxxx	Fisch-Muller *et al.* In press	GBxxxxx	Montoya-Burgos *et al.* In prep.
Guyanancistrus longispinis[1]	MHNG	GF99-204	French Guiana, Oyapock River	GBxxxxx	Fisch-Muller *et al.* In press	GBxxxxx	Montoya-Burgos *et al.* In prep.
Guyanancistrus niger[1]	MHNG	GF99-185	French Guiana, Oyapock River	GBxxxxx	Fisch-Muller *et al.* In press	GBxxxxx	Montoya-Burgos *et al.* In prep.
Lasiancistrus heteracanthus[1]	MHNG	CA 13	Peru,	GBxxxxx	Fisch-Muller *et al.* In press	GBxxxxx	Montoya-Burgos *et al.* In prep.
Lithoxus lithoides[1]	MHNG	GY04-136	Guyana, Essequibo River	GBxxxxx	Fisch-Muller *et al.* In press	GBxxxxx	Montoya-Burgos *et al.* In prep.
Pseudancistrus barbatus[1]	MHNG	GF00-074	French Guiana, Maroni River	GBxxxxx	Fisch-Muller *et al.* In press	GBxxxxx	Montoya-Burgos *et al.* In prep.
Scobinancistrus aureatus[1]	MHNG	MUS 358	Brazil, aquarium trade, Rio Xingu[2]	GBxxxxx	Montoya-Burgos *et al.* In prep.	GBxxxxx	Montoya-Burgos *et al.* In prep.
Hypancistrus zebra[1]	MHNG	MUS 420	Brazil, aquarium trade, Rio Xingu[2]	GBxxxxx	Montoya-Burgos *et al.* In prep.	GBxxxxx	Montoya-Burgos *et al.* In prep.
Megalancistrus cf. *parananus*[1]	MHNG	MUS	Brazil, aquarium trade	GBxxxxx	Montoya-Burgos *et al.* In prep.	GBxxxxx	Montoya-Burgos *et al.* In prep.
Hypostomus gymnorhynchus[1]	MHNG	72.2	French Guiana, Approuague River	GBxxxxx	Fisch-Muller *et al.* In press	GBxxxxx	Montoya-Burgos *et al.* In prep.
Peckoltia oligospila[1]	MHNG	BR98-154	Brazil, Rio Guamá	GBxxxxx	Montoya-Burgos *et al.* In prep.	GBxxxxx	Fisch-Muller *et al.* In press
Peckoltia sabaji[1]	MHNG	GY04-029	Guyana, Rupununi River	GBxxxxx	Montoya-Burgos *et al.* In prep.	GBxxxxx	Fisch-Muller *et al.* In press
Peckoltia cavatica[1]	MHNG	GY04-030	Guyana, Rupununi River	GBxxxxx	Montoya-Burgos *et al.* In prep.	GBxxxxx	Fisch-Muller *et al.* In press
Panaqolus koko[1]	MHNG	GF00-	French Guiana, Maroni River	GBxxxxx	Montoya-Burgos *et al.* In prep.	GBxxxxx	Fisch-Muller *et al.* In press

[1] outgroup

[2] according to the exporter

* specimen reidentified after publication

profile: (1) 3 min. at 94°C (initial denaturing), (2) 35 sec. at 94°C, (3) 30 sec. at 51°C, (4) 150 sec. at 72°C, and (5) 5 min. at 72°C (final elongation). Steps 2 to 4 were repeated 35 to 39 times according to the quality and concentration of DNA. The internal round of PCR was performed using 1 µl of DNA template sampled from external round PCR product, the pair of primers: An12S-1D: 5'- GTA TGA CAC TGA AGA TGT TAA G -3' and iH3059: 5'- GAA CTC AGA TCA CGT AGG -3', and the same protocol as above except for the annealing temperature that was set to 54°C. PCR products were sent to Macrogen Inc. (Seoul, Korea) for sequencing. For the complete sequencing of the 2,500 bp long mitochondrial fragment, two internal primers were used: Lor1D-1D: 5'- AGG AGC CTG TTC TAG AAC CG-3' and Lor12S-3D (Covain *et al.* 2008).

2.3 Sequence alignment and phylogenetic reconstruction.

The DNA sequences were edited and assembled using BioEdit 7.0.1 (Hall, 1999), and aligned manually (for an explanation see Rodriguez *et al*., in press). Regions with ambiguous alignments in loops regions of mitochondrial genes were excluded from the analyses. Gaps were considered as missing data, and regions impossible to amplify or to sequence were coded as ambiguities (N). Since mitochondrial DNA is presumably transmitted through maternal lineage as a single not recombining genetic unit (Meyer, 1993), a first partition corresponding to the mitochondrial genes was created. In addition, the mutational patterns in intronic and exonic regions of F-RTN4 being rather characterized by insertions/deletions in introns, and transitions/transversions in exons, two other partitions were created. Combinability between mitochondrial and nuclear markers was secondarily assessed using the Congruence Among Distance Matrices (CADM) test (Legendre and Lapointe, 2004) as implemented in ape 2.6.2 (Paradis *et al*., 2004; Paradis, 2006) in R 2.12.1 (R Development Core Team, 2009). The CADM test is a generalization to several distance matrices of the Mantel test (Mantel, 1967). This test against incongruence of all distance matrices relies on the Kendall's coefficient of concordance W (Kendall and Babington Smith, 1939) among the unfolded and rank-transformed distance matrices, and uses a Friedman's χ^2 statistic (Friedman, 1937) for its computation. An observed statistics (χ^2_{ref}) was calculated for the ordered (by rows or columns) matrices and was compared, in the upper tail, to a null hypothesis sampling distribution of randomized statistics (χ^{2*}) obtained by permuting at random all matrices, independently of one another. In case of rejection of the null hypothesis, an *a posteriori* testing procedure is available to determine witch matrices are congruent. This procedure relies on the mean of the Mantel correlations of the rank-transformed distances

(Spearman's correlation r_S) between the tested matrix and all other matrices. In this case, a single matrix is permuted at a time, and repeated for all matrices in turn. It tests the null hypothesis of incongruence of the matrix subjected to the test with respect to the other matrices. A Holm (1979) correction for multiple testing is applied for all *a posteriori* tests. In addition, pairwise Mantel correlations of the rank-transformed distances between matrices can also be computed. Pairwise maximum likelihood (ML) (Felsenstein, 1981) distances were computed with Treefinder (Jobb *et al*., 2004) version of October 2008 for each partition using a likelihood model under which the pairwise distances are optimized. Appropriate substitution models corresponding to each potential partition were accordingly estimated with the Akaike Information Criterion (Akaike, 1974) as implemented in Treefinder. The CADM test was computed using 9,999 permutations of the three ML distances matrices. Two phylogenetic reconstruction methods allowing the analysis of partitioned data were used. First, a ML reconstruction was performed with Treefinder, and robustness of the results was estimated by resampling the data set with the nonparametric bootstrap (Efron, 1979) following Felsenstein's (1985b) methodology with 2,000 pseudoreplicates. Second, a Bayesian inference analysis was conducted in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Two runs of eight chains (one cold, seven heated) were conducted simultaneously for $2x10^7$ generations, with the tree space sampled each 1000^{th} generation. Convergence between chains occurred after $2x10^6$ generations (average standard deviation of split frequencies <0.01). After a visual representation of the evolution of the likelihood scores, and checking for the stationarity of all model parameters using Tracer 1.5 (Rambaut and Drummond, 2007) (*i.e.*: potential scale reduction factor (PSRF), uncorrected roughly approached 1 as runs converged (Gelman and Rubin, 1992), and Effective Sample Size (ESS) of all parameters superior to 200), the $2x10^6$ first generations were discarded as burn-in. The remaining trees were used to compute the consensus tree. Phylogenetic reconstructions were performed on the TITAN cluster at the University of Oslo, Norway through Bioportal (Kumar *et al*., 2009).

2.4 Analysis of biological traits in Loricariinae

To explore the main evolutionary trends shaped through time in Loricariinae, a complete table mixing quantitative and discrete data was created. In addition to the morphological data set presented in Covain and Fisch-Muller (2007), and Covain *et al*. (2008), height ecomorphological, three ecological, one ethological, and one morphological variables were recorded from museum specimens, field and personal observations, or

literature (*e.g.* Evers and Seidel, 2005; Dotzer and Weidner, 2003). The taxonomical sampling of this data set was chosen in a way to include a maximum of representatives of the Loricariinae from which phylogenetic information was available, and from which we had no missing data. A total of 232 specimens representing 42 species belonging to 18 genera were measured and examined for ecomorphological and meristic data respectively. All measurements were taken with a digital caliper to the nearest 0.01 mm from specimens deposited in the ichthyological collection of MHNG. The mean value by species was then computed for each quantitative variable as mean specific estimator. Qualitative data were invariant by species. Quantitative data comprised the meristic data presented in Covain and Fisch-Muller (2007): *i.e.* number of caudal-fin rays: [caud]; number of pectoral-fin rays [pect]; number of pelvic-fin rays [pelv]; number of dorsal-fin rays [dors]; number of premaxillary teeth [nbdtsup]; number of dentary teeth [nbdtinf]; and the ecomorphological variables: Compression Index [CI] (maximum body depth divided by maximum body width), a high value indicates a more compressed fish and characterizes fishes inhabiting biotopes with slower flowing water (Watson and Balon, 1984); Relative Body Depth [RBD] (maximum body depth divided by standard length), a low value indicates a slender fish and is assumed to be inversely related to habitat water velocity (Gatz, 1979); Relative Peduncle Length [RPL] (caudal peduncle length divided by standard length), longer caudal peduncle indicates fish with better swimming abilities (Watson and Balon, 1984); Caudal Peduncle Compression index [CPC] (caudal peduncle depth divided by caudal peduncle width at the same point), high value characterizes less active swimmers (Gatz, 1979); Index of Ventral Flattening [IVF] (body depth below the midline divided by maximum body depth), a low index characterizes fishes inhabiting fast flowing waters (Gatz, 1979); Relative Eye Diameter [RED] (eye diameter divided by standard length), is directly proportional to the development of visual capabilities (Gatz, 1979); Relative Mouth Width [RMW] (mouth width divided by standard length), is expected to be related to feeding habits in Loricariinae (adapted from Gatz, 1979); Relative Mouth Height [RMH] (mouth height divided by standard length), is expected to be related to feeding habits in Loricariinae (adapted from Gatz, 1979). The qualitative variables corresponded to the one presented in Covain and Fisch-Muller (2007): abdominal cover [abd] with three modalities: 1 = absent, 2 = present incomplete, 3 = present complete; secondary organization in the abdominal cover [ssec] with two modalities: 1 = absent, 2 = present; postorbital notches [encorb] with three modalities: 1 = absent, 2 = present weak, 3 = present deep; predorsal keels [cdor] with two modalities: 1 = absent, 2 = present; lip structure [lips] with three modalities: 1 = papillose, 2 = filamentous, 3 = rather smooth;

fringed barbels [mlips] with two modalities: 1 = present, 2 = absent; mouth shape [mouth] with three modalities: 1 = elliptical, 2 = bilobate, 3 = bilobate with trapezoidal opening; tooth shape [teeth] with four modalities: 1 = pedunculated, 2 = straight bicuspid, 3 = spoon shaped size reduced, 4 = straight bicuspid size reduced; maxillary barbels [barb] with two modalities: 1 = conspicuous, 2 = inconspicuous; rostrum [rost] with two modalities: 1 = absent, 2 = present; snout shape [snout] with two modalities: 1 = pointed, 2 = rounded; with addition of the secondary sexual dimorphism [SD] with three modalities: 1 = mainly expressed through hypertrophy of odontodes, 2 = mainly expressed through characteristics of the mouth, 3 = not expressed; the three ecological variables: main habitat [hab] with three modalities: 1 = forest creek, 2 = medium river, 3 = large river; favored substrate [sub] with five modalities: 1 = rocks, 2 = stones, 3 = sand, 4 = mud, 5 = organic; water velocity [stream] with three modalities: 1 = high, 2 = medium, 3 = low; and the ethological variable: reproductive strategy [repro] with five modalities: 1 = abdomino-lip brooder, 2 = lip brooder with support, 3 = lip brooder, 4 = cavity brooder, 5 = open water brooder. Ecological data represented extra-phenotypic data whereas morphological and ethological data corresponded to intra-phenotypic data.

A first global assessment of the phylogenetic dependence of the different variables constituting this complete table was performed using the orthogram method proposed by Ollier *et al.* (2006) extended to the multivariate case. The orthogram decomposes the trait variance along a phylogenetic tree represented as an orthonormal basis. In the original paper, orthonormal basis is constructed to represent the topology of the phylogenetic tree but other alternatives are available (see Jombart *et al*., 2010). Then, a linear regression is performed with the centered trait variable as response variable, and the orthonormal basis as explanatory variables. Regression coefficients allow reconstructing the trait variable, and squared coefficients provided variance decomposition of the trait onto the orthonormal basis. The plotting of the squared coefficients and of the cumulative squared coefficients provides two graphical tools called orthogram and cumulative orthogram (Ollier *et al.*, 2006). Four permutation procedures associated to orthograms are used to test the null hypothesis of phylogenetic independence. These procedures are based on different statistics and consider different alternative hypotheses. The R2Max statistics is used to test against the alternative hypothesis that one vector explained a significant part of the trait variance (punctual effect). SkR2k is used to test against the alternative hypothesis that vectors near the tips (or the root) explained a significant part of the trait variance. SkR2k is high when the trait variance is rather explained by last vectors (towards tips) and low when explained by first vectors

(towards root). Dmax is a Kolmogorov-Smirnov-like statistic and is used to test if the vector of squared coefficients may be an ordered random sample of the uniform distribution on (0, 1). Dmax was used to test against the alternative hypothesis that some successive vectors explained a significant part of the trait variance (gradual effect). Finally, SCE is a measure of the average local variation of the orthogram and tests against the alternative hypothesis that there are significant differences in variance explained by vectors and their neighbors (precedent or subsequent) (local effect). This approach can be extended to the multivariate case using a multivariate table instead of a single quantitative response variable (Rao, 1964). This approach allows to decompose the multivariate variability (including qualitative variables coded as a table of dummy variables) on the phylogenetic basis. Prior to the computation of the multivariate orthogram test, the phylogenetic tree was restricted to the same set of 42 species, and the complete table was submitted to a Hill and Smith Analysis (HSA) (1976) to reveal its structuring. The HSA consists in a Principal Component Analysis (PCA) of a table mixing quantitative and qualitative variables. Secondarily, each variable was individually tested for phylogenetic dependence to reveal their pattern of evolution along the phylogenetic tree. For this, the orthogram method, initially devoted to the detection of phylogenetic dependence in quantitative traits, was extended to the discrete case. Distribution of the statistics under the null hypothesis and confidence limits of (cumulative) orthograms were built using 9,999 random permutations of the trait values. Orthograms (multivariate and univariate) and associated tests (Ollier *et al*., 2006) were conducted using the adephylo 1.1-0 package (Jombart *et al.,* 2010) in R 2.12.1 (R Development Core Team 2009). A control for false discovery rate for multiple testing under dependency (Benjamini and Yekutieli, 2001) was applied since all tested variables may be proved to be phylogenetically dependent.

To investigate the global pattern of evolution of the different traits along the phylogeny, and to reveal potential evolutionary associations among traits, we used the MSPA in a phylogenetic context. This analysis corresponds to a non-centered PCA of a table containing the decompositions of traits on the orthonormal basis. This table crosses traits and phylogenetic eigenvectors and contains values of squared coefficients and its analysis aims to identify traits having similar decomposition (and thus similar phylogenetic history).

Finally, in order to date the appearance of the main innovations in Loricariinae, the phylogenetic tree was calibrated. After verification that the assumption of constant molecular clock was significantly rejected using a likelihood ratio test conducted between an unconstrained topology and a clock-constrained topology under the GTR + G + I model (Tavaré, 1986), a Bayesian tree calibration method allowing relaxed molecular clock models

on partitioned data was applied. Node ages and substitution rates were estimated using an uncorrelated lognormal relaxed clock in BEAST 1.5.4 (Drummond *et al*., 2006; Drummond and Rambaut, 2007). The GTR + G model was applied on the intronic and mitochondrial partitions, and the HKY + G (Hasegawa *et al*., 1985) model on the exonic partition using a Yule tree prior and the best ML phylogenetic tree as fixed topology. The Middle Miocene rise of Eastern Cordillera (~12 Ma) that split the Magdalena drainage from the Orinoco drainage, and the Late Miocene rise of the Merida Andes (~8 Ma) that split the Maracaibo Lake from the Orinoco drainage were used as Time to Most Recent Common Ancestor (TMRCA) for sister species split by these geologic entities (for a review see Albert *et al*., 2006). Fifty millions generation were used with parameters sampling each 1,000th generation for the Markov Chain Monte Carlo (MCMC) exploration of parameters' space. A normal distribution was applied for the TMRCA priors. Other parameters were set to default. The convergence of the chain was assessed by inspection of the trace plots and ESS using Tracer 1.5. Since all parameters converged (ESS > 200), the default 10% parameters and trees were discarded as burn-in, and summarized using TreeAnnotator 1.5.4. The chronogram was edited using FigTree 1.3.1. The computation of the chronogram was performed on the TITAN cluster through Bioportal.

3. Results

3.1 Phylogenetic analysis of the subfamily Loricariinae.

We sequenced the almost complete 12S and 16S mitochondrial genes, and the partial nuclear gene F-RTN4 for 326 representatives of the Loricariinae and 16 Loricariidae belonging to Hypostominae and Neoplecostominae as outgroup (Table 1). Other sequences for 24 representatives of Loricariinae, *Ancistrus cirrhosus* and *Pseudorinelepis genibarbis* were obtained from GenBank using the accession numbers provided in Covain *et al.* (2008), Chiachio *et al.* (2008), and Rodriguez *et al.* (in press). The sequence alignment including initially 8,503 positions was restricted to 8,426 positions after removal of ambiguous regions. From these 8,426 positions, 2,545 corresponded to the mitochondrial genes (962 positions for the 12S rRNA gene, 74 for the tRNA Val gene, and 1,509 for the 16S rRNA gene), and 5,881 to the nuclear F-RTN4 gene (894 positions for the exonic regions, and 4,987 for the intronic regions). No significant conflicting phylogenetic signal was detected in the data set, as the global CADM test rejected the null hypothesis of incongruence between matrices (CADM: W

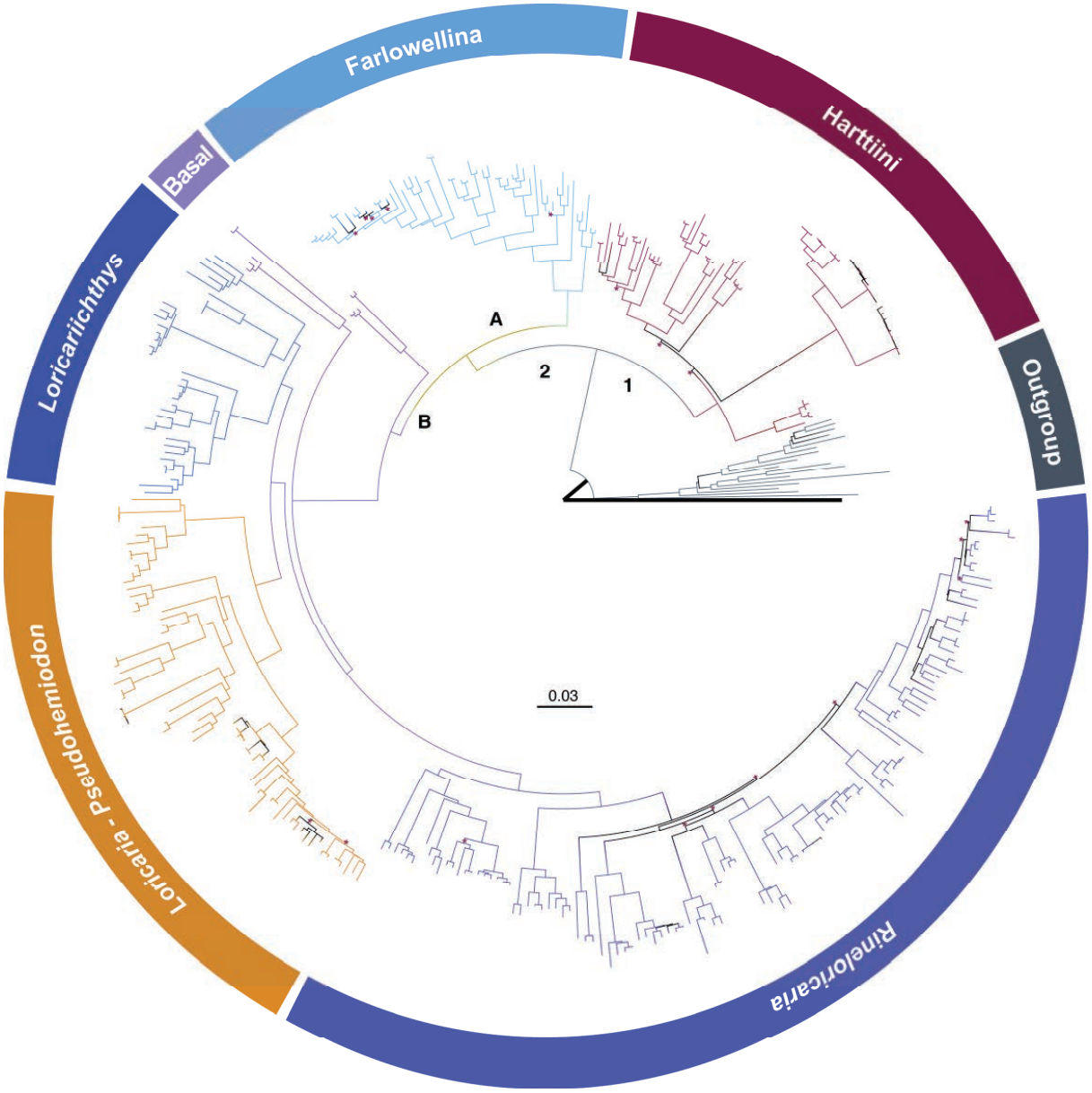


Fig. 1. Maximum Likelihood tree of the Loricariinae (-lnL = 116702.5) inferred from the combined analysis of sequences of partial 12S and 16S mitochondrial genes, and partial F-RTN4 nuclear gene. The models GTR + G for mitochondrial genes and intronic regions of F-RTN4, and HKY + G for exonic regions of F-RTN4 were applied for both ML and Bayesian reconstructions. Shaded regions indicate nodes with both bootstrap supports and posterior probabilities below 50 and 0.70 respectively. Stars indicate incongruence between ML and Bayesian reconstructions. 1: Harttiini; 2: Loricariini; A: Farlowellina, B: Loricariina. Scale indicates the number of substitutions per site as expected by the model.

= 0.7964, χ^2_{ref} = 163976.6, $p_{(\chi 2ref \geq \chi 2^*)}$ = 0.0001). The CADM *a posteriori* tests did not detect any conflicting matrix in the data ($\bar{r}_S$ mitochondrion = 0.6295, $p_{(\bar{r}_{S\ ref} \geq \bar{r}_{S\ ^*)}}$ = 0.0003; $\bar{r}_S$ exons = 0.7239, $p_{(\bar{r}_{S\ ref} \geq \bar{r}_{S\ ^*)}}$ = 0.0003; $\bar{r}_S$ introns = 0.7304, $p_{(\bar{r}_{S\ ref} \geq \bar{r}_{S\ ^*)}}$ = 0.0003). The sequences were consequently concatenated, and three partitions corresponding to mitochondrial genes, exonic parts of F-RTN4, and intronic parts of F-RTN4 were used to reconstruct the tree. The models GTR + G (Tavaré, 1986) for mitochondrial genes and intronic regions of F-RTN4, and HKY + G (Hasegawa *et al*., 1985) for exonic regions of F-RTN4 displayed the smallest AIC and fitted accordingly our data the best as indicated by Treefinder. Bayesian and ML phylogenetic reconstructions lead to equivalent tree topologies, both comparable in broad outline to the one obtained by Covain *et al.* (2008), and Rodriguez *et al.* (in press). The best ML tree (-lnL = 116702.5) and Bayesian tree (Fig. 1) split the Loricariinae into two highly supported lineages by both bootstrap values and posterior probabilities: the Harttiini (clade 1), and the Loricariini (clade 2). The Loricariini was divided in turn into two strongly supported clades: the Farlowellina (clade A); and the Loricariina (clade B). Within the Loricariina three main groups were found with high supports, one constituting the *Loricariichthys* group (*sensu* Covain and Fisch-Muller, 2007), a second comprising *Spatuloricaria* in a sister position to the *Loricaria* plus *Pseudohemiodon* groups (*sensu* Covain and Fisch-Muller, 2007), and a third comprising all *Rineloricaria* representatives. *Metaloricaria* is recovered at the base of the Loricariina, and the second diverging group comprised *Dasyloricaria* and *Fonchiiloricaria*, these three genera constituting basal Loricariina.

3.1.1 Harttiini

The Harttiini tribe constituted a monophyletic group and included the genera *Harttia, Cteniloricaria*, and *Harttiella* (Fig. 2). *Cteniloricaria* and *Harttiella* were found monophyletic with high statistical supports. *Cteniloricaria* included two species, *C. napova* and *C. platystoma* (type species). *Harttiella* comprised six species, *H. crassicauda* (type species), *H. parva*, *H. pilosa*, *H. longicauda*, *H. intermedia*, and *H. Lucifer*. *Harttiella intermedia* was found nested within *H. longicauda*. Relationships among other Harttiini belonging to *Harttia* were partly unresolved. Guianese *Harttia* comprising *H. guianensis*, *H. surinamensis*, *H. fluminensis*, and *H. tuna* did not group with other *Harttia* or with another genus, except in the Bayesian reconstruction where they formed the sister group of *Cteniloricaria* with very low posterior probabilities (0.53). The only relationship strongly supported in *Harttia* was the

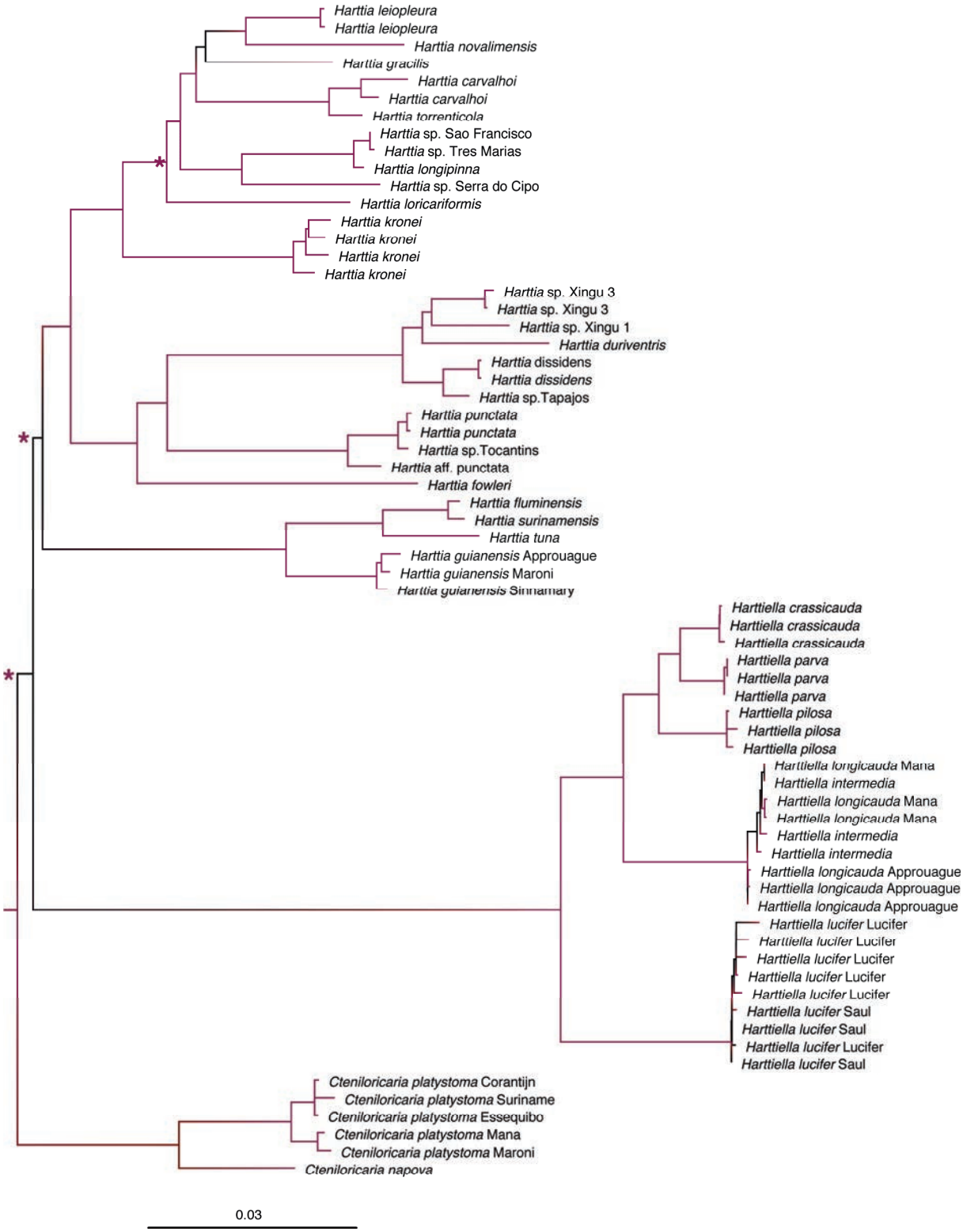


Fig. 2. Best ML tree, labelled subtree of the Harttiini tribe. Shaded regions indicate nodes with both bootstrap supports and posterior probabilities below 50 and 0.70 respectively. Stars indicate incongruence between ML and Bayesian reconstructions. Scale indicates the number of substitutions per site as expected by the model.

clade including Amazonian representatives (*H. punctata*, *H. duriventris*, etc…) plus the Guianese *H. fowleri* in a sister position to representatives from South east Brazil (including *H. loricariformis*, type species of the genus and *H. leiopleura* type species of *Quiritixys*). Deeper relationships among genera were not statistically supported.

3.1.2 Loricariini, Farlowellina

The Loricariini tribe was found monophyletic (Fig. 3). Within Loricariini, the subtribe Farlowellina also constituted a monophyletic assemblage, and comprised *Lamontichthys*, *Pterosturisoma*, *Sturisoma*, *Farlowella, Aposturisoma*, and *Sturisomatichthys*. Interspecific

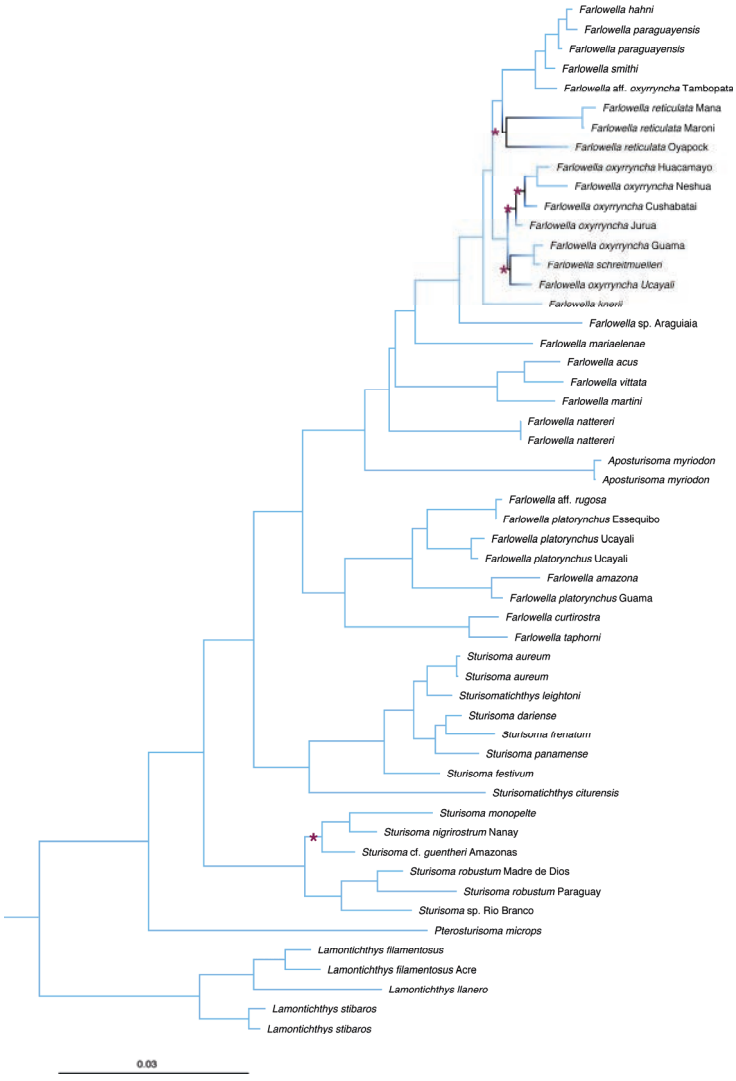


Fig. 3. Best ML tree, labelled subtree of the Loricariini tribe: Farlowellina subtribe. Shaded regions indicate nodes with both bootstrap supports and posterior probabilities below 50 and 0.70 respectively. Stars indicate incongruence between ML and Bayesian reconstructions. Scale indicates the number of substitutions per site as expected by the model.

relationships were congruent between both analyses. *Lamontichthys* (including *L. filamentosus*, type species) was monophyletic and connected with high support at base of the subtribe. The second diverging genus was the monotypic *Pterosturisoma microps* that formed

the sister genus of the remaining Farlowellina. The third group comprised all cis-Andean representatives of *Sturisoma* of this study in a sister position to other representatives of *Sturisoma* plus *Sturisomatichthys*, *Farlowella* and *Aposturisoma*; a position also strongly supported. The subsequent highly supported group comprised a mix of representatives of *Sturisomatichthys* (including *S. leightoni*, type species) and the trans-Andean *Sturisoma* rendering both genera paraphyletic. The last group comprised all representatives of *Farlowella* plus *Aposturisoma*. The base of this group was made of massive forms of *Farlowella* comprising *F. platorynchus*, *F. amazona*, *F.* aff. *rugosa*, *F. taphorni* and *F. curtirostra*. The next strongly supported diverging species was the monotypic *Aposturisoma myriodon* in a sister position to all remaining *Farlowella* (including *F. acus*, type species). This topological situation rendered *Farlowella* paraphyletic.

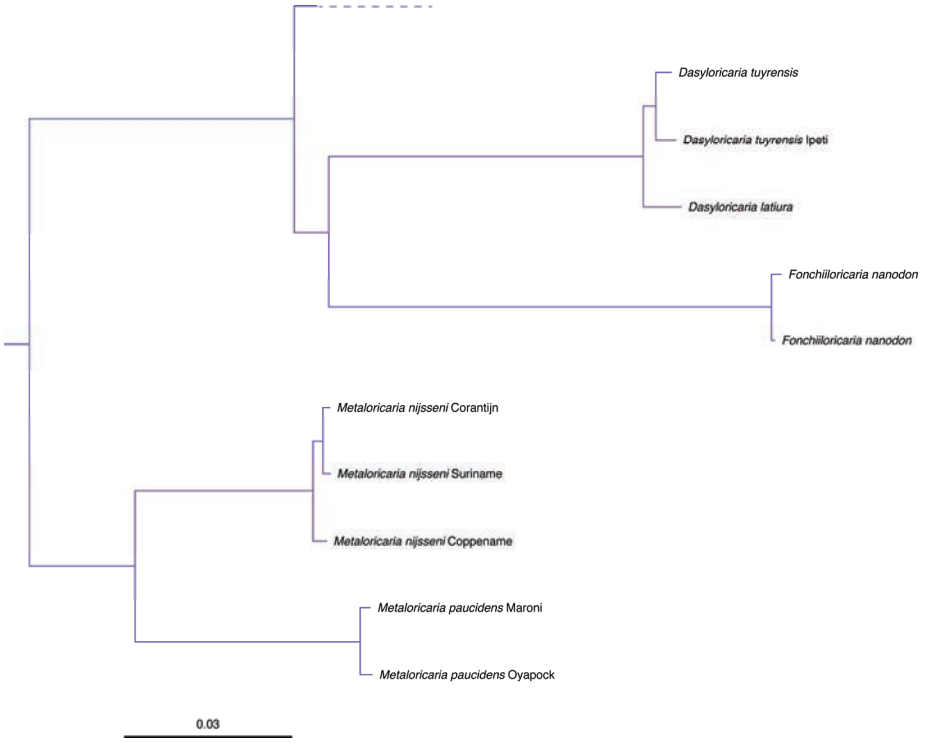


Fig. 4. Best ML tree, labelled subtree of the Loricariini tribe: Loricariina subtribe, basal Loricariina group. Scale indicates the number of substitutions per site as expected by the model.

3.1.3 Loricariini, basal Loricariina

The subtribe Loriicariina was also found monophyletic as sister group of Farlowellina (Fig. 4). The base of the subtribe comprised the representatives of *Metaloricaria* (including *Metaloricaria paucidens*, type species) in a sister position to all Loricariina, a position strongly supported by bootstrap supports and posterior probabilities. The second diverging group comprised *Dasyloricaria* representatives in a sister position to the monotypic

Fonchiiloricaria nanodon, both in turn forming the sister group of the remaining Loricariina. The sister relationship between *Dasyloricaria* and *Fonchiiloricaria* was however not supported by bootstrap values. The sister group of these two genera split into two groups with on one side representatives of *Rineloricaria* and *Ixinandria*, and on the other side members of the *Loricaria-Pseudohemiodon* and *Loricariichthys* groups.

3.1.4 Loricariini, Loricariina, Rineloricaria

The genus *Rineloricaria* (including *Fonchiiichthys*, *Hemiloricaria*, and *Leliella*) formed the most species rich group of the subfamily and constituted a monophyletic assemblage (including *Ixinandria steinbachi*, type species of *Ixinandria*) with high statistical support (Fig. 5). The first diverging group of *Rineloricaria* comprised the trans-Andean *R. altipinnis* in a sister relationship to the cis-Andean *R. stewarti, R. fallax, R. formosa, R. melini, R. teffeana, R. morrowi,* and several undescribed species. The second diverging group comprised different populations of *R. lanceolata* and *R. hoehnei*. The latter species was nested within *R. lanceolata* and all internal relationships were fully resolved and highly supported. These two species formed the sister group of all remaining *Rineloricaria* representatives. Concerning the sister group of the *R. lanceolata* clade, the different reconstructions provided two alternative hypotheses. The Bayesian reconstruction recovered the monophyly of the South-eastern species of *Rineloricaria* plus *Ixinandria steinbachi* (nested within *Rineloricaria* as sister species of *R. misionera*) which formed the sister group of a second monophyletic group comprising the representatives of *Rineloricaria* from the Amazon, Orinoco, and trans-Andean region (except *R. altipinnis*), whereas the ML reconstruction recovered the species *R. osvaldoi* and relatives forming the sister group of all the remaining *Rineloricaria* plus *Ixinandria*. Then the species from the Amazon, Orinoco, and the trans-Andean region diverged and formed the sister group of Amazonian species including *R. wolfei* in a sister position to the South-eastern clade (including *I. steinbachi*, type species of *Ixinandria*). However, the Bayesian reconstruction leaded to a better resolution of the phylogeny with all posterior probabilities greater than 0.6, whereas bootstrap values only supported the monophyly of the South-eastern clade. In both reconstructions, the type species of *Ixinandria* was nested within South-eastern *Rineloricaria*. The species *R. uracantha* (type species of *Fonchiiichthys*), *R. heteroptera* (type species of *Leliella*), and *R. eigenmanni* and relatives from Orinoco basin (potentially close relatives of *R. caracasensis*, type species of *Hemiloricaria*) were all found nested within the clade Amazon, Orinoco, and trans-Andean region, in positions strongly supported by

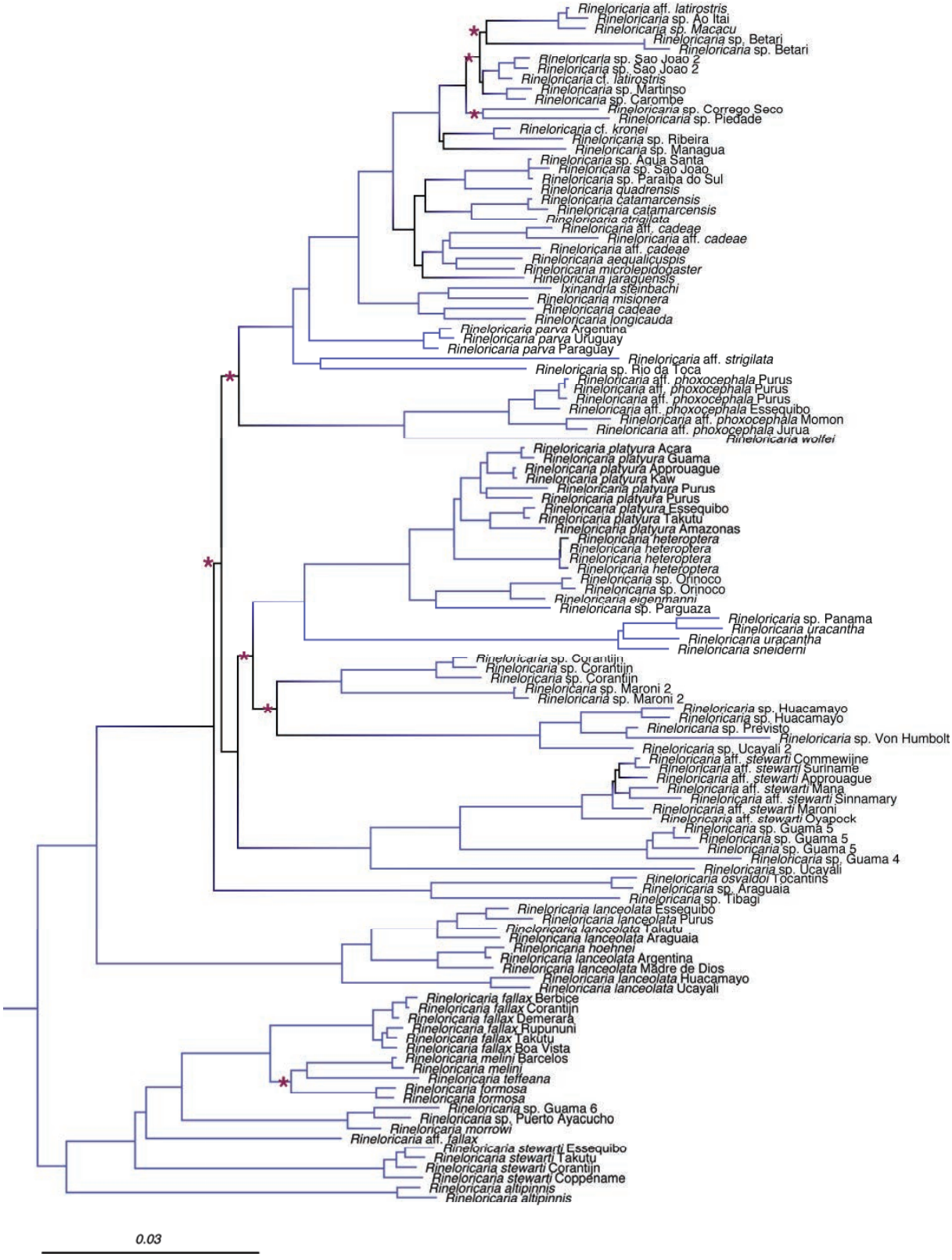


Fig. 5. Best ML tree, labelled subtree of the Loricariini tribe: Loricariina subtribe, *Rineloricaria* group. Shaded regions indicate nodes with both bootstrap supports and posterior probabilities below 50 and 0.70 respectively. Stars indicate incongruence between ML and Bayesian reconstructions. Scale indicates the number of substitutions per site as expected by the model.

bootstrap values and posterior probabilities. The genus *Rineloricaria sensu lato* constituted the sister group of the *Loricariichthys* group on one side, and of the *Loricaria-Pseudohemiodon* group on the other side.

3.1.5 Loricariini, Loricariina, Loricariichthys *group*

Within Loricariina, members of the *Loricariichthys* group formed a strongly supported natural grouping comprising *Pseudoloricaria*, *Limatulichthys*, *Loricariichthys*, and *Hemiodontichthys* (Fig. 6) and formed the sister group of the *Loricaria-Pseudohemiodon* group. *Loricariichthys* (including *L. maculatus*, type species) was found monophyletic and constituted the sister genus of all other members of its groups. The second diverging genus was made of the different populations of the monotypic *Hemiodontichthys acipenserinus* in a sister position to two other monotypic sister genera *Pseudoloricaria* and *Limatulichthys*. All internal relationships within the *Loricariichthys* group were congruent in both reconstructions and fully resolved with high statistical support.

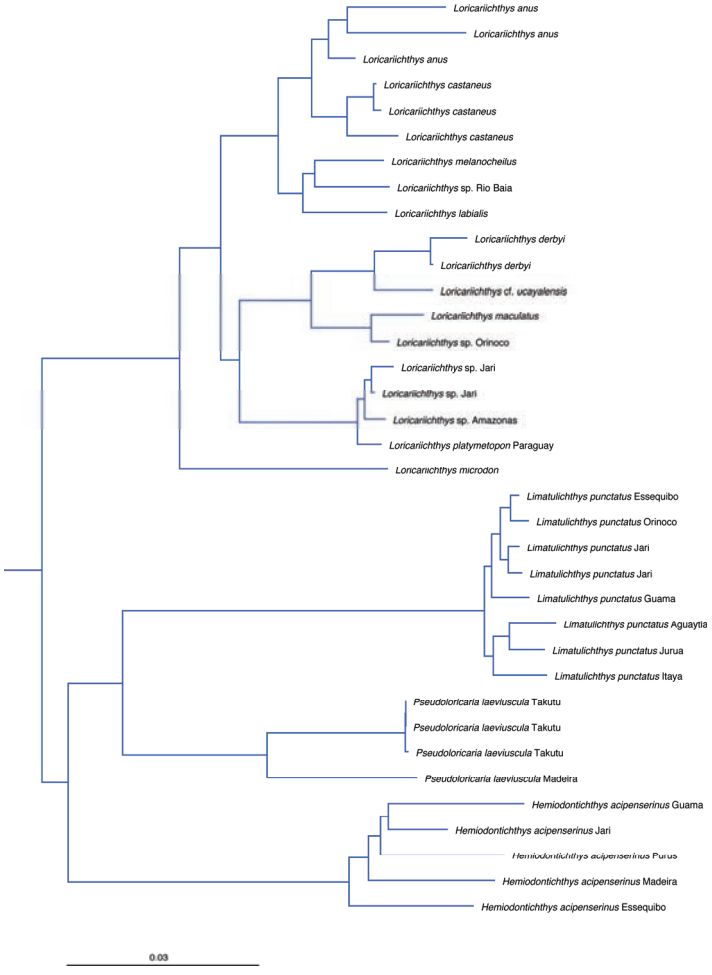


Fig. 6. Best ML tree, labelled subtree of the Loricariini tribe: Loricariina subtribe, *Loricariichthys* group. Scale indicates the number of substitutions per site as expected by the model.

3.1.6 Loricariini, Loricariina, Loricaria-Pseudohemiodon *group*

The *Loricaria-Pseudohemiodon* group formed a strongly supported clade comprising the genera *Spatuloricaria*, *Loricaria* (including *Proloricaria*), *Brochiloricaria*, *Paraloricaria*, *Planiroricaria*, *Crossoloricaria*, *Pseudohemiodon*, *Apistoloricaria*, and *Rhadinoloricaria*, and formed accordingly the most genera rich group (Fig. 7). Interspecific relationships were congruent between both reconstructions except for the species and populations closely related to *L. cataphracta*. *Spatuloricaria* was found monophyletic and formed the sister genus of all other genera of the group. The remaining members of the *Loricaria-Pseudohemidon* group split into two strongly supported clades corresponding to the *Loricaria* group (*sensu* Covain and Fisch-Muller, 2007) on one side and the *Pseudohemiodon* group (*sensu* Covain and Fisch-Muller, 2007) on the other side. The *Loricaria* group was strongly supported and comprised *Loricaria* (including *L. cataphracta* type species), *Brochiloricaria*, and *Paraloricaria*. With exceptions of *L. prolixa* (type species of *Proloricaria*) and *L. apeltogaster*, *Loricaria* formed a monophyletic group statistically highly supported. *Loricaria* formed the sister genus of all other representatives of its group. The sister group of *Loricaria* comprised *Loricaria prolixa* in a sister position to *Brochiloricaria* representatives, both in turn forming the sister group of *L. apeltogater* as sister species of representatives of *Paraloricaria*. However, except for their exclusion of *Loricaria*, the positions of *L. prolixa* and *L. apeltogaster* were not statistically supported. The *Pseudohemiodon* group was also strongly supported and comprised the trans-Andean representatives of *Crossoloricaria* (including *C. variegata*, type species) at base of the group, a position strongly supported by bootstrap supports and posterior probabilities. The second diverging group corresponded to the monotypic *Planiloricaria cryptodon* in a sister position to the remaining genera of the group. The third diverging group comprised the representatives of *Pseudohemiodon* which were found monophyletic with high statistical support. The sister group of *Pseudohemiodon* was also strongly supported and comprised a mix of representatives of *Rhadinoloricaria*, *Apistoloricaria* and the cis-Andean *Crossoloricaria*, where *Rhadinoloricaria* was found paraphyletic. All internal relationships were however fully resolved with strong support.

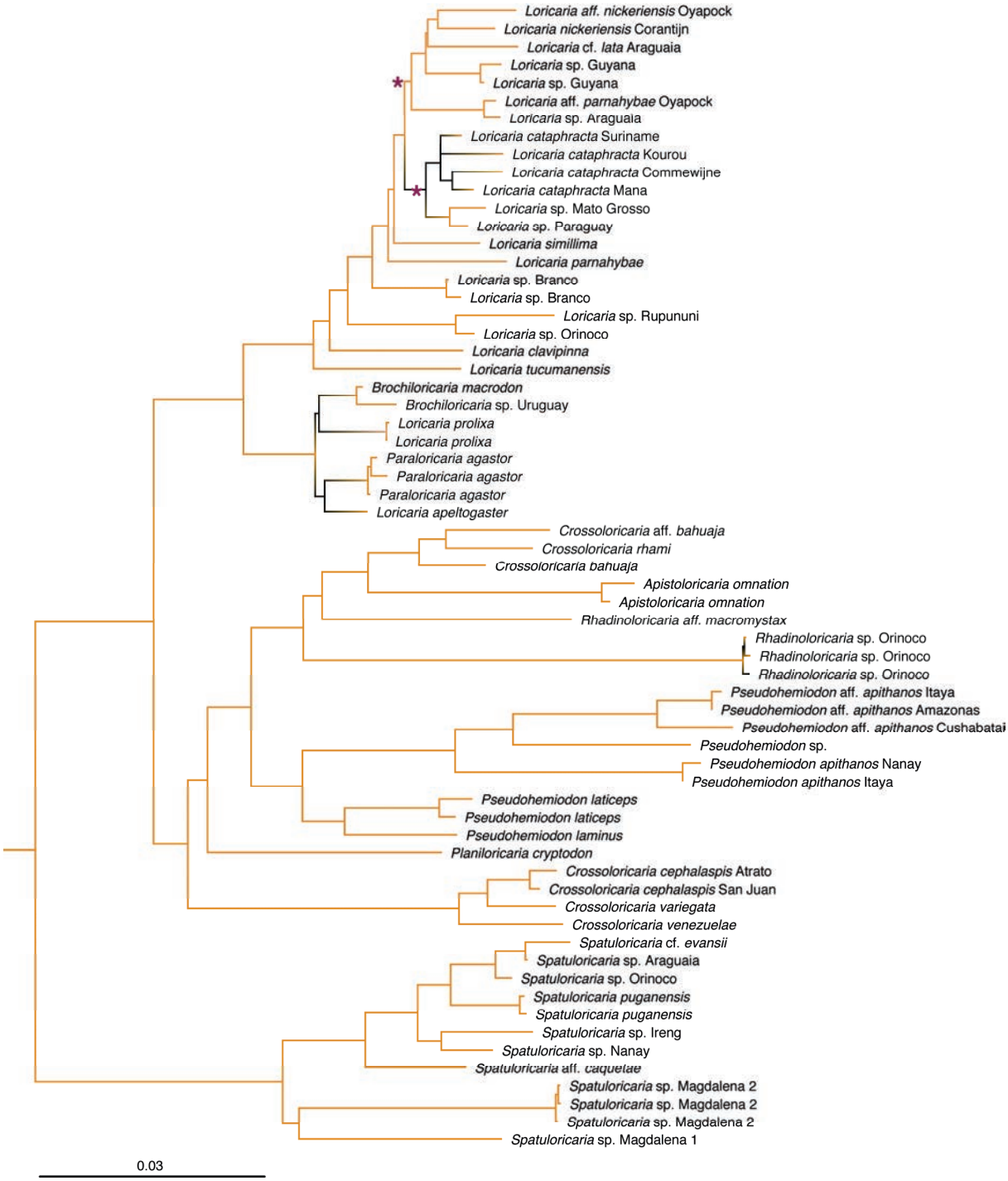


Fig. 7. Best ML tree, labelled subtree of the Loricariini tribe: Loricariina subtribe, *Loricaria-Pseudohemiodon* group. Shaded regions indicate nodes with both bootstrap supports and posterior probabilities below 50 and 0.70 respectively. Stars indicate incongruence between ML and Bayesian reconstructions. Scale indicates the number of substitutions per site as expected by the model.

Table 2. Biological traits recorded for 42 species of the Loricariinae. N: number of measured specimens for each species. Discrete quantitative meristic data: **caud** to **nbdtinf**; **caud**: number of caudal-fin rays (inc. spines); **pect**: number of pectoral-fin rays (inc. spine); **pelv**: number of pelvic-fin rays (inc. spine); **dors**: number of dorsal-fin rays (inc. spine); **nbdtsup**: number of premaxillary teeth (per premaxilla); **nbdtinf**: number of dentary teeth (per dentary); continuous quantitative ecomorphometric variables: **CI** to **RMH**; **CI**: Compression Index; **RBD**: Relative Body Depth; **RPL**: Relative Peduncle Length; **CPC**: Caudal Peduncle Compression index; **IVF**: Index of Ventral Flattening; **RED**: Relative Eye Diameter; **RMW**: Relative Mouth Width; **RMH**: Relative Mouth Height; qualitative morphological variables: **abd** to **SD**; **abd**: abdominal cover with three modalities: 1 = absent, 2 = present incomplete, 3 = present complete; **ssec**: secondary organization in the abdominal cover with two modalities: 1 = absent, 2 = present; **encorb**: postorbital notches with three modalities: 1 = absent, 2 = present weak, 3 = present deep; **cdor**: predorsal keels with two modalities: 1 = absent, 2 = present; **lips**: lip structure with three modalities: 1 = papillose, 2 = filamentous, 3 = rather smooth; **mlips**: fringed barbels with two modalities: 1 = present, 2 = absent; **mouth**: mouth shape with three modalities: 1 = elliptical, 2 = bilobate, 3 = bilobate with trapezoidal opening; **teeth**: tooth shape with four modalities: 1 = pedunculated, 2 = straight bicuspid, 3 = spoon shaped size reduced, 4 = straight bicuspid size reduced; **barb**: maxillary barbels with two modalities: 1 = conspicuous, 2 = inconspicuous; **rost**: rostrum with two modalities: 1 = absent, 2 = present; **snout**: snout shape with two modalities: 1 = pointed, 2 = rounded; **SD**: secondary sexual dimorphism with three modalities: 1 = mainly expressed through hypertrophy of odontodes, 2 = mainly expressed through characteristics of the mouth, 3 = not expressed; qualitative ecological variables: **hab** to **stream**; **hab**: main habitat with three modalities: 1 = forest creek, 2 = medium river, 3 = large river; **sub**: favored substrate with five modalities: 1 = rocks, 2 = stones, 3 = sand, 4 = mud, 5 = organic; **stream**: water velocity with three modalities: 1 = high, 2 = medium, 3 = low; qualitative ethological variable: **repro**: reproductive strategy with five modalities: 1 = abdomino-lip brooder, 2 = lip brooder with support, 3 = lip brooder, 4 = cavity brooder, 5 = open water brooder.

sp	N	caud	pect	pelv	dors	nbdtsup	nbdtinf	CI	RBD	RPL	CPC	IVF	RED	RMW	RMH	abd	ssec	encorb	cdor	lips	mlips	mouth	teeth	barb	rost	snout	SD	hab	sub	stream	repro
Apistoloricaria omnation	11	12	7	6	7	2	5	0.65	0.08	0.54	0.41	0.60	0.02	0.09	0.08	2	2	2	2	2	2	3	3	1	1	1	2	2	3	2	1
Brochiloricaria macrodon	2	12	7	6	7	4	5	0.57	0.10	0.49	0.38	0.50	0.03	0.08	0.10	3	1	2	2	2	2	2	2	1	1	2	2	3	3	2	1
Crossoloricaria bahuaja	1	12	7	6	7	5	8	0.55	0.09	0.49	0.32	0.65	0.04	0.10	0.08	2	2	2	2	2	2	3	3	1	1	1	2	3	3	2	1
*Crossoloricaria venezuelae**	1	12	7	6	7	3	5	0.55	0.08	0.49	0.39	0.72	0.05	0.11	0.11	2	2	2	2	2	2	3	3	1	1	1	2	3	3	2	1
Farlowella oxyrryncha	11	13	7	6	6	28	23	0.90	0.05	0.49	0.48	0.43	0.02	0.05	0.06	3	2	1	1	1	1	1	1	2	2	2	1	2	5	2	5
Farlowella platorynchus	9	13	7	5	6	22	19	0.88	0.05	0.48	0.50	0.41	0.02	0.05	0.06	3	2	1	1	1	1	1	1	2	2	2	1	3	5	2	5
Farlowella vittata	3	12	7	5	6	39	33	0.88	0.05	0.53	0.58	0.36	0.02	0.04	0.06	3	2	1	1	1	1	1	1	2	2	2	1	2	5	2	5
Harttia guianensis	9	14	7	6	7	88	80	0.50	0.10	0.48	0.43	0.38	0.05	0.12	0.12	1	1	1	1	1	1	1	1	2	1	1	1	3	1	1	5
Hemiodontichthys acipenserinus	10	12	7	6	7	0	9	0.51	0.08	0.47	0.34	0.59	0.04	0.08	0.10	3	2	3	2	3	1	2	4	2	2	2	2	3	3	2	3
Ixinandria steinbachi	1	12	7	6	7	12	12	0.67	0.14	0.40	0.53	0.65	0.03	0.12	0.12	1	1	2	1	1	1	1	2	2	1	1	1	2	2	1	4
Lamontichthys filamentosus	2	14	8	6	7	34	33	0.86	0.12	0.57	0.44	0.45	0.03	0.07	0.07	3	1	1	1	1	1	1	1	2	1	1	1	3	1	1	5
Lamontichthys llanero	2	14	8	6	7	30	36	0.70	0.09	0.55	0.45	0.43	0.04	0.08	0.09	3	1	1	1	1	1	1	1	2	1	1	1	3	1	1	5
Limatulichthys punctatus	10	12	7	6	7	9	11	0.63	0.08	0.54	0.45	0.63	0.04	0.06	0.07	3	1	2	1	3	1	2	4	2	1	2	2	3	3	2	2
Loricaria cataphracta	8	12	7	6	7	3	6	0.78	0.10	0.55	0.38	0.58	0.03	0.06	0.07	3	1	3	2	2	2	2	2	1	1	2	2	3	4	3	1
Loricaria simillima	9	12	7	6	7	3	7	0.74	0.10	0.53	0.36	0.55	0.03	0.07	0.07	3	1	3	2	2	2	2	2	1	1	2	2	2	3	3	1
Loricaria sp. Colombia	1	12	7	6	7	3	7	0.79	0.11	0.54	0.38	0.59	0.03	0.06	0.06	3	1	3	2	2	2	2	2	1	1	2	2	2	3	2	1
Loricaria sp. Paraguay	13	12	7	6	7	3	6	0.64	0.10	0.53	0.38	0.55	0.03	0.06	0.07	3	1	3	2	2	2	2	2	1	1	2	2	3	4	3	1

Loricariichthys platymetopon	9	12	7	6	7	8	11	0.76	0.12	0.48	0.50	0.63	0.04	0.09	0.11	3	2	3	1	3	1	2	4	2	1	2	2	3	4	3	3
Paraloricaria vetula	3	12	7	6	7	5	6	0.58	0.11	0.49	0.35	0.55	0.03	0.07	0.08	2	1	2	2	2	2	2	2	1	1	2	2	3	3	1	1
Pseudohemiodon aff. *apithanos*	6	12	7	6	7	3	5	0.61	0.10	0.50	0.40	0.57	0.04	0.11	0.08	3	1	2	2	2	2	3	3	1	1	1	2	2	3	2	1
Pseudohemiodon apithanos	8	12	7	6	7	3	5	0.59	0.11	0.47	0.34	0.59	0.04	0.10	0.09	3	1	2	2	2	2	3	3	1	2	2	2	2	3	2	1
Pseudohemiodon laminus	2	12	7	6	7	3	6	0.49	0.07	0.51	0.35	0.64	0.02	0.09	0.07	3	1	2	2	2	2	3	3	1	2	2	2	2	3	2	1
Pseudohemiodon laticeps	1	12	7	6	7	5	7	0.44	0.09	0.44	0.31	0.44	0.03	0.10	0.08	3	1	2	2	2	2	3	3	1	2	2	2	3	3	2	1
Pseudohemiodon sp.	4	12	7	6	7	3	6	0.50	0.07	0.51	0.37	0.67	0.02	0.10	0.08	3	1	2	2	2	2	3	3	1	2	2	2	2	3	2	1
Pseudoloricaria laeviuscula	12	12	7	6	7	10	11	0.58	0.08	0.51	0.45	0.64	0.04	0.07	0.09	3	1	2	1	3	1	2	2	2	1	2	2	3	3	2	2
Pterosturisoma microps	3	14	7	6	7	43	41	0.79	0.12	0.51	0.30	0.39	0.02	0.08	0.08	3	1	1	1	1	1	1	1	2	1	1	3	3	1	1	5
Rineloricaria aff. *latirostris*	3	12	7	6	7	8	8	0.69	0.10	0.45	0.48	0.77	0.04	0.10	0.10	3	1	3	2	1	1	2	2	2	1	2	1	2	2	1	4
Rineloricaria eigenmanni	2	12	7	6	7	8	8	0.74	0.10	0.53	0.44	0.65	0.04	0.07	0.08	3	1	3	2	1	1	2	2	2	1	2	1	1	3	2	4
Rineloricaria fallax	10	12	7	6	7	8	7	0.70	0.09	0.56	0.41	0.69	0.04	0.06	0.07	3	1	3	2	1	1	2	2	2	1	2	1	1	3	2	4
Rineloricaria lanceolata	10	12	7	6	7	6	6	0.73	0.10	0.53	0.36	0.67	0.04	0.07	0.08	3	1	3	2	1	1	2	2	2	1	2	1	1	3	2	4
Rineloricaria melini	1	12	7	6	7	5	7	0.90	0.11	0.55	0.44	0.55	0.04	0.05	0.06	3	1	3	2	1	1	2	2	2	1	2	1	1	3	2	4
Rineloricaria parva	3	12	7	6	7	8	8	0.69	0.09	0.55	0.38	0.58	0.03	0.06	0.07	3	1	3	2	1	1	2	2	2	1	2	1	2	2	2	4
Rineloricaria platyura	7	12	7	6	7	9	8	0.76	0.10	0.56	0.37	0.63	0.04	0.06	0.07	3	1	3	2	1	1	2	2	2	1	2	1	2	3	2	4
Rineloricaria aff. *stewarti*	11	12	7	6	7	6	7	0.68	0.08	0.55	0.42	0.72	0.03	0.06	0.08	3	1	3	2	1	1	2	2	2	1	2	1	1	2	2	4
Rineloricaria sp. Puerto Ayacucho	2	12	7	6	7	8	8	0.65	0.08	0.54	0.42	0.58	0.03	0.06	0.06	3	1	3	2	1	1	2	2	2	1	2	1	2	3	2	4
Rineloricaria sp. Ucayali 1	2	12	7	6	7	6	8	0.71	0.09	0.55	0.37	0.61	0.04	0.05	0.07	3	1	3	2	1	1	2	2	2	1	2	1	2	3	2	4
Rineloricaria teffeana	1	12	7	6	7	9	10	0.70	0.09	0.61	0.44	0.64	0.03	0.04	0.07	3	1	3	2	1	1	2	2	2	1	2	1	2	3	2	4
Spatuloricaria sp. Nanay	1	12	7	6	7	4	3	0.64	0.09	0.49	0.34	0.62	0.05	0.09	0.10	2	1	2	2	1	2	1	2	2	1	2	1	3	2	1	4
Sturisoma aureum	7	14	7	6	7	29	26	0.85	0.09	0.56	0.52	0.54	0.03	0.06	0.07	3	1	1	1	1	1	1	1	2	2	2	1	2	5	2	5
Sturisoma festivum	4	14	7	6	7	27	31	0.93	0.11	0.59	0.38	0.55	0.04	0.07	0.07	3	1	1	1	1	1	1	1	2	2	2	1	2	5	2	5
Sturisoma nigrirostrum	9	14	7	6	7	24	22	0.73	0.10	0.50	0.39	0.59	0.03	0.06	0.07	3	1	1	1	1	1	1	1	2	2	2	1	2	5	2	5
Sturisomatichthys leightoni	2	14	7	6	7	35	30	0.85	0.11	0.56	0.41	0.67	0.04	0.07	0.07	3	1	1	1	1	1	1	1	2	1	2	1	2	5	2	5

* quantitative data recorded on its close relative: *C. cephalaspis*

3.2 Characterization of phylogenetic dependence in biological traits of the Loricariinae.

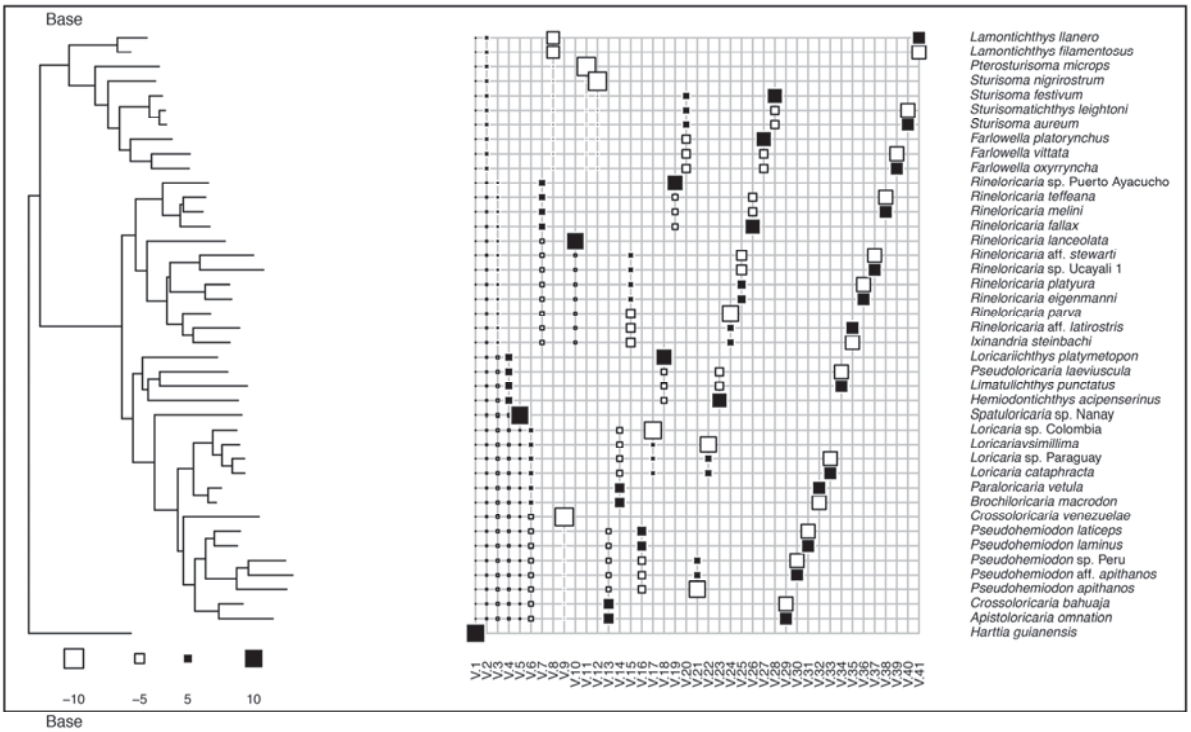
To allow the computation of the orthogram tests, the molecular phylogeny was restricted to the subset of species (n = 42) corresponding to those included in the table of biological traits (Tab. 2). The tree topology together with the vectorial basis (Fig. 8) allowed the identification of the ranking of the nodes, and consequently to see which vector accounted for which node.

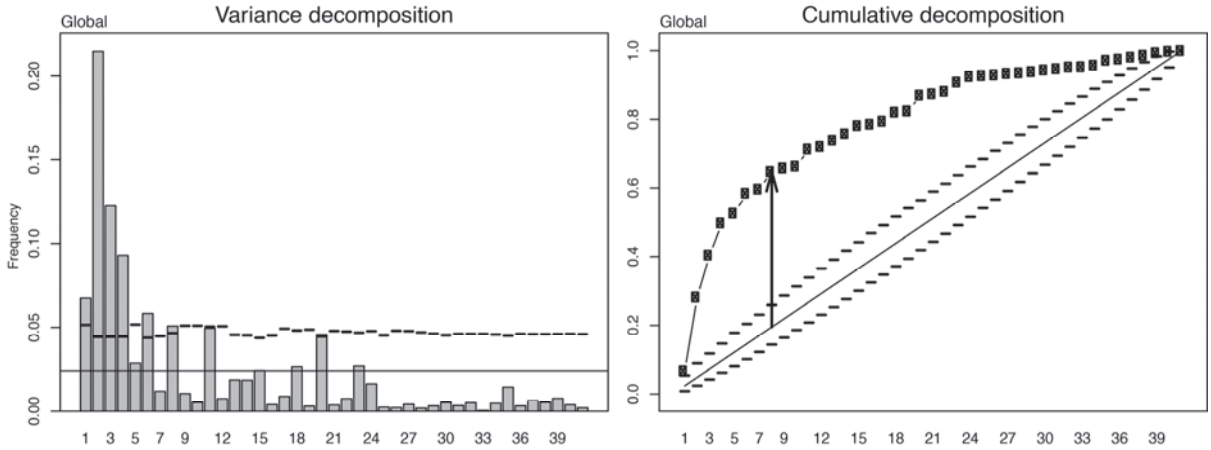
3.2.1 Global pattern of phylogenetic dependence of the complete table

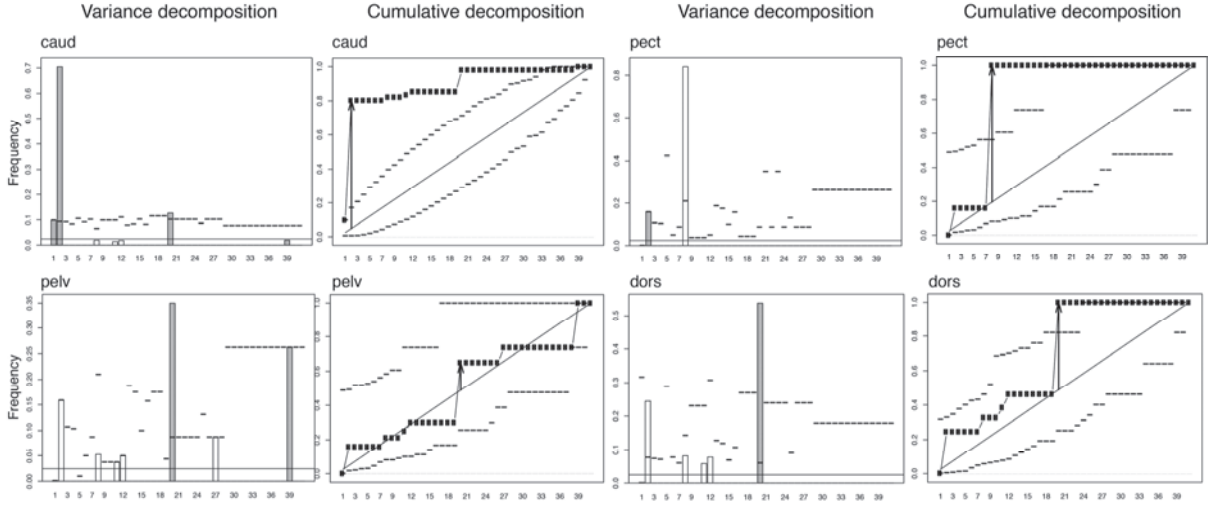
The multivariate orthogram test (Fig. 8-Global), indicated that several vectors explained the greatest part of the variance. Eleven vectors showed indeed departure from the expected value under the hypothesis of absence of phylogenetic dependence (given by the solid line in Fig. 8), and vectors 1, 2, 3, 4, 6, and 8 peaked outside of the confidence limit (given by the dashes). The cumulative orthogram (Fig. 8) confirmed predominance of numerous vectors in the variance distribution. A significant departure from H0 was registered for several vectors, and this pattern was preserved for several successive vectors. The maximum deviation from the expected value was given for the sum of the height first vectors (vertical arrow in Fig. 8) meaning that maximum variation was registered on these height vectors. All statistical tests were also significant. The small value of SkR2k indicated that the variance distribution was rather skewed towards the root (Table 3; SkR2k: $p(X \leq X_{obs}) = 0.0001$), indicating that the deepest nodes of the phylogeny explained the variance distribution. R2Max was also significant (Table 3; $p(X \geq X_{obs}) = 0.0001$). This result confirmed the predominance of few vectors in the variance distribution. These results suggested that the set of biological traits recorded have been shaped deep in the phylogeny and underwent sudden diversification events suggesting a rather punctual evolutionary pattern of traits in the phylogeny.

3.2.2 Patterns of phylogenetic dependence in meristic data

The meristic data corresponded to a set of six discrete quantitative variables. The orthogram of the number of caudal-fin rays [caud] (Fig. 8-caud), indicated that vector 2, and in less proportions vector 20, explained the greatest part of the variance. The cumulative orthogram (Fig. 8-caud) confirmed predominance of vectors 2 and 20 in the variance distribution. The maximum deviation from the expected value under absence of phylogenetic


Base
Lamontichthys llanero
Lamontichthys filamentosus
Pterosturisoma microps
Sturisoma nigrirostrum
Sturisoma festivum
Sturisomatichthys leightoni
Sturisoma aureum
Farlowella platorynchus
Farlowella vittata
Farlowella oxyrryncha
Rineloricaria sp. Puerto Ayacucho
Rineloricaria teffeana
Rineloricaria melini
Rineloricaria fallax
Rineloricaria lanceolata
Rineloricaria aff. stewarti
Rineloricaria sp. Ucayali 1
Rineloricaria platyura
Rineloricaria eigenmanni
Rineloricaria parva
Rineloricaria aff. latirostris
Ixinandria steinbachi
Loricariichthys platymetopon
Pseudoloricaria laeviuscula
Limatulichthys punctatus
Hemiodontichthys acipenserinus
Spatuloricaria sp. Nanay
Loricaria sp. Colombia
Loricariavsimillima
Loricaria sp. Paraguay
Loricaria cataphracta
Paraloricaria vetula
Brochiloricaria macrodon
Crossoloricaria venezuelae
Pseudohemiodon laticeps
Pseudohemiodon laminus
Pseudohemiodon sp. Peru
Pseudohemiodon aff. apithanos
Pseudohemiodon apithanos
Crossoloricaria bahuaja
Apistoloricaria omnation
Harttia guianensis
−10
−5
5
10
Base


Global
Variance decomposition
Frequency
Global
Cumulative decomposition


Variance decomposition
Cumulative decomposition
Variance decomposition
Cumulative decomposition
caud
caud
pect
pect
Frequency
pelv
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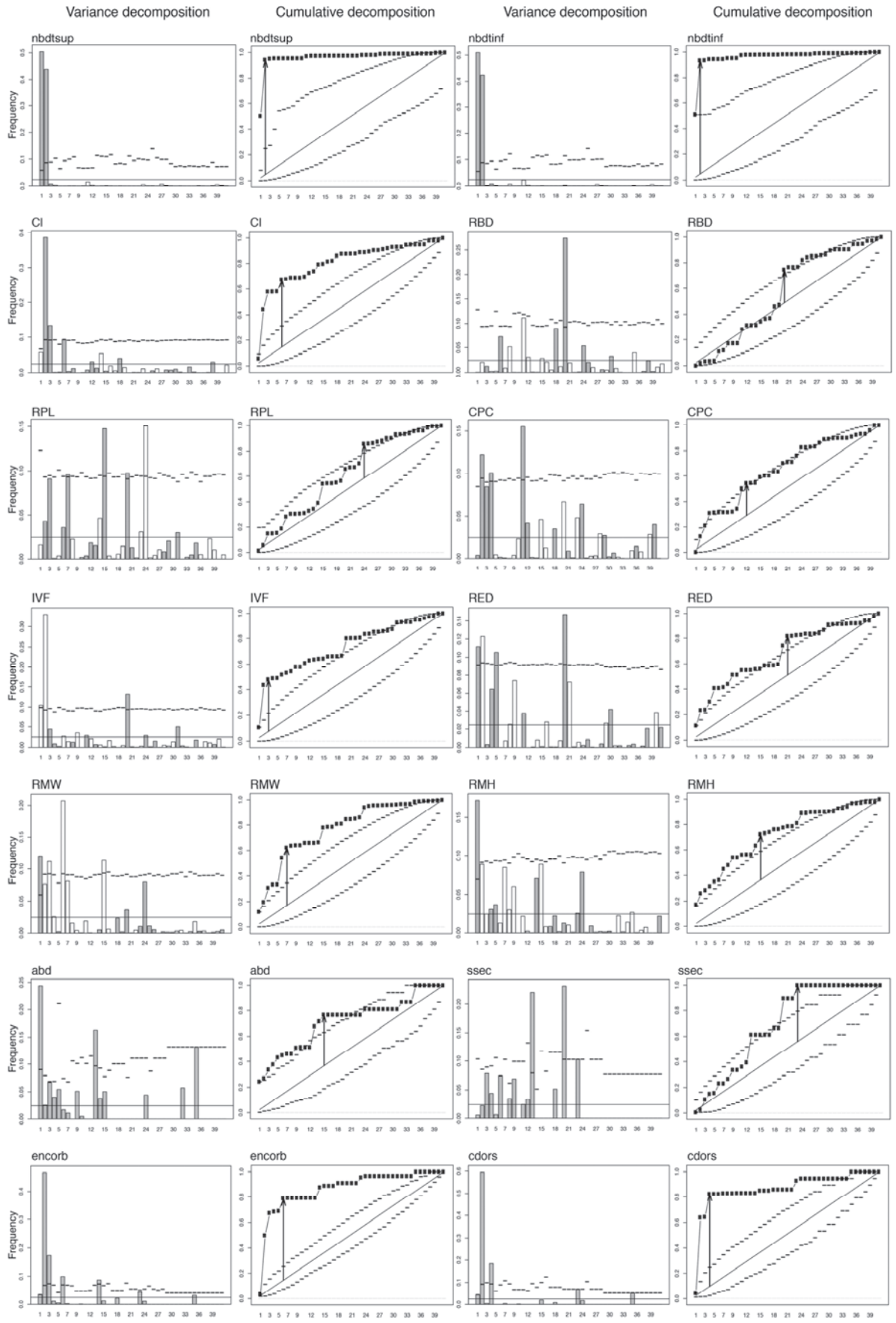
dors
Frequency


Variance decomposition
Cumulative decomposition
Variance decomposition
Cumulative decomposition
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nbdtsup
nbdtinf
nbdtinf
CI
CI
RBD
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CPC
CPC
IVF
IVF
RED
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RMH
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abd
ssec
ssec
encorb
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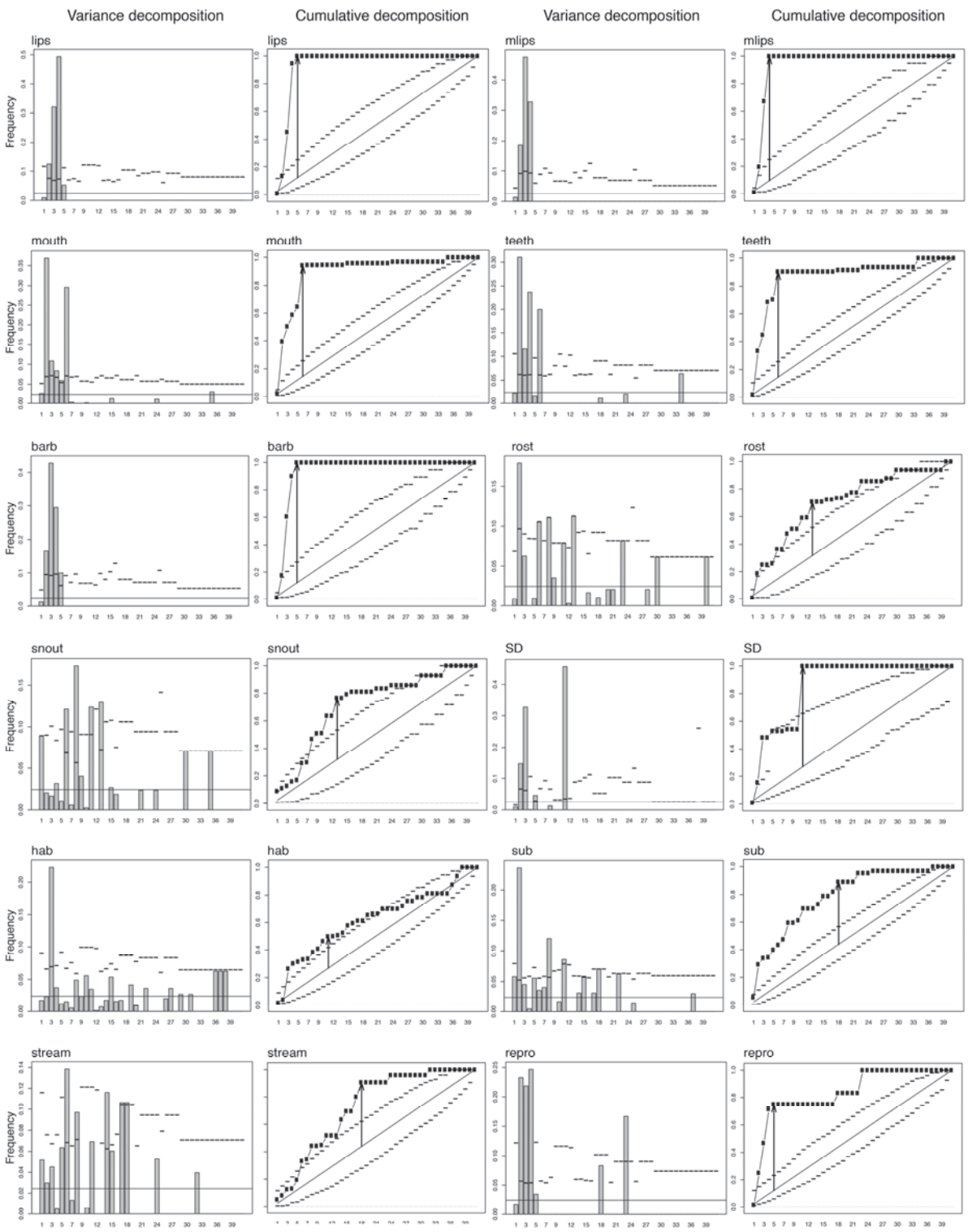
cdors
Frequency


Variance decomposition
Cumulative decomposition
Variance decomposition
Cumulative decomposition
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lips
mlips
mlips
mouth
mouth
teeth
teeth
barb
barb
rost
rost
snout
snout
SD
SD
hab
hab
sub
sub
stream
stream
repro
repro
Frequency

Fig. 8. Variance decomposition of biological traits across the orthonormal basis defined by the phylogenetic tree topology. Base: Phylogenetic tree (left) and description of the topology of the tree by the orthonormal vectors V.1 to V.41 which represent nodes and descendent tips (right). The indicative scale show squares with sizes proportional to the values of the orthonormal vectors (white and black for negative and positive values, respectively). **Global**: multivariate orthogram: variance decomposition of the multivariate dataset using the orthogram plot (left panel) and the cumulative orthogram plot (right panel). **caud** to **repro**: univariate orthograms: each plot represents variance decomposition for a single variable at a time using the orthogram (left panel) and the cumulative orthogram (right panel) plots. Titles and details for each variable are provided table 1. In the orthogram plots, the abscise gives the number of the vectors associated to nodes while the ordinate shows the contribution of the vector to the variance of the trait given by the squared regression coefficient (white and grey for positive and negative coefficients, respectively); dashes correspond to the upper confidence limit at 5 % deduced from 9,999 Monte Carlo permutations; solid line represents the mean value. In the cumulative orthogram plots the ordinate shows the cumulated contribution of successive vectors to the variance; black squares represent the observed value of cumulated squared regression coefficients; solid diagonal line represents expected value under absence of phylogenetic dependence; dashes correspond to the bilateral 95% confidence interval. Vertical arrow indicates the position of maximum deviation from the expected value (diagonal line).

dependence was given for the sum of the two first vectors (vertical arrow) meaning that maximum variation was registered on these vectors. All four statistical tests were also significant, particularly R2Max (Table 3; Corrected p-value: $Cp(X \geq X_{obs}) = 0.0013$), indicating that a punctual modification of the number of caudal-fin rays occurred at a particular node and that it stayed unchanged afterwards. Moreover, the variance distribution was rather skewed towards the root (Table 3; SkR2k: $Cp(X \leq X_{obs}) = 0.0007$), indicating that the deepest nodes of the phylogeny explained the variance distribution. These results suggested that this trait has been shaped deep in the phylogeny, and that a major punctual event occurred at node 2, between Farlowellina and Loricariina lineages, with a reduction of the number of caudal-fin rays in Loricariina (12 versus 14). In addition a second event occurred at node 20, between *Farlowella* on one side and *Sturisoma* and *Sturisomatichthys* on the other side, with a reduction of the number of caudal-fin rays in *Farlowella* (13 versus 14). The orthogram of the number of pectoral-fin rays [pect] (Fig. 8-pect), identified a single major punctual event that occurred at node 8 between *Lamontichthys* and other Farlowellina (orthogram and cumulative orthogram pointed out vector 8 as explaining the major part of the variance distribution). Moreover R2Max was highly significant (Table 3; $Cp(X \geq X_{obs}) = 0.0055$) implying that few or even a single vector was responsible of the trait variance. In addition SkR2k ($Cp(X \leq X_{obs}) = 0.0403$) was small suggesting that the variance was rather skewed toward the root. Dmax ($Cp(X \geq X_{obs}) = 0.0107$) and overall SCE ($Cp(X \geq X_{obs}) = 0.0235$) detected a local effect. A single punctual event explained thus the increase of the number of pectoral-fin rays in *Lamonticthys* (8 versus 7 in all other Loricariinae). The

orthogram plot of the number of pelvic-fin rays [pelv] (Fig. 8-pelv), pointed out vector 19 as explaining the major part of the trait variance. However, no vector peaked outside of the confidence limit in the cumulative orthogram, and none of the four statistics were significant. The decrease of the number of pelvic-fin rays in two species of *Farlowella* (5 versus 6) corresponded thus to randomly distributed events independent of the phylogeny. The orthogram of the number of dorsal-fin rays [dors] (Fig. 8-dors), also indicated vector 19 as explaining major part of the variance distribution. However, contrary to the preceding case, the cumulative orthogram shows a strong departure from the value under absence of phylogenetic dependence with vector 19 peaking out of the confidence limit (vertical arrow on vector 19). In addition, only R2Max was significant (Table 3; $Cp(X \geq X_{obs}) = 0.0360$) implying a rather unique punctual event as explaining the decrease of the number of dorsal-fin rays in all members of Farlowella (6 versus 7). The orthograms of the number of premaxillary [nbdtsup] and dentary [nbdtinf] teeth displayed almost identical orthograms. The orthogram plots (Figs. 8-nbdtsup and 8-nbdtinf) pointed vectors 1 and 2 as explaining the major part of the variance distribution. Cumulative orthograms confirmed this fact with a maximum departure from the expected value under absence of phylogenetic dependence registered for the sum of two first vectors (arrow on vector 2). Out of the four statistics tested (Table 3), only R2Max was not significant meaning that a rather gradual effect was responsible of the variance distribution. Moreover, this distribution was skewed towards the root (Table 3, SkR2k: $Cp(X \leq X_{obs}) = 0.0007$ for both variables). Consequently, these two traits have been also shaped rather deep in the phylogeny. Two major successive events can be reconstructed in the overall gradual trend: a first decrease in the number of premaxillary and dentary teeth between Harttiini and Loricariini lineages (Figs. 8-nbdtsup and 8-nbdtinf, vector 1), and a second decrease between Farlowellina and Loricariina lineages (Figs. 8-nbdtsup and 8-nbdtinf, vector 2).

3.2.2 Patterns of phylogenetic dependence in morphometric data

The ecomorphometric data corresponded to a set of eight continuous quantitative variables. The orthogram of the Compression Index [CI] (Fig. 8-CI), pointed out vector 2 and 3 as explaining the major part of the variance. The cumulative orthogram confirmed predominance of vector 6 in the variance distribution. The maximum deviation from the expected value under absence of phylogenetic dependence was thus given for the sum of the six first vectors (vertical arrow). All four statistical tests were also significant. The significance of R2Max

Table 3. Tests against the absence of phylogenetic dependence for the multivariate dataset and for each variable. Variables are titled as in table 1. Global: multivariate dataset; dark grey: quantitative data; light grey: qualitative data. R2Max, SkR2k, Dmax, and SCE tests: tests against phylogenetic dependence as defined by Ollier *et al.* (2006). Pvalues: uncorrected significances of the tests using 9,999 random permutations. C Pvalues: corrected Pvalues using the control for false discovery rate for multiple testing under dependency (Benjamini and Yekutieli, 2001). Bold types indicate significant tests for $\alpha = 5\%$.

	Global	caud	pect	pelv	dors	nbdtsup	nbdtinf	CI	RBD	RPL	CPC	IVF	RED	RMW	RMH
R2Max test	0.2147	0.7045	0.8400	0.3500	0.5385	0.5042	0.5100	0.3868	0.2743	0.1507	0.1553	0.3318	0.1478	0.2073	0.1725
P value (X ≥ X obs.)	**0.0001**	0.0001	0.0006	0.4013	0.0045	0.0427	0.0436	0.0002	0.0233	0.5778	0.4771	0.0008	0.3371	0.0313	0.3600
P value (X ≤ X obs.)	1.0000	1.0000	0.9995	0.5988	0.9956	0.9574	0.9565	0.9999	0.9768	0.4223	0.5230	0.9993	0.6630	0.9688	0.6401
C Pvalue (X ≥ X obs)	-	**0.0013**	**0.0055**	1.0000	**0.0360**	0.2613	0.2613	**0.0022**	0.1745	1.0000	1.0000	**0.0068**	1.0000	0.2084	1.0000
C P value (X ≤ X obs.)	-	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SkR2k test	9.3633	5.3148	7.0388	21.3513	13.4635	2.4191	2.3916	8.8212	18.4860	16.5208	15.0892	11.7447	13.8664	10.0977	12.2109
P value (X ≥ X obs.)	1.0000	1.0000	0.9917	0.5548	0.9575	1.0000	1.0000	1.0000	0.8242	0.9519	0.9862	1.0000	0.9988	1.0000	0.9996
P value (X ≤ X obs.)	**0.0001**	0.0001	0.0084	0.4453	0.0426	0.0001	0.0001	0.0001	0.1759	0.0482	0.0139	0.0001	0.0013	0.0001	0.0005
C Pvalue (X ≥ X obs)	-	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
C P value (X ≤ X obs.)	-	**0.0007**	**0.0403**	1.0000	0.1891	**0.0007**	**0.0007**	**0.0007**	0.7269	0.2063	0.0641	**0.0007**	**0.0078**	**0.0007**	**0.0033**
Dmax test	0.4523	0.7544	0.8049	0.1622	0.5122	0.8943	0.8856	0.5271	0.2587	0.2712	0.2497	0.4073	0.3053	0.4526	0.3575
P value (X ≥ X obs.)	**0.0001**	0.0001	0.0017	0.5767	0.0169	0.0001	0.0001	0.0001	0.0411	0.0295	0.0504	0.0007	0.0061	0.0001	0.0044
P value (X ≤ X obs.)	1.0000	1.0000	0.9984	0.4234	0.9832	1.0000	1.0000	1.0000	0.9590	0.9706	0.9497	0.9994	0.9940	1.0000	0.9957
C Pvalue (X ≥ X obs)	-	**0.0007**	**0.0107**	1.0000	0.0779	**0.0007**	**0.0007**	**0.0007**	0.1759	0.1309	0.2083	**0.0049**	**0.0318**	**0.0007**	**0.0240**
C P value (X ≤ X obs.)	-	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SCE test	4.1082	7.9127	7.4794	0.2982	2.2223	11.2733	11.2529	4.7354	0.6015	0.6913	1.0890	2.6506	1.5726	3.5431	2.2563
P value (X ≥ X obs.)	**0.0001**	0.0001	0.0047	0.9968	0.0435	0.0001	0.0001	0.0001	0.1484	0.1056	0.0317	0.0002	0.0037	0.0001	0.0007
P value (X ≤ X obs.)	1.0000	1.0000	0.9954	0.0033	0.9566	1.0000	1.0000	1.0000	0.8517	0.8945	0.9684	0.9999	0.9964	1.0000	0.9994
C Pvalue (X ≥ X obs)	-	**0.0007**	**0.0235**	1.0000	0.1931	**0.0007**	**0.0007**	**0.0007**	0.6133	0.4520	0.1461	**0.0014**	**0.0202**	**0.0007**	**0.0047**
C P value (X ≤ X obs.)	-	1.0000	1.0000	0.3955	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Table 3. End.

	abd	ssec	encorb	cdor	lips	mlips	mouth	teeth	barb	rost	snout	SD	hab	sub	stream	repro
R2Max test	0.2439	0.2316	0.4656	0.5948	0.4933	0.4754	0.3686	0.3104	0.4278	0.1792	0.1732	0.4565	0.2225	0.2358	0.1385	0.2468
P value (X ≥ X obs.)	0.0878	0.0254	0.0001	0.0001	0.0001	0.0001	0.0002	0.0001	0.0001	0.0625	0.0971	0.0467	0.0003	0.0001	0.0546	0.0001
P value (X ≤ X obs.)	0.9123	0.9747	1.0000	1.0000	1.0000	1.0000	0.9999	1.0000	1.0000	0.9376	0.9030	0.9534	0.9998	1.0000	0.9455	1.0000
C Pvalue (X ≥ X obs)	0.4384	0.1791	**0.0013**	**0.0013**	**0.0013**	**0.0013**	**0.0022**	**0.0013**	**0.0013**	0.3257	0.4655	0.2665	**0.0030**	**0.0013**	0.2974	**0.0013**
C P value (X ≤ X obs.)	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SkR2k test	12.5362	13.3044	6.3714	6.3035	3.4566	3.1168	5.0414	6.0828	3.3051	12.8731	12.3094	6.6438	16.2266	9.5568	11.8825	7.6321
P value (X ≥ X obs.)	0.9965	0.9961	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9984	0.9990	1.0000	0.9926	1.0000	0.9999	1.0000
P value (X ≤ X obs.)	0.0036	0.0040	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0017	0.0011	0.0001	0.0075	0.0001	0.0002	0.0001
C Pvalue (X ≥ X obs)	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
C P value (X ≤ X obs.)	**0.0196**	**0.0208**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0097**	**0.0069**	**0.0007**	**0.0375**	**0.0007**	**0.0014**	**0.0007**
Dmax test	0.4008	0.4390	0.6420	0.7296	0.8780	0.9024	0.7966	0.7578	0.8780	0.3894	0.4486	0.7317	0.2312	0.4525	0.4686	0.6280
P value (X ≥ X obs.)	0.0091	0.0018	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0019	0.0012	0.0001	0.0161	0.0001	0.0001	0.0001
P value (X ≤ X obs.)	0.9910	0.9983	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9982	0.9989	1.0000	0.9840	1.0000	1.0000	1.0000
C Pvalue (X ≥ X obs)	**0.0454**	**0.0108**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0108**	**0.0080**	**0.0007**	0.0772	**0.0007**	**0.0007**	**0.0007**
C P value (X ≤ X obs.)	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SCE test	2.2356	2.1686	6.7332	7.0930	10.5149	10.8364	8.4051	7.4323	10.5844	2.0923	2.5217	6.7142	0.8299	3.9123	2.6969	5.5610
P value (X ≥ X obs.)	0.0046	0.0032	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0019	0.0013	0.0001	0.0060	0.0001	0.0001	0.0001
P value (X ≤ X obs.)	0.9955	0.9969	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9982	0.9988	1.0000	0.9941	1.0000	1.0000	1.0000
C Pvalue (X ≥ X obs)	**0.0235**	**0.0183**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0007**	**0.0114**	**0.0082**	**0.0007**	**0.0288**	**0.0007**	**0.0007**	**0.0007**
C P value (X ≤ X obs.)	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

(Table 3; $Cp(X \geq X_{obs}) = 0.0022$) implied that few vectors explained the major part of the trait variance (punctual effect). The variance was moreover skewed toward the root (Table 3, SkR2k: $Cp(X \leq X_{obs}) = 0.0007$) meaning that this trait was shaped deep in the phylogeny. In summary, the Compression Index underwent a sudden modification between Farlowellina and Loricariina lineages, the latter being generally less deep and wider in body shape, followed by a second event between *Rineloricaria* and the remaining Loricariina, and finally between the *Loricaria* and *Pseudohemiodon* groups, the latter being extremely depressed and wide. Following the interpretation of the CI, members of Farlowellina and *Rineloricaria* are inhabitant of biotopes with slower flowing waters. The orthograms of the Relative Body Depth [RBD], Relative Peduncle Length [RPL], and Caudal Peduncle Compression index [CPC] displayed variations for several vectors that appeared independent from the phylogeny (none of the tests were significant). The variations in body shape potentially related to swimming abilities as suggested by these three descriptors corresponded thus to rather random events in Loricariinae. The orthogram of the Index of Ventral Flattening [IVF] pointed out vectors 1, 2 and 20 as explaining the major part of the variance (Fig. 8-IVF). The cumulative orthogram confirmed the predominance of the three first vectors in the distribution of the trait variance (vertical arrow on vector 3). The maximum deviation from the expected value under absence of phylogenetic dependence was thus given for the sum of the three first vectors. A second peak was also observed for the twentieth vector. The significance of the four statistics, especially R2Max (Table 3; $Cp(X \geq X_{obs}) = 0.0068$) and SkR2k (Table 3, $Cp(X \leq X_{obs}) = 0.0007$) indicated rather punctual events shaped deep in the phylogeny to explain the variation in body shape. Two successive punctual events occurred between Harttiini and Loricariini lineages with an increase in body depth in Loricariini, and between Farlowellina and Loricariina lineages, the latter being usually deeper. A last event occurred between *Farlowella* and the trans-Andean *Sturisoma* + *Sturisomatichthys* with an index usually greater in the latter. Three major punctual events explained thus the adaptation to fast flowing waters in Harttiini, and Farlowellina. The orthograms of the Relative Eye Diameter [RED], Relative Mouth Width [RMW], and Relative Mouth Height [RMH] displayed comparable patterns of phylogenetic dependence (Figs. 8-RED, 8-RMW, and 8-RMH). A rather gradual effect was indeed responsible of traits variance (as suggested by the non significance of R2Max tests, Table 3), and these traits were shaped deep in the phylogeny (small SkR2k tests, Table 3). However, different vectors were responsible of the variance distribution. Orthogram of the Relative Eye Diameter (Fig. 8-RED) pointed out vectors 1, 2, 5, and 21 as explaining the major part of variance distribution, that is to say between Harttiini

and Loricariini lineages (Harttiini having larger eye), between Farlowellina and Loricariina (Farlowellina with usually smaller eye), and between members of *Rineloricaria* consisting in *R.* sp. Puerto Ayacucho and its sister group comprising *R. fallax*, *R. melini*, and *R. teffeana* (the latter with larger eye). The orthogram of the Relative Mouth Width (Fig. 8-RMW) pointed out vectors 1, 3, 6, and 15 as explaining the greatest part of the trait variance, *i.e.* between Harttiini and Loricariini lineages (Harttiini having wider mouth), between *Rineloricaria* and all other Loricariina (consisting in *Loricariichthys*, *Loricaria* and *Pseudohemiodon* groups) the latter having usually a wider mouth, between *Loricaria* and *Pseudohemiodon* groups (*Pseudohemiodon* with wider mouth), and between Southeastern and Northern *Rineloricaria* (Southeastern species with wider mouth). The orthogram of the Relative Mouth Height (Fig. 8-RMH) pointed out the first vector as explaining the major part of the variance distribution, *i.e.* between Harttiini and Loricariini, *Harttia* having the highest mouth. However several other vectors also showed departure from the expected value in absence of phylogenetic dependence, but did not peak out of the confidence limit.

3.2.3 Patterns of phylogenetic dependence in qualitative data

The qualitative data corresponded to a set of 16 variables comprising 12 morphological, one ethological, and three ecological variables, the latter being interpretable as ordinal. Among these 16 variables, six displayed rather diffuse patterns of phylogenetic dependence even though rather deeply shaped in the phylogeny (R2Max test not significant and small SkR2k; table 3). These were the presence or absence of an abdominal cover [abd], of a secondary organization in the abdominal cover [ssec], and of a rostrum [rost], the snout shape [snout], the secondary sexual dimorphism [SD], and the water velocity [stream]. The orthogram of the presence or absence of an abdominal cover (Fig. 8-abd) pointed out vectors 1 and 13 as explaining the major part of the trait variance, that is to say between *Harttia guianensis* (without cover) and the Loricariini (usually covered), and between *Crossoloricaria* + *Apistoloricaria* (incompletely covered) and *Pseudohemiodon* (covered). Several vectors also showed departure from H0 that explained this gradual trend in the evolution of the trait, but did not peak outside of the confidence limit (e.g. vectors 2, 3, 4, 5, 9, 14, 15, 24, 32, and 36). The orthogram of the presence or absence of a secondary organization of the abdominal cover (Fig. 8-ssec) showed a very similar pattern of the distribution of the trait variance and pointed out vectors 13 and 20 as explaining the major part of the variance. These vectors described important modification in the trait between *Crossoloricaria* + *Apistoloricaria* (with secondary

organization consisting in a medial row of plates on the abdomen) and *Pseudohemiodon* (without distinct organization in the abdominal cover), and between trans-Andean *Sturisoma* + *Sturisomatichthys* (abdominal cover indistinctly organized) and *Farlowella* (organized in two or three rows). The orthogram of the presence or absence of a rostrum (Fig. 8-rost) revealed numerous vectors showing departure from the hypothesis of absence of phylogenetic dependence (vectors 2, 3, 6, 8, 9, 11, 13, 24, 30, 40) suggesting that this trait appeared or disappeared successively in several lineages. However, only vector 2 peaked out of the confidence interval, that was between Loricariina and Farlowellina, the latter often having a rostrum. The orthogram of the snout shape (Fig. 8-snout) pointed out vectors 6, 8, 11, and 13 as explaining the major part of the trait variation implying multiple appearance events for a given shape. The snout was indeed often rounded among members of the *Pseudohemiodon* group compared to members of the *Loricaria* group (vector 6). Among the former, *Apistoloricaria* and cis-Andean *Crossoloricaria* indeed often displayed a rounded snout (vector 13). Among Farlowellina, *Lamonticthys* (vector 8) and *Pterosturisoma* (vector 11) also possessed a rounded snout compared to *Sturisoma* or *Farlowella*. Concerning the orthogram of the sexual dimorphism (Fig. 8-SD), vectors 2, 3, 5, and 11 explained the major part of the trait variance. All members of the Farlowellina, compared to members of Loricariina (vector 2) displayed a secondary sexual dimorphism mostly expressed through the hypertrophy of odontodes in males, except in *Pterosturisoma* that did not expressed such features (vector 11). Members of *Rineloricaria* also exhibited the sexual dimorphism through the hypertrophy of odontodes compared to the remaining Loricariina that expressed a secondary sexual dimorphism through the characteristics of the mouth (vector 3), except for *Spatuloricaria* in which SD is also expressed through odontodes (vector 5). The orthogram of the water velocity (Fig. 8-stream) pointed out numerous vectors in the distribution of the variance of this ecological parameter (1, 2, 3, 5, 6, 8, 11, 14, 15, 17, 18, 24, and 32) among which vectors 6, 8, 14, 17, and 18 explained the greatest part of the parameter variation by peaking outside of the confidence interval. Different successive adaptations to water velocity occurred in different lineages such as between *Loricariichthys* (mostly adapted to quiet areas) and the remaining members of its group (mainly inhabiting waters of medium velocity) (vector 18), or between *Lamontichthys* + *Pterosturisoma* (members of the rheophilic fauna) and the remaining Farlowellina (mostly living in medium speed waters) (vectors 8 and 11). The nine remaining qualitative traits displayed rather deep punctual effects in the distribution of the variance with few vectors explaining this distribution (R2Max test significant and small SkR2k; table 3). The orthogram of the presence or absence of a postorbital notch [encorb]

(Fig. 8-encorb) pointed out vectors 2, 3, 6 and 14 as explaining the major part of the variance. The postorbital notch appeared between Farlowellina (absent) and Loricariina (usually present) (vector 2). A second modification of the trait occurred between *Rineloricaria* (usually with deep postorbital notches) and the remaining Loricariina (in which the postorbital notch can be deep or week) (vector 3). The vector 6 explained the modification of the trait between members of the *Loricaria* group (usually with deep postorbital notch) and the *Pseudohemiodon* group (with weak postorbital notch). The last vector (14) explained the modification of the postorbital notch between *Loricaria* (deep) and *Brochiloricaria* + *Paraloricaria* (weak). The orthogram of the presence or absence of predorsal keels [cdor] (Fig. 8-cdor) displayed a very similar evolutionary pattern compared to that of the postorbital notch by pointing out vectors 2 and 4 as explaining the variance distribution. The predorsal keels appeared between Farlowellina (absent) and Loricariina (usually present) lineages. This feature disappeared in most members of the *Loricariichthys* group compared to the remaining Loricariina (vector 4). The orthogram of the structure of the lip surface [lips] (Fig. 8-lips) also pointed out vectors 2, 3 and 4 as explaining the major part of the variance of the trait. From papillose in Harttiini, Farlowellina, and *Rineloricaria* compared to other Loricariina (vectors 2 and 3) the lip surface became smooth in members of the *Loricariichthys* group and filamentous in members of the *Pseudohemiodon* and *Loricaria* (except *Spatuloricaria* that has papillose lips) groups (vector 4). The appearance of fringed barbels [mlips] at the margin of the lower lip displayed exactly the same pattern with an orthogram (Fig. 8-mlips) pointing out vectors 2, 3 and 4 as explaining the major part of the variance. From absent or inconspicuous in Harttiini, Farlowellina, and *Rineloricaria* and members of the *Loricariichthys* group, the fringed barbels developed conspicuously in members of the *Pseudohemiodon* and *Loricaria* groups (vectors 2, 3, and 4). The mouth shape [mouth] underwent five successive modifications as suggested by the orthogram that pointed out vectors 2 to 6 as explaining the major part of the variance, and as confirmed by the cumulative orthogram that displayed a vertical arrow on the sixth vector meaning that the maximum of variation was registered for the sum of six first vectors (Fig. 8-mouth). In summary, from elliptical in Harttiini and Farlowellina, the mouth became bilobate in Loricariina (vector 2). The mouth stayed bilobate in *Rineloricaria*, in members of the *Loricariichthys* and *Loricaria* groups (vectors 3, 4, and 5) to finally display a trapezoidal opening in members of the *Pseudohemiodon* group (vector 6). The tooth shape [teeth] showed a very similar pattern of phylogenetic dependence and followed a similar evolutionary trend as suggested by the orthogram (Fig. 8-teeth). From pedunculated in Harttiini and Farlowellina, the tooth became indeed straight bicuspid in most

of the Loricariina (vector 2). Then from straight bicuspid in *Rineloricaria* (vector 3), two sudden modifications occurred: first a reduction in size in members of the *Loricariichthys* group (vector 4), and second the appearance of a spoon-shaped crown in members of the *Pseudohemiodon* group (vector 6). The size of the maxillary barbels [barb] also followed this pattern of evolution as indicated by the orthogram (Fig. 8-barb) that pointed out vectors 2 to 5 as explaining the variance distribution. This was also confirmed by the cumulative orthogram that placed the vertical arrow on the fifth vector (major part of the variance explained by the sum of the five first vectors). From inconspicuous in Harttiini, Farlowellina, *Rineloricaria*, and members of the *Loricariichthys* group (vectors 2, 3, and 4), the maxillary barbel became conspicuously developed in members of the *Loricaria* and *Pseudohemiodon* groups (vector 5). Among the ecological variables, the adaptation to a particular type of habitat [hab] displayed a single significant event as explaining the variance distribution. The orthogram (Fig. 8-hab) indeed pointed out vector 3 as explaining most of the distribution of the trait variance. This vector described the adaptation to forest creeks of numerous members of *Rineloricaria* compared to other Loricariinae that seemed to prefer medium to large rivers. Adaptation to a favored substrate [sub] followed a more complex pattern. The orthogram (Fig. 8-sub) pointed out vectors 2, 8, and 11 as explaining the greatest part of the variance by peaking out of the confidence interval, event though numerous vectors showed departure from the null hypothesis of the absence of phylogenetic dependence. These vectors contrasted Farlowellina to Loricariina (vector 2), and particularly within Farlowellina its members of the rheophilic fauna that were *Lamontichthys* (vector 8) and *Pterosturisoma* (vector 11) that live on rocks to the remaining *Farlowella*, *Sturisoma*, and *Sturisomatichthys* that often live on organic substrate such as submerged branches and leaves. The orthogram of the reproductive strategy [repro] (Fig. 8-repro) pointed out vectors 3, 4, 5 and 22 as explaining the major part of the trait variance. Most of the variance was however explained by the sum of the five first vectors as attested by the cumulative orthogram. From open water brooders in Harttiini and Farlowellina, the reproduction evolved toward different strategies in Loricariina (vector 2). If *Rineloricaria* and *Ixinandria* members evolved toward a cavity brooding strategy, the remaining Loricariina adapted to mouth brooding strategies (vector 3). Members of the *Loricariichthys* group evolved toward lip brooding strategies whereas members of the *Loricaria* and *Pseudohemiodon* groups evolved toward an abdomino-lip brooding strategy (vector 4). Within the *Loricariichthys* group from strictly lip brooders, *Pseudoloricaria* and *Limatulichthys* adapted toward an alternative strategy consisting in lip brooding using a support.

3.2.4 Exploration of co-evolution among traits using the MSPA

Biological traits were mainly structured on the first two axes of MSPA (Fig. 9d) that accounted for 61.18% of the total variation (42.17% for axis 1 and 19.01% for axis 2). The principal axes were mainly represented by deepest nodes of the phylogeny (vectors 2 and in less proportion 1 for the first axis, and vectors 3 and in less proportion 4, 5, and 6 for the second axis). Axis 1 mainly described the splitting between Harttiini and Loricariini (V.1) and overall Farlowellina and Loricariina (V.2) lineages. Axis 2 mostly described the main splitting events between Loricariina lineages, especially the splitting between *Rineloricaria* and the remaining Loricariina (V.3), between the *Loricariichthys* group and *Spatuloricaria* plus the *Loricaria-Pseudohemiodon* groups (V.4), between *Spatuloricaria* and the *Loricaria-Pseudohemiodon* groups (V.5), and between the *Loricaria* and the *Pseudohemiodon* group (V.6) (Figs. 8, 9 a and b). The traits displaying the most important variations and close between them and to the vectors shared the same evolutionary history (*i.e.* they underwent evolutionary events for the same nodes of the phylogeny following the same evolutionary process as described by the orthograms). On the first axis these traits corresponded, in absolute decreasing scores, to: absence of postorbital notches, open water brooder reproductive strategy, pedunculated teeth, elliptical mouth shape, 13 to 14 caudal-fin rays, adaptation to organic substrate, the absence or presence of predorsal keels, and numerous (usually $n > 20$) premaxillary and dentary teeth (Fig. 9 a and c). For all these traits, the pattern of their respective orthogram was highly similar with vector 2 (and 1 for the premaxillary and dentary teeth) explaining major part of the variance. On the second axis the traits displaying the most important scores (in decreasing order) were: cavity brooder reproductive strategy, sexual dimorphism mainly expressed through hypertrophy of odontodes and through characteristics of the mouth, papillose lips, presence or absence of fringed barbels, abdomino-lip brooder reproductive strategy, filamentous lips, conspicuous or inconspicuous maxillary barbels, rather smooth lips, and adaptations to large rivers or forest creeks (Fig. 9 a and c). All those traits displayed similar patterns of their orthograms implying mainly vectors 3 and 4 in the explanation of the variance distribution.

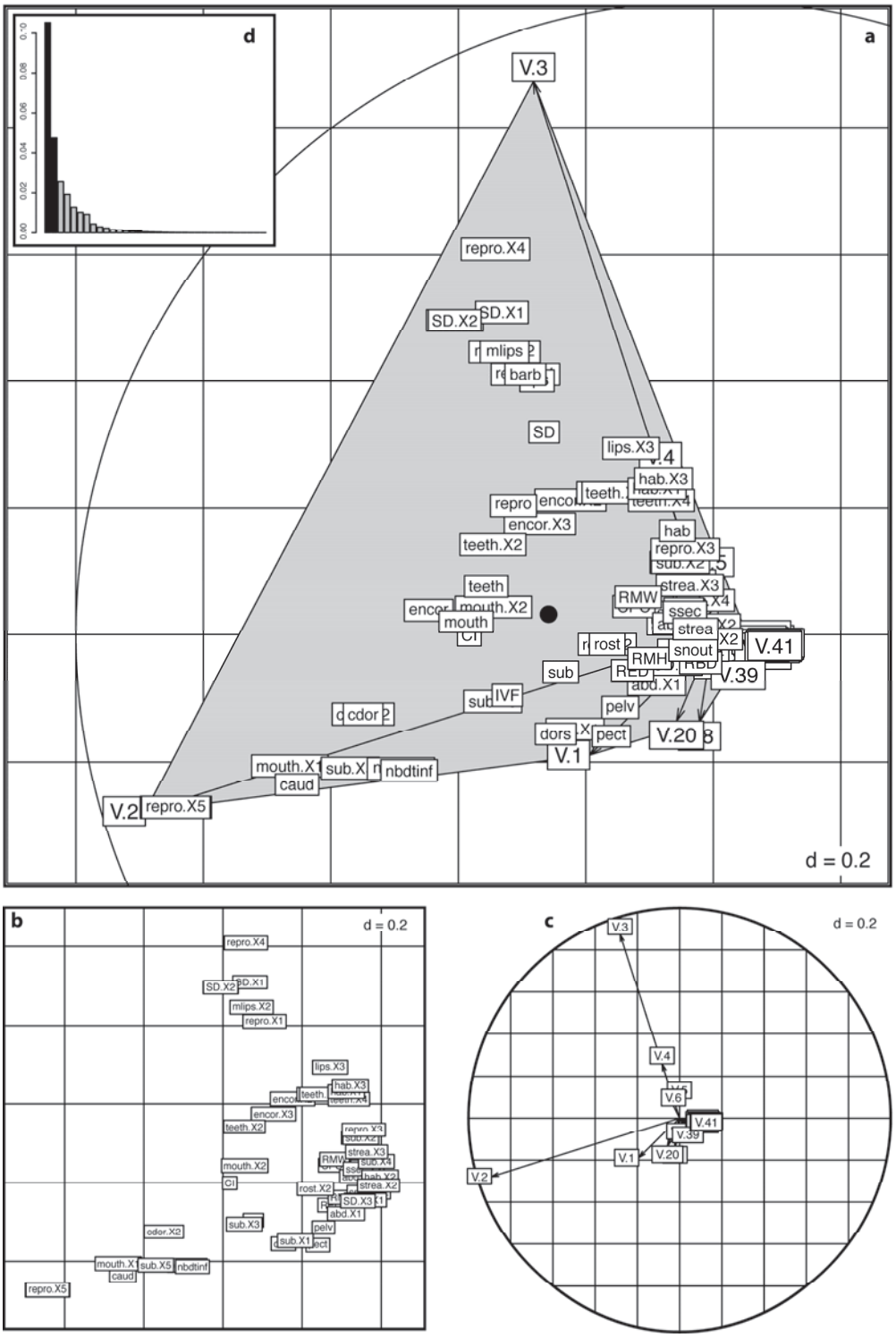

Fig. 9. Multi-Scale Pattern Analysis of biological traits in Loricariinae using the orthonormal basis describing the phylogenetic tree topology as proximity matrix. a: MSPA biplot axes 1-2: superimposition of vectors describing the phylogenetic tree and biological traits; arrows represent phylogenetic vectors (V.1 to V.41); boxes represent biological traits (qualitative variables located at the average of the coordinates of their modalities); longest arrows indicate the most important regions of the phylogenetic tree explaining the traits evolution, and boxes close to these arrows indicate the traits that underwent similar evolutionary changes for these same regions of the tree. b: projection of the biological traits in the first MSPA plane (qualitative variables represented only by their different modalities). c: projection of the phylogenetic vectors in the unitary radius circle axes 1-2. d: eigenvalues of the MSPA.

3.2.5 Molecular dating of the main innovations in Loricariinae

The likelihood ratio test of constant molecular clock was significantly rejected ($L_{clock} > L_{non\ clock}$; $2\Delta \ln L = 2724.1855$; D.F. = 369, p-value < 0.0001), implying local clocks. Relaxed molecular clock methods were accordingly applied. According to phylogenetic results, two calibration points (TMRCA) were used: one located at the node splitting the representatives of *Spatuloricaria* from Magdalena River from those from Orinoco and Amazon Rivers and estimated to -12 Ma, and a second located at the node splitting *Farlowella curtirostra* and *F. taphorni* both from Maracaibo basin, from Amazonian *Farlowella* and estimated to -8 Ma. The Bayesian calibration of the tree estimated the origin of the Loricariinae during the Eocene period around 43.5 Ma. ago (Fig. 10). Most of the morphological, ethological, and ecological characteristics highlighted by the MSPA appeared quickly in the deepest node of the phylogeny during Oligocene and Miocene periods, *i.e.* between -33.90 and -15.68 Ma. Particularly, at the level of vector 2 (-31.80 Ma.), sudden modifications affected the traits with the appearance of postorbital notches on the orbital rim in Loricariina, the breeding strategy evolved from open water brooder toward alternative strategies, the teeth originally pedunculated and numerous underwent a reduction in number and modifications in shape, the mouth shape also modified from elliptical to bilobate, the number of caudal-fin rays decreased, adaptations to organic substrates occurred in Farlowellina, and predorsal keels appeared in Loricariina. These modifications took place between -33.90 and -31.80 Ma. Along vector 3 (-24.71 Ma.), alternative breeding strategies appeared such as cavity brooding or mouth brooding, the sexual dimorphism previously mostly expressed through hypertrophy of odontodes reoriented toward mouth and teeth characteristics, the lips transformed from papillose to filamentous or smooth, and fringed barbels appeared as well as conspicuous maxillary barbels. These modifications occurred from vector 3 to vector 6, *i.e.* between -24.71 and -15.68 Ma.

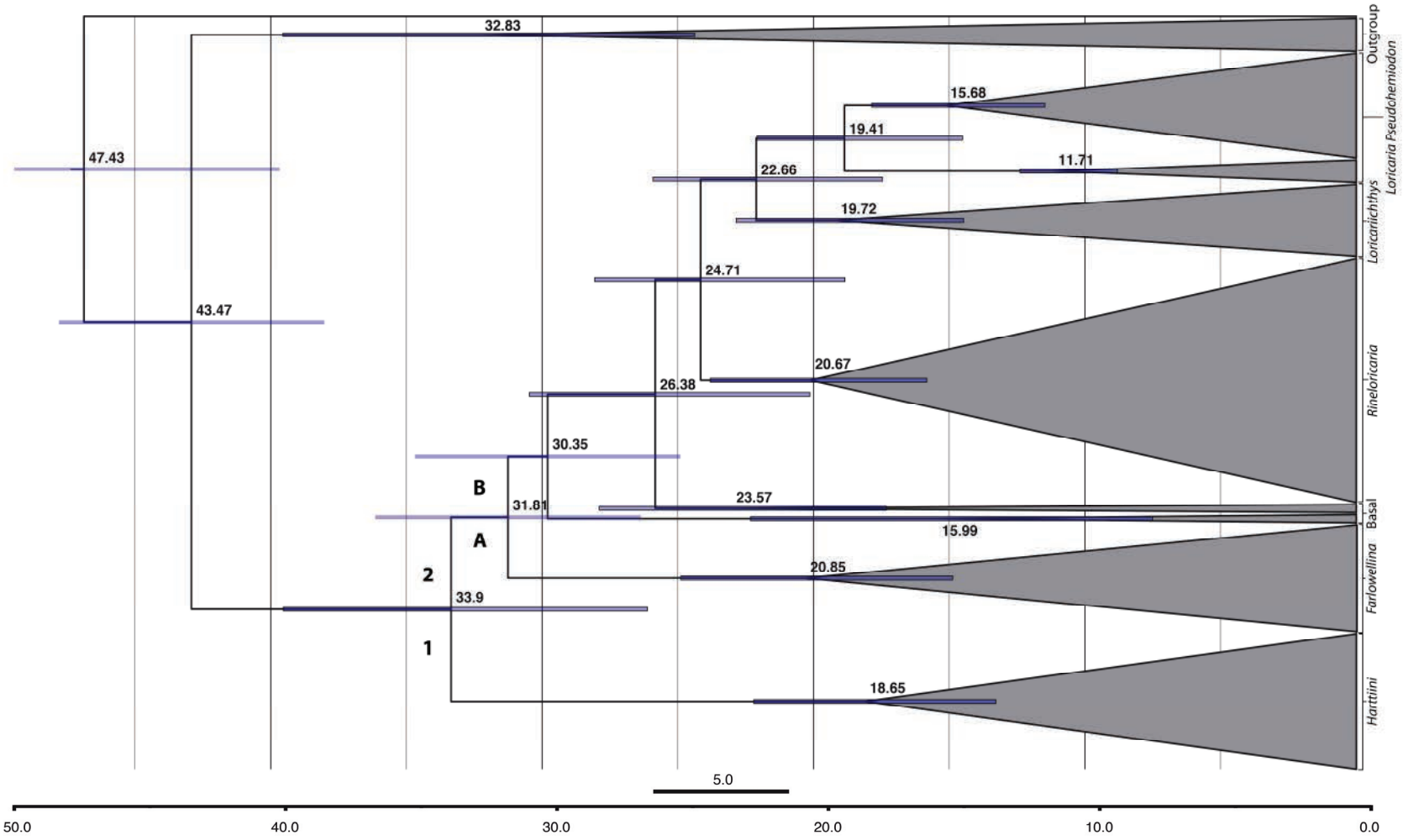

Fig. 10. Molecular dating for the main evolutionary innovations in Loricariinae. Bayesian maximum clade credibility chronogram computed on the partitioned data set using the GTR + G model for mitochondrial and intronic sequences, and the HKY + G model for the exonic regions. Numbers above branches indicate the inferred dates for the nodes. Bars indicate confidence intervals for the aging of the nodes. Scale represents time in Ma. Following results of the MSPA, only nodes involving the most important traits are represented. Other nodes are collapsed for readability. The size of the clades is proportional to the number of included species. 1: Harttiini; 2: Loricariini; A: Farlowellina, B: Loricariina.

4- Discussion

The present study aimed to reconstruct the evolutionary history of the Loricariinae, a highly specialized group of Neotropical catfishes, and in deciphering their main evolutionary trends shaped through time. A first step consisted thus to reconstruct an exhaustive phylogeny of the subfamily to provide the correct necessary framework for a comprehensive evolutionary study of this group. Then, we applied the new tests developed herein to detect phylogenetic dependence in qualitative data and for a complete table. This generalization of the orthogram function and associated tests (Ollier *et al*., 2006) to data of different nature provides a unifying procedure relying on the same assumptions, and makes accordingly the results of the different tests directly comparable, whatever the statistical nature of the data under study, including univariate or multivariate data. In the method developed by Abouheif (1999), the author had indeed to adapt the tests to the statistical nature of the data. The TFSI test for quantitative data corresponds strictly to a Moran I test (Pavoine *et al*., 2008), and provides a measure of autocorrelation (the more closely related the species the more similar the tip values), whereas the RUNS test looks for the randomness of the distribution of the data (similar character states are located in the same region of the phylogeny). The significant result of a TFSI will thus be "the data are positively or negatively autocorrelated", whereas the result of a RUNS test will be "the data are not randomly distributed". In a general testing procedure, these differences in the assumptions of the tests may not be important, since what was expected is the detection of a phylogenetic signal in the data. To the contrary, when one wants to compare the results of the tests, the fact to use two distinct statistics makes direct comparisons impossible. The complete development of the orthograms fills this gap, and makes patterns of phylogenetic dependence among traits comparable. Nevertheless, comparisons among trait evolutionary patterns become fastidious with the progressive increase and complexity of traits under study. The new multivariate method proposed here and relying on the MSPA (Jombart *et al*., 2009) using the phylogenetic vectorial basis of orthograms as matrix of proximity, accounts for this issue and provides a powerful multivariate tool to explore co-evolutionary patterns among multiple traits. The MSPA describes the correlation structure among a set of biological traits at different level of the phylogeny and can be applied to both quantitative and qualitative traits. The MSPA provides a graphical output allowing a direct interpretation of associations among traits for different nodes of the phylogenetic tree. The factorial map of variables (Fig. 9 a and b), reveals the contribution of each vector of the vectorial basis to the axes, and identifies the nodes defined

by these vectors. The graph of eigenvalues (Fig. 9d) identifies the axis explaining the major part of the information, and informs about the possible existence of several structures in the data (*i.e.* different level of the tree explaining the distribution of evolutionary patterns of traits), as well as the existence of axes containing evolutionary "noise" which are discarded from further interpretation. The factorial map of traits (Fig. 9 a and c) identifies the traits displaying similar evolutionary patterns for a given node. Thus, the MSPA provides an ordination of the essential nodes of the tree together with the traits displaying the strongest phylogenetic variation for these nodes (*i.e.* traits that underwent evolutionary events at the same node).

4.1 Systematic of the Loricariinae

Prior to the evolutionary study, we reconstructed the phylogeny of the subfamily using mitochondrial and nuclear markers. The phylogenetic results confirmed the monophyly of the subfamily, and its splitting into two tribes, the Harttiini, and the Loricariini. Corroborating previous results (Montoya-Burgos *et al*., 1998; Covain *et al.*, 2008; Rodriguez *et al*., in press), the Harttiini are restricted to *Harttia* (type genus), *Harttiella* and *Cteniloricaria*. Deeper relationships within Harttiini are not resolved due to very short internal branches suggesting explosive radiation of the main lineages between -18.65 and -16.46 Ma. The nested position of *H. leiopleura*, type species of *Quiritixys*, within the South-eastern species of *Harttia* that also included *H. loricariformis*, the type species of the genus, renders *Harttia* paraphyletic with the necessity to describe several new genera (considering our sampling, a total of four to render each lineage monophyletic). To prevent this taxonomic issue, a conservative approach consists thus to place *Quiritixys* into the synonymy of *Harttia*. A second problem concerns the position of *Harttiella intermedia* nested within *H. longicauda*. A rapid overview of this situation would probably lead to the placement of *H. intermedia* into the synonymy of *H. longicauda*. However, based on morphometric analyses, Covain *et al*. (in press) demonstrated that the former was perfectly distinct from the latter, and even belonged to another morphological group named *crassicauda* group and comprising all stockier species (contrary to *H. longicauda* that belonged to the *longicauda* group that comprised all slender species). In the same study, the barcode sequence of *H. intermedia* was also found identical to that of *H. longicauda*, and the authors hypothesised introgressive hybridization or a recent founder effect in an isolated population to explain this phenomenon, both species being present in the same basin. The use of the nuclear F-RTN4 gene in the present study, and the topological

result identical to that obtained using barcode sequences, infirm the hypothesis of introgressive hybridization. Since the establishment of reciprocal monophyly between two sister taxa is a function of time (Hubert *et al*., 2008), when not enough time passed to accumulate mutations able to differentiate sister species, a paraphyletic grouping may be observed with one species nested within a second one (*i.e.* the coalescent of the first species is contained within the coalescent of the second) (Meyer and Paulay, 2005). *Harttiella intermedia* represents thus a rather recent vicariant form of *H. longicauda* isolated in the Trinité Massif in French Guiana, and corroborates the hypothesis of Covain *et al*. (in press) of a morphologically fast evolving species not yet genetically distinguishable from its ancestor following the example of the East African lacustrine cichlid species flock (*e.g*. Won *et al*., 2005).

Within Loricariini, the phylogeny of Farlowellina revealed unexpected results. All genera but *Lamontichthys* and *Pterosturisoma* appeared paraphyletic. The nested position of *Aposturisoma* within *Farlowella* renders indeed the latter polyphyletic. If one considers *Aposturisoma* as a valid genus based on its particular body shape, ecological habits, and restricted distribution to the Huacamayo-Aguaytia drainage, members of the *F. amazona* species group (*sensu* Retzer and Page, 1997) should be placed in a new genus. However, the lack of significant distinctive features between the *F. amazona* group and other *Farlowella*, and the close relatedness of *Aposturisoma* and *Farlowella*, may imply that *Aposturisoma* corresponds to a local form of *Farlowella* adapted to rheophilic habits. This corroborates the hypothesis of Covain and Fisch-Muller (2007) that saw the morphological characteristics of *Aposturisoma* as adaptations to stream habitat rather than an intermediary shape between *Farlowella* and *Sturisoma* as supposed by Isbrücker *et al*. (1983). If this hypothesis applies, *Aposturisoma* should be considered a junior synonym of *Farlowella*. Nevertheless, this question still deserves further evidences before statement. The second highlighted paraphyly concerns the genera *Sturisoma* and *Sturisomatichthys*. Contrary to the preceding case, a strong geographical structure is present in this result with one group of *Sturisoma* comprising all cis-Andean species, and a second group comprising all trans-Andean members of *Sturisoma* and *Sturisomatichthys*. Moreover, the type species of *Sturisoma*, *S. rostrata*, is described from Brazilian rivers, whereas the type species of *Sturisomatichthys*, *S. leightoni*, is described from the Magdalena River in Colombia. For these reasons, *Sturisoma* is here restricted to the species occurring in the cis-Andean region whereas *Sturisomatichthys* comprised all former trans-Andean species of *Sturisoma* and *Sturisomatichthys*. Moreover, the diagnostic feature provided by Isbrücker and Nijssen (in Isbrücker, 1979) to distinguish *Sturisomatichthys* from

Sturisoma, *i.e*. the absence of a rostrum in *Sturisomaticthys*, is not phylogenetically informative as attested by the orthogram of this feature (see vector 12 in Fig. 8-rost).

The basal Loricariina comprises particular forms of the Loricariinae that can be seen as relictual species due to their particular morphological characteristics, restricted distributions, and long branches rendering the phylogenetic signal noisy. *Metaloricaria* connects indeed at base of the subtribe and possesses a very particular morphology reminiscent to that of *Harttia* with which it shares the same habitat (stream waters in riffles). This resemblance probably resulted to the initial description of *M. nijsseni* as a member of *Harttia* (Boeseman, 1976), despite clear autapomorphic features such as an horse-shoe like mouth shape, teeth pedunculated yet reduced in size and number, or 13 caudal-fin rays, that initiate the future trends of the Loricariina (strong modifications in mouth, lips, and teeth characteristics, decrease of the number of caudal-fin rays…). *Metaloricaria* is restricted to the Guiana Shield in rivers flowing through Suriname and French Guiana. In the same way, *Dasyloricaria* is restricted to the Pacific slope of the Andes, unique pattern of distribution within the subfamily, although it shares a mosaic of morphological characteristics with representatives of other Loricariina mainly distributed on the Atlantic slope. Along with members of *Rineloricaria*, it shares papillose lips and hypertrophied odontodes along the sides of the head in breeding males. With some representatives of the *Loricariichthys* group (*sensu* Covain and Fisch-Muller, 2007), it shares deep postorbital notches, an abdominal cover strongly structured, and a similar mouth shape, including the hypertrophied lower lip of breeding males (Steindachner, 1878). Finally, with some representatives of the *Loricaria* group, it shares a triangular head, strong predorsal keels, and the upper caudal fin ray produced into a long whip. The last basal Loricariina, *Fonchiiloricaria*, is restricted to the Upper Huallaga River. It possesses 14 caudal-fin rays, and no postorbital notches, two features characteristic for Harttiini and Farlowellina. In addition it also possesses autapomorphic features such as an extreme reduction in size and number of premaxillary teeth (when not missing) relative to dentary teeth (Rodriguez *et al*., in press). All those relictual species exhibit features that will be successively lost or maintained in other Loricariina lineages. In this case the observed autapomorphic features could correspond to the retention of ancestral characters, considering moreover that these ancient lineages are poorly diversified.

Rineloricaria constitutes by far the most species rich genus of the Loricariinae, including 66 valid species and 60 to 80 estimated undescribed. Several attempts have been made to split this genus into different genera. Isbrücker and Nijssen (1976) proposed the revalidation of *Hemiloricaria* Bleecker, 1862 (type species: *Hemiloricaria caracasensis*), but they finally left

it in the synonymy of *Rineloricaria* by lack of obvious characters to split these two genera. In an aquarist hobbyist journal, Isbrücker (in Isbrücker *et al*., 2001) changed his mind and finally revalidated *Hemiloricaria* based on the disposition of breeding odontodes in males, and assigned 24 species to this genus (*e.g*. *R. altipinnis*, *R. eigenmanni*, *R. lanceolata*, *R. parva*, *R. platyura*, *R. wolfei*…), most of them belonging to different lineages considering the present results. Moreover, the breeding odontodes on the predorsal area of males are not always present in the species assigned to this group (*e.g. R. platyura*). In the same publication, Isbrücker and Michel described *Fonchiiichthys* (type species: *Loricaria uracantha*), and Isbrücker described *Leliella* (type species: *Rineloricaria heteroptera*) on the basis of subtle differences in the sexual dimorphism. However, our phylogenetic reconstruction found *R. uracantha, R. heteroptera* and *R. eigenmanni* (a very close relative of *R. caracasensis* following the examination of type specimens) within the same clade. For these reasons, *Hemiloricaria*, *Fonchiiichthys,* and *Leliella* are here placed in the synonymy of *Rineloricaria*. In addition, the nested position of *Ixinandria steinbachi* in a sister position to *R. misionera* within Southeastern representatives of *Rineloricaria*, renders *Rineloricaria* paraphyletic. To circumvent this issue, we equally place here *Ixinandria* in the synonymy of *Rineloricaria*. The diagnostic feature given by Isbrücker and Nijssen (in Isbrücker, 1979) for *Ixinandria*, a naked belly and particular sexual dimorphism, appeared phylogenetically uninformative (*e.g.* orthogram of the abdominal cover, vector 18 in Fig. 8-abd), and should be considered as specific characters. This is reinforced by the appearance, in close relatives of *R. steinbachi* from South-East Brazil or Argentina, of a gradual increase in the abdominal platting, rendering thus the belly partly covered (*e.g*. *R. maquinensis*, *R. aequalicuspis* or *R. misionera*). Finally, the nested position of *R. hoehnei* within *R. lanceolata* renders the latter paraphyletic. For this reason, and for lack of distinctive character, we thus place here *R. hoehnei* (Miranda Ribeiro, 1912) in the synonymy of *R. lanceolata* (Günther, 1868). However, considering the branches length, *R. lanceolata* may be proved to host a species complex.

The *Loricariichthys* group appears more structured and homogeneous, with all genera found monophyletic and strongly supported. With the exception of the nominal genus, this group comprised surprisingly mostly monotypic genera (*Limatulichthys*, *Pseudoloricaria*, and *Hemiodontichthys*, with addition of *Furcodontichthys* following results of Covain and Fisch-Muller, 2007). However, given their broad geographic range, and long branches among populations, *Hemiodontichthys acipenserinus* and *Pseudoloricaria laeviuscula* could comprise species complexes. Isbrücker and Nijssen (1974) reported indeed variations in

morphometric features of *H. acipenserinus*, with populations from the Amazonian region tending to be slender than those from the Paraguay and Guaporé Rivers. Conversely, despite a nomenclatural imbroglio (see Covain and Fisch-Muller, 2007), *Limatulichthys* displays much shorter branches among its populations than previous genera. Consequently it may correspond to a single widespread species.

Within the *Loricaria* group, the nominal genus is found paraphyletic. *Loricaria prolixa* connects indeed in a sister position to representatives of *Brochiloricaria*, and *L. apeltogater* in a sister position to *Paraloricaria*. *Loricaria prolixa* was designated by Isbrücker (in Isbrücker *et al*., 2001) as type species of a new genus *Proloricaria*, based on a flattened and anteriorly broad body. The weakness of these supposed diagnostic features whose are also valid for other genera (*e.g*. *Pyxiloricaria*, *Pseudohemiodon*) leaded several authors to consider *Proloricaria* as a junior synonym of *Loricaria* (Ferraris in Reis *et al*., 2003; Covain and Fisch-Muller, 2007). Our results sustain however the validity of *Proloricaria* that is here revalidated. The sister position of *L. apeltogater* to *Paraloricaria* needs further investigation before statement. The specimen collected was indeed not preserved, and we can not certify that it belonged to the species. However, in the description of *P. agastor*, Isbrücker (1979) already noticed the close resemblance of both species (the smallest syntype of *L. apeltogaster* was even subsequently identified as *P. agastor*), distinguishing them on the basis of the dentition. *Paraloricaria* possesses small teeth on both jaws whereas *L. apeltogater* possesses the typical dentition for *Loricaria* with premaxillary teeth two times longer than dentary ones.

Within the *Pseudohemiodon* group, the trans-Andean *Crossoloricaria* which includes *C. variegata*, type species, connects at base of the group in a sister position to all other genera, whereas the cis-Andean *Crossoloricaria*, are nested within the remaining members of the *Pseudohemiodon* group, rendering *Crossoloricaria* paraphyletic. *Crossoloricaria* is poorly diagnosed, its only distinctive character (incomplete abdominal cover consisting of a double median row of plates) being shared by *Apistoloricaria* and *Rhadinoloricaria*. Moreover, *Crossoloricaria rhami* possesses a complete abdominal plate development (Isbrücker and Nijssen, 1983), thus rendering the diagnostic feature of *Crossoloricaria* invalid. In addition, *Apistoloricaria* is also not well diagnosed and is distinguished from *Rhadinoloricaria* primarily by the presence or absence of the iris operculum (absent or vestigial in *Apistoloricaria* versus present in *Rhadinoloricaria*), a more conspicuous rostrum in *Rhadinoloricaria*, and by the number of fringed barbels (14 in *Apistoloricaria* versus 12 in *Rhadinoloricaria*). Based on the phylogenetic results and the weakness of these diagnostic features (see orthograms of the presence or absence of an abdominal cover, of a secondary

organization in the abdominal cover, and of a rostrum, vector 29 in Figs. 8-abd, 8-ssec and 8-rost), *Crossoloricaria* is here restricted to the trans-Andean region, whereas the cis-Andean *Crossoloricaria* and *Apistoloricaria* are placed in the synonymy of *Rhadinoloricaria*.

4.2 Evolutionary trends of the Loricariinae

In a recent evaluation of the phylogenetic dependence of the morphological traits used as diagnostic features for the definition of the different genera of Loricariinae, Covain *et al.* (2008) highlighted a significant phylogenetic signal in three quantitative, and eight qualitative traits using TFSI and RUNS tests. Subsequently, the authors revealed using orthograms, a rather gradual pattern of evolution for the number of premaxillary and dentary teeth, and a single punctual event in the decrease of caudal-fin ray number. Since the method was only available for quantitative data, the authors used maximum likelihood ancestral state reconstructions (Lewis, 2001) to characterize the evolutionary patterns in qualitative data. Covain *et al.* (2008) observed a similar pattern of evolution for the traits linked to the mouth, and hypothesised co-evolution in traits related to the mouth such as mouth shape, tooth shape, lips structure, and barbels, due to identical selective pressure acting on this organ. They tentatively explained this co-evolution by the ecology of the species that colonized a large number of ecological niches, as illustrated by the fact that rheophilic species such as *Harttia* or *Lamontichthys* which live on stones possess elliptical mouth with papillose lips whereas sand dwellers that live in medium speed flowing waters such as *Loricaria* or *Pseudohemiodon* possess a bilobate mouth with filamentous lips. To evaluate the hypothesis that ecological habits (*e.g*. use of trophic and spatial resources) explained the evolution of the mouth structures, we tested ecomorphometric and ecological variables for phylogenetic dependence. The ecomorphology aims to identify relationships between morphology and ecology at different levels (individuals, populations, species, guilds, and communities) (Peres-Neto, 1999). At the level of communities, the ecomorphological analyses are supposedly powerful to identify cases of evolutionary convergences in phylogenetically distant species, or to the contrary to identify cases of adaptative divergences between closely related taxa (Casatti and Castro, 2006), this last hypothesis applying to our problematic. Different studies conducted on Neotropical communities revealed strong ecomorphological patterns related to feeding and/or locomotion making ecomorphological variables good predictors of species habits (*e.g*. Casatti and Castro, 2006; Oliveira *et al*., 2010; Gibran, 2010). Within loricariids, Casatti and Castro (2006) illustrated the observed modifications in shape between Hypoptopomatinae

(represented by *Hisonotus* sp.), Hypostominae (represented by *Hypostomus garmani*), and Loricariinae (represented by *Harttia* sp.) by the exploitation of micro-habitats in fast flowing waters, implying thus locomotion abilities. *Harttia* sp., contrary to other species, was indeed found able to exploit areas with stronger current because of its extremely depressed body and long caudal peduncle (IVF and RPL). Oliveira *et al*. (2010) corroborate this result in other loricariids (*Hypostomus* spp., *Loricariichthys platymetopon*, *Rhinelepis aspera*, and *Pterygoplichthys ambrosettii*) by characterizing these benthic detritivores in having developed caudal peduncles and pectoral fins, and shallow bodies. These features are essential to these species for the stabilisation on the substrate and for short displacements in lotic environments. In these turbulent areas, the body suffers the effect of different forces. To maintain their position on the substrate, loricariids possess a flat ventral surface, and an anteriorly elevated body. This shape makes the water flowing along the upper surface faster than the water flowing along the ventral surface, facilitating the adherence to the substrate by Bernoulli Effect. Additionally, loricariids posses a sucker mouth used for adherence to the substrate and displacement against the current. The sucker mouth of loricariids represents a key innovation of the family due to the decoupling of biomechanical constraints of muscles acting on jaws, allowing scrapping and adherence (Schaeffer and Lauder, 1986). This decoupling (biomechanical relaxed condition) rendering each half upper and lower jaws independently movable is hypothesised to be one of the innovations responsible for the great specific diversity of Loricariidae (Schaeffer and Lauder, 1996). The ecomorphological hypothesis stipulates that morphological attributes of each species should reflect its ecology, and can accordingly be used as indicators of its habits and adaptations to different habitats (Gibran, 2010). We thus hypothesised that, if the evolution of the mouth structures and the different ecological and ecomorphological variables followed a similar evolutionary pattern (*i.e*. they have similar orthograms), this implies that these intra and extra-phenotypic components are potentially linked, and thus evolution in one component induced evolutionary changes in the second (*i.e*. the ecology constrained the evolution of mouth characteristics). The multivariate orthogram suggested that most of the traits were shaped deep in the phylogeny with the eight first vectors explaining the variance distribution (Fig. 8-Global). The univariate orthograms confirmed this result, with most of the traits linked to the mouth that are effectively explained by the first vectors (number of premaxillary and dentary teeth, tooth and mouth shapes, lip surface, maxillary and fringed barbels) with the addition of the number of caudal-fin rays, and the presence or absence of predorsal keels and postorbital notches (Figs. 8-nbdtsup, 8-nbdtinf, 8-teeth, 8-mouth, 8-lips, 8-barb, 8-mlips, 8-caud, 8-cdor, and 8-encorb), corroborating thus

the findings of Covain *et al*. (2008). However, the patterns highlighted by the orthograms for ecomorphological and ecological data appeared different. Out of the eight ecomorphometric variables, only the Compression Index and the Index of Ventral Flattening displayed comparables patterns involving the first vectors. Other variables displayed a more diffuse pattern of phylogenetic dependence, and even three variables displayed variations independent of the phylogeny. Concerning the ecological variables, only the favoured habitats and substrates possessed comparable evolutionary patterns in regard of the mouth characteristics. The MSPA perfectly confirms these results and revealed strong associations among mouth features and the deepest nodes of the phylogeny, confirming thus that all these structures are linked. However, very few correlations were observed with the ecological and ecomorphological variables (see variables sub, hab, and IVF in Fig. 9 a and c), rejecting thus the hypothesis of the main influence of the ecology of the species in the great diversity in mouth characteristics observed in Loricariinae. The MSPA (and univariate orthograms) highlighted in fact that such modifications were related to sexual characteristics that are the reproductive strategies and the sexual dimorphism. The co-evolution among traits related to the mouth was thus shaped by behavioural constraints suggesting sexual selection. This hypothesis is reinforced by the co-variation with the secondary sexual dimorphism which can be exuberant in certain species (*e.g*. *H. leiopleura*, *Spatuloricaria* spp., *R.* aff. *latirostris*…). In a recent study, Geerinckx *et al*. (2011) solved the paradox of respiration in regard to adherence of the sucker mouth. They demonstrated the key role of the pre-valvular cavity in this phenomenon as well as the importance of the oral valve and maxillary barbels in water flow. From the initial condition related to respiration and feeding, the mouth evolved in Loricariidae toward new functions related to adherence to the substrate and locomotion (in fast flowing water, the fish uses its mouth for short displacements against the current, R. C. pers. obs.). In Loricariinae the mouth (and related features) evolved from this secondary function toward a third function related to reproduction, especially in *Loricariichthys*, *Loricaria*, and *Pseudohemiodon* group members. Surprisingly, these new innovations were concomitant with the loss of pronounced secondary sexual dimorphism. The hypertrophy of odontodes that is sometimes extreme in open and cavity brooders, disappeared in mouth brooders. If the appearance of a rounded crown tooth in mouth brooding males could be explained by a higher risk for eggs and embryos to be damaged by pointed crowns, the loss of hypertrophied odontodes on the snout margin and pectoral fins does not have direct interpretation. Males of open and cavity brooders stay struck to the fry when guarding eggs and embryos (R. C. pers. obs.) and this behaviour is not more risky for the fry than having the

eggs in the mouth and against the abdomen such as in abdomino-lip brooders. Moreover, the hypertrophied odontodes are seasonal and only expressed during the reproduction period. We can tentatively explain this phenomenon by the action of predation. In open and cavity brooders, the fry is often hidden in caves or exposed in fast flowing waters, and actively defended by the male. This guarding behaviour preserve the fry from predators by inaccessibility of the fry (hidden or laid in difficult to access places), and let the male free for the defence of eggs and embryos. Even in case of death of the male, the fry may thus be prevented from predation. In open and cavity brooders, the hypertrophied odontodes may play a role in the defence of the fry, but also prove to females the reproductive value of the male, larger males bearing larger odontodes accessing more easily to reproduction (R.C. pers. obs.). In mouth brooders, the situation is inverted. Any predation activity against the male, definitely compromise the success of reproduction. In this case, bearing external attributes rendering brooding males identifiable may represent a signal for predators, and thus represents a severe disadvantage. The hypertrophy of odontodes may thus have been sexually selected by females, but its subsequent loss may be the result of natural selection carried out by predators.

All these innovations appeared during the Oligocene period (~ 30Ma.) and evolved throughout Miocene. This period is characterized by major geological events that affected the whole subcontinent (Lundberg *et al*., 1998, 2010; Hoorn and Wesslingh, 2010). The uplift of the Andes initiated during the Middle Cretaceous about 90 Ma. ago by the low-elevation of the proto-cordillera (Lundberg *et al*., 1998), and underwent a major orogenic phase during Oligocene around -30 Ma. At this period, tectonic activity was responsible for the uplift of the Central Cordillera (Central and Northern Andes), and for the onset uplift of the Eastern Cordillera (Northern Andes). The main rivers flowed in a south-north direction in the area corresponding to the modern western Amazon, and major drainages divided in central-eastern Amazonia (Hoorn and Wesslingh, 2010). During the entire Miocene, the Andes continued to uplift, and extensive lakes and inland seas appeared in the foreland basin in western Amazonia and northward (Lundberg *et al*., 1998), leading to the formation of the Pebas megawetland in western Amazonia during the middle Miocene (~11-16 Ma.) (Hoorn and Wesslingh, 2010). During the late Miocene (~7-11 Ma.), the uplift of the Central Andes accelerated, the Eastern Cordillera and Mérida Andes, the Western Amazon portal, the Vuapes Arch uplifted, leading to the establishment of the west-east transcontinental Amazon drainage system (Hoorn and Wesslingh, 2010). The South American fossil records strongly suggest a pre-Miocene diversification of the fish fauna, as attested by the presence of

stingrays, lungfish, *Arapaima*, characids, cichlids and sciaenids (Lundberg *et al*., 2010). These fossils reveal that the ichthyofauna was essentially modern by the late Miocene. Lundberg *et al*. (2010) hypothesized thus a Cretaceous and tertiary diversification of fishes favoured by the uplift of the Andes and fluctuating global sea levels. Our results corroborate this hypothesis. If diversification events occurred at the specific level during the quaternary, most of the contemporaneous genera were already present before -11 Ma. The morphological and behavioural innovations characteristics for the Loricariinae were already acquired before the late Miocene, and could thus be linked to these major events.

This study revealed the main evolutionary trends shaped through time in Loricariinae. Major innovations were constrained by reproductive behaviour and appeared during the tertiary, a period characterized by orogenesis of the Andes and progressive establishment of the modern Amazon and Orinoco. The orthograms herein generalized to any type of data, have proven to be relevant and efficient tools for the characterization of the patterns of phylogenetic dependence of the data. In addition, the MSPA not only revealed co-evolution among traits but also highlighted the region of the tree that underwent these changes. This powerful analysis is thus able to detect among multiple traits of different nature, which can all be under phylogenetic dependence, those that underwent similarly evolutionary changes at different level of a phylogeny. This analysis highlights thus the importance of the evolutionary patterns in the comparison of multiple traits, all phylogenetically constraints traits not being necessarily linked at the same level.

Acknowledgments

We are grateful to M. Sabaj Perez, and J. G. Lundberg, ANSP; E. Bermingham, and G. R. Reina, STRI; M. Lucena, MCP; J. Armbruster, AUM; O. T. Oyakawa, MZUSP; J. Casciota, and A. Almirón, MLP**;** Y.P. Cardoso, AUGM-UNLP; M. S. Rodriguez, LIRP; G. Costa Silva, LBP; P. Keith, R. Causse, and P. Pruvost, MNHN; M. van Oijen and K. van Egmond, RMNH; R. Vonk and H. Praagman, ZMA; J. Maclaine, BMNH; H. Wellendorf**,** NMW; S. Schaeffer, AMNH; M. A. Rogers, FMNH; D. Catania, CAS; G. M. Gonzo, UNSA; L. Rapp Py-Daniel and M. Rocha, INPA; C. Dhlouy, San Lorenzo; and S. Rubin and L. Moissonier, France; for the loan of specimens and/or tissue samples. We acknowledge H. Ortega, M. Hidalgo and V. Meza Vargas, MUSM; T. Pequeño and collaborators from CIMA; L. Rodriguez (Conservación de Recursos Naturales); P. de Rham, Lausanne, P. Gaucher, CNRS Guyane; R. Vigouroux and P. Cerdan, Hydreco Guyane; C. Weber, and A. Merguin, MHNG;

M. Dewynter, ONF Guyane; F. Melki, Biotope France; K. Wan Tong You, Paramaribo; P. Ouboter, NZCS; C. Bernard, CSBD; the North Rupununi District Development Board; and the Iwokrama organization for their field and logistic assistance; the G. and A. Claraz Foundation for their financial support for the missions in Suriname in 2001, 2005, 2007 and 2008, and in French Guiana in 2006; the Académie Suisse des Sciences Naturelles (ScNat) for their financial support for the missions in Guyana 2004 and French Guiana 2006; The C. Topali Found for their financial contribution to the acquisition of field material for the mission Suriname 2007, and lab material in 2009; the All Catfish Species Inventory (NSF-DEB 0315963) for the financing of collection consultancies to RC (NMW and ANSP in 09/2007 and 12/2007), and open access in Zootaxa to Covain and Fisch-Muller (2007); and the A. Lombard found for data acquisition in 2010. We are also grateful to A. Huser, Sallmann-Fehr AG for the gift of gill nets for the mission in Suriname in 2005 and 2007; the Guyana Environmental Protection Agency, and Ministry of Amerindian affairs; the French Guiana Diren, and Préfecture; and the Surinamese Ministry of Agriculture, Animal Husbandry and Fisheries for the different necessary authorizations and collecting permits. Part of this project was supported by the Fond National Suisse de la Recherche Scientifique (JIMB 3100A0–104005). The figures were finalized by Florence Marteau, MHNG. Jan Pawlowski and José Fahrni, University of Geneva, are acknowledged for laboratory facilities; this work represents part of the PhD dissertation of the first author, who would particularly acknowledge Denis Duboule, University of Geneva, Sonia Fisch-Muller, MHNG, and Stéphane Dray, University of Lyon for their supervision.

References

Abouheif, E., 1999. A method to test the assumption of phylogenetic independence in comparative data. *Evolutionary Ecology Research* **1**: 895–909.

Akaike, H., 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19 (6)**: 716–723.

Albert, J.S., Lovejoy, N.R., and Crampton, W.G.R., 2006. Miocene tectonism and the separation of cis- and trans-Andean river basins: Evidence from Neotropical fishes. *Journal of South American Earth Sciences* **21**: 14–-27.

Alves-Gomes, J.A., Orti, G., Haygood, M., Heiligenberg W., and Meyer, A., 1995. Phylogenetic analysis of the South American electric fishes (order Gymnotiformes) and the evolution of their electrogenic system-a synthesis based on morphology, electrophysiology, and mitochondrial sequence data. *Molecular Biology and Evolution* **12**: 298–318.

Benjamini, Y., and Yekutieli, D., 2001. The control of the false discovery rate in multiple testing under dependency. *Annals of Statistics* **29**: 1165–1188.

Blomberg, S.P., Garland, Jr. T., and Ives, A.R., 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

Boeseman, M., 1976. A short review of the Surinam Loricariinae; with additional information on Surinam Harttiinae, including the description of a new species (Loricariidae, Siluriformes). *Zoologische Mededelingen (Leiden)* **50 (11)**: 153-177, Pls. 1-8.

Bruggeman, J., Heringa, J., and Brandt, B.W., 2009. PhyloPars: estimation of missing parameter values using phylogeny. *Nucleic Acids Research* **37**: W179-W184.

Casatti, L., and Castro, R.M.C., 2006. Testing the ecomorphological hypothesis in a headwater riffles fish assemblage of the rio São Francisco, southeastern Brazil. *Neotropical Ichthyology* **4(2)**: 203-214.

Cheverud, J.M., Dow, M.M., and Leutenegger, W., 1985. The quantitative assessment of phylogenetic constraints in comparative analyses: sexual dimorphism in body weight among primates. *Evolution* **39**: 1335–1351.

Chiachio, M.C., Oliveira, C., and Montoya-Burgos, J.I., 2008. Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). *Molecular Phylogenetics and Evolution* **49**: 606-617.

Covain, R., and Fisch-Muller, S., 2007. The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: Loricariidae): a practical key and synopsis. *Zootaxa* **1462**: 1–40.

Covain, R., Dray, S., Fisch-Muller, S., and Montoya-Burgos, J.I., 2008. Assessing phylogenetic dependence of morphological traits using co-inertia prior to investigate character evolution in Loricariinae catfishes. *Molecular Phylogenetics and Evolution* **46**: 986-1002.

Covain, R., Fisch-Muller, S., Montoya-Burgos J.I., Mol J., Le Bail, P-Y., and Dray, S., (in press). The Harttiini (Siluriformes, Loricariidae) from the Guianas: a multi-table approach to assess their diversity, evolution, and distribution. *Cybium*.

de Carvalho Paixão, A., and Toledo–Piza, M., 2009. Systematics of *Lamontichthys* Miranda-Ribeiro (Siluriformes: Loricariidae), with the description of two new species. *Neotropical Ichthyology* **7**: 519-568.

Diniz-Filho, J.A.F., de Sant'Ana, C.E.R., and Bini, L.M., 1998. An eigenvector method for estimating phylogenetic inertia. *Evolution* **52**: 1247–1262.

Dolédec, S., and Chessel, D., 1994. Co-inertia analysis: an alternative method for studying species-environment relationships. *Freshwater Biology* **31**: 277–294.

Dotzer, P., and Weiner, T., 2003. *Harttia guianensis*, Die Pflege und die Zucht sind swar schwierig, aber doch möglich. *Das Aquarium* **407 (5)**: 20-26.

Drummond, A.J., and Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**:214.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., and Rambaut, A., 2006. Phylogenetics and dating with confidence. *PLoS Biology* **4**: e88.

Efron, B., 1979. Bootstrap methods: Another look at the jackknife. *Annals of Statistics* **7**: 1–26.

Evers, H-G., and Seidel, I., 2005. Catfish Atlas Vol. 1: South American Catfishes of the Families Loricariidae, Cetopsidae, Nematogenyidae and Trichomycteridae. 943 p. Melle: Mergus Verlag Gmbh.

Felsenstein, J., 1981. Evolutionary trees from DNA-sequences - a maximum-likelihood approach. *Journal of Molecular Evolution* **17**: 368–376.

Felsenstein, J., 1985a. Phylogenies and the comparative method. *American Naturalist* **125**: 1–15.

Felsenstein, J., 1985b. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Felsenstein, J., 2008. Comparative methods with sampling error and within-species variation: Contrasts revisited and revised. *American Naturalist* **171**: 713–725.

Ferraris, Jr. C.J., 2007. Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. *Zootaxa* **1418**: 1-628.

Ferraris, Jr. C.J., 2003. Subfamily Loricariinae (Armoured catfishes). *In:* Reis, R.E., Kullander, S.O., and Ferraris, Jr. C.J., (Eds.), *Check list of the freshwater fishes of South and Central America*, Edipucrs, Porto Alegre, Brazil, pp. 330-350.

Fichberg, I., and Chamon, C.C., 2008. *Rineloricaria osvaldoi* (Siluriformes: Loricariidae): a new species of armored catfish from rio Vermelho, Araguaia basin, Brazil. *Neotropical Ichthyology* **6**: 347-354.

Frekleton, R.P., 2009. The seven deadly sins of comparative analysis. *Journal of Evolutionary Biology* **22**: 1367–1375.

Friedman, M., 1937. The use of ranks to avoid the assumption of normality implicit in the analysis of variance. *Journal of the American Statistical Association* **32**: 675-701.

Gatz, Jr. A. J., 1979. Ecological morphology of freshwater stream fishes. *Tulane Studies in Zoology and Botany* **21**: 91-124.

Geerinckx, T., Herrel, A., and Adriaens, D., 2011. Suckermouth armored catfish resolve the paradox of simultaneous respiration and suction attachment: a kinematic study of *Pterygoplichthys disjunctivus*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* **315A (3)**: 1932-5231.

Gelman, A., and Rubin, D.B., 1992. Inference from Iterative Simulation Using Multiple Sequences. *Statistical Science* **7(4)**: 457-511.

Ghazzi, M.S., 2008. Nove espécies novas do gênero *Rineloricaria* (Siluriformes,Loricariidae) do rio Uruguai, do sul do Brasil. *Iheringia, Série Zoologia* **98**: 100-122.

Gibran, F.Z., 2010. Habitat partitioning, habits and convergence among coastal nektonic fish species from the São Sebastião Channel, southeastern Brazil. *Neotropical Ichthyology* **8(2)**: 299-310.

Gillespie, J.H., 1986. Rates of molecular evolution. *Annual Review of Ecology, Evolution and Systematics* **17**: 637–665.

Gittleman, J.L., and Kot, M., 1990. Adaptation: statistics and null model for estimating phylogenetic effects. *Systematic Zoology* **39**: 227–241.

Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* **53**: 325–338.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.

Harvey, P.H., and Pagel, M.D., 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford, UK, 248 pp.

Hasegawa, M., Kishino, H., and Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA**.** *Journal of Molecular Evolution* **22 (2)**: 160–74.

Hill, M.O., and Smith, A.J.E., 1976. Principal component analysis of taxonomic data with multi-state discrete characters. *Taxon* **25**: 249-255.

Holm, S., 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6**: 65–70.

Hoorn, C., and Wesselingh, F., (eds.) 2010. Amazonia, Landscape and Species Evolution: A Look into the Past. Blackwell Publishing, London, UK, 464 pp.

Hubert, N., Hanner, R., Holm, E., Mandrak, N.E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J., and Bernatchez, L., 2008. Identifying Canadian freshwater fishes through DNA barcodes. *Plos One* **3**: e2490.

Huelsenbeck, J.P., and Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**:754-755.

Huelsenbeck, J.P., Nielsen, R., and Bollback, J., 2003. Stochastic mapping of morphological characters. *Systematic Biology* **52**: 131–158.

Ingenito, L.F.S., Ghazzi, M.S., Duboc, L.F., and Abilhoa, V., 2008. Two new species of *Rineloricaria* (Siluriformes: Loricariidae) from the rio Iguaçu basin, southern Brazil. *Neotropical Ichthyology* **6**: 355-366.

Isbrücker, I.J.H., 1979. Description préliminaire de nouveaux taxa de la famille des Loricariidae, poissons-chats cuirassés néotropicaux, avec un catalogue critique de la sous-famille nominale (Pisces, Siluriformes). *Revue Française d'Aquariologie et Herpétologie* **5**: 86-116.

Isbrücker, I.J.H., and Nijssen, H., 1974. On *Hemiodontichthys acipenserinus* and *Reganella depressa*, two remarkable mailed catfishes from South America (Pisces, Siluriformes, Loricariidae). *Beaufortia* **22**: 193-222.

Isbrücker, I.J.H., and Nijssen, H., 1976. *Rineloricaria heteroptera,* a new species of mailed catfish from Rio Amazonas near Manaus, Brazil (Pisces, Siluriformes, Loricariidae). *Zoologischer Anzeiger* **196**: 109-124.

Isbrücker, I.J.H., and Nijssen, H., 1983. *Crossoloricaria rhami* n. sp., un nouveau poisson-chat cuirassé du Rio Huacamayo, Pérou (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpétologie* **10**: 9-12.

Isbrücker, I.J.H., Britski, H.A., Nijssen, H., and Ortega, H., 1983. *Aposturisoma myriodon*, une espèce et un genre nouveaux de Poisson-Chat cuirassé, tribu Farlowellini Fowler, 1958 du Bassin du Rio Ucayali, Pérou (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpétologie* **10**: 33-42.

Isbrücker, I.J.H., Seidel, I., Michels, J.P., Schraml, E., and Werner, A., 2001. Diagnose vierzehn neuer Gatungen der Familie Loricariidae Rafinesque, 1815 (Teleostei, Ostariophysi). *In:* R. Stawikowski (ed.), *Harnischwelse 2*, Die Aquarien und Terrarien Zeitschrift, Sonderheft, Eugen Ulmer, Stuttgart, Germany, p. 17-24

Jobb, G., von Haeseler, A., and Strimmer, K., 2004. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology* **4**: 18.

Jombart, T., Balloux, F., and Dray, S., 2010. adephylo: new tools for investigating the phylogenetic signal in biological traits. *Bioinformatics* **26**: 1907-1909.

Jombart, T., Dray, S., and Dufour, A.-B., 2009. Finding essential scales of spatial variation in ecological data: a multivariate approach. *Ecography* **32**: 161-168.

Kendall, M.G., and Babington Smith, B., 1939. The problem of m rankings. *Annals of Mathematical Statistics* **10**: 275-287.

Kumar, S., Skjæveland, Å., Orr, R.J.S., Enger, P., Ruden, T., Mevik, B.-H., Burki, F., Botnen, A., and Shalchian-Tabrizi, K., 2009. AIR: A batch-oriented web program package for construction of supermatrices ready for phylogenomic analyses. *BMC Bioinformatics* **10**:357.

Legendre, P., and Lapointe, F-J., 2004. Assessing congruence among distance matrices: single malt Scotch whiskies revisited. *Australian and New Zealand Journal of Statistics* **46**: 615-629.

Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* **50**: 913–925.

Lundberg, J.G., Sabaj Pérez, M.H., Dahdul, W.M., and Aguilera, O.A., 2010. The Amazonian Neogene fish fauna. *In*: Hoorn, C.M., and Wesselingh, F.P., (eds.) *Amazonia, Landscape and Species Evolution: A Look into the Past*. Blackwell Publishing, London, UK, pp. 281–301.

Lundberg, J.G., Marshall, L.G., Guerrero, J., Horton, B., Malabarba, M.C.S.L., and Wesselingh, F., 1998. The stage for Neotropical fish diversification: a history of tropical South American Rivers. *In*: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena Z.M.S. and Lucena, C.A.S. (eds.) *Phylogeny and Classification of Neotropical Fishes*. Edipucrs Porto Alegre, Porto Alegre, Brazil, pp. 13–48.

Lynch, M., 1991. Methods for the analysis of comparative data in evolutionary biology. *Evolution* **45**: 1065–1080.

Mantel, N., 1967. The detection of desease clustering and a generalized regression approach. *Cancer Research* **27**: 209-220.

Meyer, C.P., and Paulay, G., 2005. DNA barcoding: error rates based on comprehensive sampling. *PLoS Biology* **3**: 2229–2238.

Meyer, A., 1993. Evolution of mitochondrial DNA in fishes. *In*: Hochachka, P.W., and Mommsen, P., (eds) *Biochemistry and Molecular Biology of Fishes, Volume 2: Molecular Biology Frontiers*. Elsevier Science, Amsterdam, the Netherlands, pp. 1-38.

Montoya-Burgos, J.I., Muller, S., Weber, C., and Pawlowski, J., 1998. Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences. *In*: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M.S., and Lucena, C.A.S., (eds.) *Phylogeny and Classification of Neotropical Fishes*. Edipucrs Porto Alegre, Porto Alegre, Brazil, pp. 363-374.

Moran, P.A.P., 1950. Notes on continuous stochastic phenomena. *Biometrika* **37**: 17–23.

Oliveira, E.F., Goulart, E., Breda, L., Minte-Vera, C.V., Paiva, L.R.S., and Vismara, M.R., 2010. Ecomorphological patterns of the fish assemblage in a tropical floodplain: effects of trophic, spatial and phylogenetic structures. *Neotropical Ichthyology* **8 (3)**: 569-586.

Ollier, S., Couteron, P., and Chessel, D., 2006. Orthonormal transform to decompose the variance of a life-history trait across a phylogenetic tree. *Biometrics* **62**: 471–477.

Pagel, M., 1999a. The maximum likelihood approach to reconstructing Ancestral character states of discrete characters on phylogenies. *Systematic Biology* **48**: 612–622.

Pagel, M., 1999b. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.

Pagel, M., Meade, A., and Barker, D., 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology* **53**: 673–684.

Paradis, E., 2006. Analysis of Phylogenetics and Evolution with R. Springer, New York, 211 pp.

Paradis, E., and Claude, J., 2002. Analysis of comparative data using generalized estimating equations. *Journal of theoretical Biology* **218**: 175–185.

Paradis, E., Claude, J., and Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289-290.

Pavoine, S., Baguette, M., and Bonsall, M.B., 2010. Decomposition of trait diversity among the nodes of a phylogenetic tree. *Ecological Monographs* **80**: 485-507.

Pavoine, S., Ollier, S., Pontier, D., and Chessel, D., 2008. Testing for phylogenetic signal in life history variable: Abouheif's test revisited. *Theoretical Population Biology* **73**: 79–91.

Peres-Neto, P.R., 1999. Alguns métodos e estudos em ecomorfologia de peixes de riachos. *In*: Caramaschi, E.P., Mazzoni, R., and Peres-Neto, P.R. (eds.) *Ecologia de peixes de riachos*. Série Oecologia Brasiliensis, PPGE-UFRJ, Rio de Janeiro, Brazil, volume VI, pp. 209-236.

Pinna, M.C.C. de, 1998. Phylogenetic relationships of Neotropical Siluriformes (Teleostei: Ostariophysi): historical overview and synthesis of hypotheses. *In*: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M., and Lucena, C.A.S., (eds.) *Phylogeny and classification of Neotropical fishes*. Edipucrs Porto Alegre, Porto Alegre, Brazil, pp. 279-330.

R Development Core Team, 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rambaut, A, and Drummond, A.J., 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer

Rao, C.R., 1964. The use and interpretation of principal component analysis in applied research. *Sankhya* **A26**: 329-359.

Rao, C.R., 1982. Diversity and dissimilarity coefficients: a unified approach. *Theoretical Population Biology* **21**: 24-43.

Rapp Py-Daniel, L., and Fichberg, I., 2008. A new species of *Rineloricaria* (Siluriformes: Loricariidae: Loricariinae) from rio Daraá, rio Negro basin, Amazon, Brazil. *Neotropical Ichthyology* **6**: 339-346.

Reis, R.E., Kullander, S.O., and Ferraris, Jr. C.J., (eds.) 2003. Check list of the freshwater fishes of South and Central America. Edipucrs Porto Alegre, Porto Alegre, Brazil, 742 pp.

Retzer, M.E., and Page, L.M., 1997. Systematics of the stick catfishes, *Farlowella* Eigenmann & Eigenmann (Pisces, Loricariidae). *Proceedings of the Academy of Natural Sciences, Philadelphia* **147**: 33-88.

Rholf, F.J., 2001. Comparative methods for the analysis of continuous variables: geometric interpretations. *Evolution* **55**: 2143–2160.

Rodriguez, M.S., and Miquelarena, A., 2008. *Rineloricaria isaaci* (Loricariidae: Loricariinae), a new species of loricariid catfish from the Uruguay River basin. *Journal of Fish Biology* **73**: 1635–1647.

Rodriguez, M.S., and Reis, R.E., 2008. Taxonomic Review of *Rineloricaria* (Loricariidae: Loricariinae) from the Laguna dos Patos drainage, Southern Brazil, with the descriptions of two new species and the recognition of two species groups. *Copeia* **2008**: 333-349.

Rodriguez, M.S., Cramer, C.A., Bonatto, S.L., and Reis, R.E., 2008. Taxonomy of *Ixinandria* Isbrücker and Nijssen (Loricariidae: Loricariinae) based on morphological and molecular data. *Neotropical Ichthyology* **6**: 367-378.

Rodriguez, M.S., Ortega, H., and Covain, R., (in press). Intergeneric phylogenetic relationships in Loricariinae catfishes (Siluriformes: Locariidae), with description of *Fonchiiloricaria nanodon*: a new genus and species from Peru. *Journal of Fish Biology*.

Ronquist, F., and Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**:1572-1574.

Sanderson, M.J., 1997. A nonparameteric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution* **14**: 1218–1231.

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* **19**: 101–109.

Schaefer, S.A., and Lauder, G.V., 1986. Historical transformation of functional design: Evolutionary morphology of feeding mechanisms in loricarioid catfishes. *Systematic Zoology* **35**:489-508.

Schaefer, S.A., and Lauder, G.V., 1996. Testing historical hypotheses of morphological change: biomechanical decoupling in Loricarioid catfishes. *Evolution* **50 (4)**: 1661-1675.

Schaefer, S.A., and Stewart, D.J., 1993. Systematics of the *Panaque dentex* species group (Siluriformes: Loricariidae), wood-eating armored catfishes from tropical South America. *Ichthyological Exploration of Freshwaters* **4**: 309–342.

Schluter, D., Price, T., Mooers, A.O., and Ludwig, D., 1997. Likelihood of ancestor states in adaptive radiation. *Evolution* **51**: 1699–1711.

Sokal, R.R., and Rohlf, F.J., 1995. Biometry, 3rd ed. W.H. Freeman & Co. (eds), New York, 887 pp.

Steindachner, F., 1878. Zur Fischfauna des Magdalenen-Stromes. *Anzeiger der Mathematisch- Naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften in Wien* **15**: 88-91.

Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *In*: Miura, R.M., (ed.) *Some mathematical questions in biology-DNA sequence analysis.* American Mathematics Society, Providence, RI, pp 57–86.

Thomas, M.R., and Rapp Py-Daniel, L.H., 2008. Three new species of the armored catfish genus *Loricaria* (Siluriformes:Loricariidae) from river channels of the Amazon basin. *Neotropical Ichthyology* **6**: 379-394.

Thomas, M.R., and Sabaj Pérez, M.H., 2010. A new species of whiptail catfish, genus *Loricaria* (Siluriformes: Loricariidae), from the Rio Curuá (Xingu Basin), Brazil. *Copeia* **2010**: 274-283

Thorne, J.L., and Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology* **51**: 689–702.

von Neumann, J., Kent, R.H., Bellinson, H.R., and Hart, B.I., 1941. The mean square successive difference. *Annals of Mathematical Statistics* **12**: 153–162.

Watson, D.J., and Balon, E.K., 1984. Ecomorphological analysis of fish taxocenes in rainforest streams of northern Borneo. *Journal of Fish Biology* **25**: 371-384.

Wiley, D.F., Amenta, N., Alcantara, D.A., Ghosh, D., Kil, Y.J., Delson, E., Harcourt-Smith, W., Rohlf, F.J., St. John, K., and Hamann, B., 2005. Evolutionary morphing. *In*: Proceedings of the IEEE Visualization 2005 (VIS '05), Minneapolis, pp. 431–438.

Won Y.-J., Sivasundar, A., Wang, Y., and Hey, J., 2005. On the origin of Lake Malawi cichlid species: A population genetic analysis of divergence. *Proceedings of the National Academy of Sciences* **102**: 6581–6586.

General conclusions and perspectives

Phylogenetic reconstructions are now playing a major role in biology and represent a prerequisite for a comprehensive study of organisms' evolution. A first issue about phylogenies concerns their ability to resolve species interrelationships. This might appear as evidence but recovering the correct systematic context of a biological study remains a fundamental prior to any analysis implying comparisons between several species or individuals. Without a clear evolutionary direction allowing the correct interpretation of the results, any interpretation remain possible, only relying on personal assumptions, knowledge, and referential. For example, molecular phylogenies were extensively used in a recent past to evaluate ancient evolutionary hypotheses mostly relying on *a priori*. As an illustration, Delsuc *et al*. (2006) demonstrated that urochordates (*i.e.* the tunicates) formed in fact the sister group of vertebrates contrary to the *a priori* well accepted cephalochordates (*i.e* the lancelets).

The first step of the present thesis was thus to reconstruct the correct systematic frame of the Loricariinae. Monophyly of the Loricariinae has already been demonstrated by both morphological (Schaefer, 1987; Armbruster, 2004) and molecular (Montoya-Burgos *et al*., 1998) analyses. However internal relationships of this group stayed unexplored. Probably due to the extreme morphological diversification observed in Loricariinae, and relative apparent stability of the diagnostic characters used to define tribal and generic ranks, both coupled to the extensive works of Isbrücker on this subfamily, the systematics of this group stayed for long largely accepted. However, these hypotheses never benefited from a real phylogenetic evaluation. The results presented in the different chapters of this thesis demonstrate that the systematics of this group was only partly correct. Particularly, the definition of the Harttiini tribe was erroneous. Isbrücker (1979) defined the Harttiini as having the dorsal fin originating approximately opposite to the pelvic-fin origin, the caudal fin with 12 (rarely 11) soft rays, no orbital notch, and little variability in tooth and lip structures, and placed *Sturisoma*, *Harttia*, *Lamontichthys*, *Harttiella*, *Pterosturisoma*, *Cteniloricaria*, *Sturisomatichthys*, and *Metaloricaria* within Harttiini. Based on the same diagnostic characters Covain and Fisch-Muller (2007) (Annex 2) recovered partly this grouping using a partitioning hierarchical analysis, with *Metaloricaria* and *Farlowella* branching out of the tribe due to diverging features. However, for identification purposes these authors followed the classification of Isbrücker (1979). Molecular phylogenies reconstructed using mitochondrial (Chapter 1) and a

combined dataset mixing mitochondrial and nuclear data (Chapters 2, 4, and 5) demonstrated that this grouping was not natural, and that Harttiini was restricted to only three genera *Harttia*, *Harttiella*, and *Cteniloricaria* (Chapters 3 and 4). Other genera but *Metaloricaria* were placed in a new subtribe of the Loricariini named Farlowellina, and *Metaloricaria* formed the sister genus of all other Loricariina (Chapters 1, 2, 4, and 5). Moreover the exhaustive phylogeny provided in Chapter 5 revealed complex evolutionary patterns in Farlowellina with *Farlowella*, *Sturisoma*, and *Sturisomatichthys* found paraphyletic despite their highly derived morphology making them resemble sticks of wood. Different synonymies (*Ixinandria* and *Apistoloricaria*) and revalidations (*Proloricaria*) were also highlighted in Loricariina. In addition, nine new species (six *Harttiella*, two *Harttia*, and one *Cteniloricaria*), and the new genus and new species *Fonchiiloricaria nanodon* were revealed and described (Chapters 2 and 3) increasing the total number of valid species to 230 distributed in 31 genera. All different chapters developed throughout this work provide thus significant updates to our knowledge and understanding of the complex systematics of the Loricariinae.

The significant modifications in the structure of the phylogenetic tree were the natural consequence of incorrectly defined diagnostic characters. The characters provided to diagnose tribal and generic ranks were accordingly evaluated in regards to the phylogeny. In the first chapter, we demonstrated that these features were in general sufficient to define naturally tribal and particularly sub-tribal ranks (including parts of the morphological groups proposed by Covain and Fisch-Muller, 2007; Annex 2), but were clearly insufficient at the generic level. For this we used the co-inertia analysis (CIA) (Dolédec and Chessel, 1994) to extract the joint structure between the phylogeny (previously converted into a distance matrix) and a table of diagnostic morphological traits (quantitative and qualitative). In this case, CIA highlighted the traits that possess the maximum covariation with the phylogeny as well as phylogenetic associations among traits. This way to proceed using the CIA represents a valid possibility for a multivariate exploration of a table of traits in regards to a phylogeny, and consequently allows to detect phylogenetic dependence in multiple traits. This first attempt represents a convincing result of the power of the multi-table methods in comparative biology that allowed the extension of this approach. We thus naturally experienced the multi-table methods in different evolutionary problematics (*i.e.* at least one of the tables always represented a phylogeny).

In a diversity study conducted on Harttiini within the Guianas, the multiple co-inertia analysis (MCOA) (Chessel and Hanafi, 1996) united morphometry, genetics, and ecology-

distribution in the same analysis (Chapter 3). The MCOA highlighted unrevealed associations among these three types of data, and provided strong evidences for the validity of three genera of Harttiini differing in combination of these different data. The analysis also demonstrated that the real diversity was twice as previously recorded. This tremendous diversity was shaped by (or oriented toward) an intraphenotypic component made of morphological adaptations and genetic divergences, and an extraphenotypic component made of ecology and distribution of the species. The morphological adaptations included important modifications in size and shape particularly at the level of the caudal peduncle. These morphological modifications were correlated with the genetic divergence and environmental parameters such as the type of colonized biotope (forest creek or main river), and the temperature, as well as with distributional gradients represented by altitude and longitude.

In the fourth chapter we evaluated the ability of the RLQ analysis (Dolédec *et al.,* 1996) to detect co-structures into two independent phylogenies constrained by their species distribution. The strength of the RLQ relies on the link table L providing the hypothesis constraining the analysis. The co-structures revealed are thus directly interpretable in the light of the emitted hypothesis, all other apparently visible co-structure being potentially related to unrevealed factors. Results of Chapter 3 demonstrated indeed that the evolution of a group was by essence multifactorial implying both intra and extra phenotypic parameters. Thus visual interpretation of potential co-structures observed in the branching order of phylogenies is hazardous and should be avoided as much as possible, other alternative evolutionary constraints potentially explaining independently such patterns. Freshwater fishes represent a group of high interest in this comparative phylogeographic approach due to biological and physiological adaptations constraining their abilities to dispersion. Contrary to marine or terrestrial organisms, freshwater fishes are only able to disperse within a river basin, or between adjacent basins in a stepping stone manner. Major climatic and geological events shaped the modern South-American Rivers through the entire Miocene and Pleistocene (Lundberg *et al*., 1998, 2010; Hoorn and Wesslingh, 2010), providing opportunities for vicariance and/or dispersion of species through headwaters or estuaries secondary contacts. Given that the history of the contemporaneous rivers is tightly linked to these underlying geological events, the chronology of river connections, and accordingly species' dispersion, may be track back in time (Hubert *et al*., 2007). Thus assuming the hypothesis of co-dispersion of species, *i.e*. that contemporaneous species may be present in the same basin because they simultaneously colonized this basin due to the same historical events (*e.g*. headwater capture, estuary secondary contact, geological fracture), we explored the

phylogeny of Harttiini and the one of *Hypostomus* previously published by Montoya-Burgos (2003). The RLQ perfectly detected a strong and significant spatial phylogenetic co-structure of both trees implying co-dispersion between species from the Amazonian and Sao Francisco basins. This result was reinforced by the fourthcorner testing procedure developed by Legendre *et al.* (1997) and extended by Dray and Legendre (2008) to allow the combination of different models in the global testing procedure, and by Dray (in prep.) to test the individual link between each variables of R and Q (here the PCOs of each phylogeny) and the axes of the RLQ analysis (the compromise established between the phylogenies and the co-distribution of species). The observed phylogenetic spatial co-structure was thus not due to chance. The dating provided for this co-dispersion in *Hypostomus* was accordingly applied to the phylogeny of Harttiini and the history of dispersion and diversification of this tribe was revealed at the subcontinental scale. The dating obtained for the phylogeny of Harttiini perfectly met those provided for *Hypostomus* suggesting a common temporal context of diversification. The sudden diversification of Harttiini and *Hypostomus* reveals an explosive radiation pattern at base of both lineages, each clade in both phylogenies appearing at the same period. These concomitant cladogenetic events suggest a global common factor explaining the origin of the different lineages such as sea level fluctuations during the Miocene period.

The multi-table methods used in Chapters 1, 3, and 4 rely on the representation of a phylogenetic distance matrix using principal coordinates (Gower, 1966) that are not always the best descriptors for a phylogeny (*e.g.* when the tree possesses strong imbalance). Moreover, first PCOs often characterize deepest nodes implying more distant relationships. These nodes display more variations onto axes, and consequently possess a greater weight in the analysis. Following results of Ogden and Rosenberg (2006) who demonstrated that balanced reconstructed topologies were much more robust to alignment inaccuracy than pectinate topologies (until 50% inaccuracy in the alignment, in mean did not impact the reconstructed phylogenetic tree topology for balanced, ultrametric, equal branch length tree shapes), we demonstrated in Chapter 2 that our manually aligned sequences data, even though containing inaccuracies, provided better results. The tree obtained using all available information was found to be more robust (smaller Colless' index (Colless, 1982) implying a more balanced topology and greater mean nodal support), and provided therefore a good estimator of the phylogeny in the multi-table analyses. Moreover, PCOs have also been efficiently used to describe phylogenies for the study of coevolution between hosts and their parasites (Legendre *et al.,* 2002). The ParaFit method indeed tests the significance of a global

hypothesis of coevolution between parasites and their host using the phylogenetic trees of both parasites and hosts beforehand described by their respective PCOs, and an host-parasite binary coding association matrix as link (see discussion about RLQ above). Another alternative multivariate method was proposed in chapter 5 and is based on the representation of the topological properties of the phylogenetic tree via an orthonormal basis.. We extend the orthogram method developed by Ollier *et al*. (2006) to deal with categorical variables and multivariate data including tables mixing qualitative and quantitative data, providing therefore a new global test of phylogenetic autocorrelation. These new tools give thus a clear prominence to the phylogenetic dependence of a table at different levels (global or local) using the same statistical frame. This unifying structure, making each test directly comparable, subsequently allowed the development of a new multivariate method for the exploration of patterns of co-evolution among traits along a phylogeny. This new approach adapts the multi-scale pattern analysis (MSPA) technique developed for the analysis of spatial data (Jombart *et al*., 2009) into a phylogenetic context. The method corrects for the possible artifact introduced by the use of principal coordinates in other multi-table methods by using a topology-based orthonormal basis representing the phylogeny (Ollier *et al*., 2006). The multivariate orthogram computed on a dataset mixing quantitative (continuous and discrete), qualitative (binary, multimodal, and ordinal), intraphenotypic (morphological and ethological) and extraphenotypic (ecological) variables revealed that the data were strongly autocorrelated with the phylogeny and implied the deepest nodes in the explanation of the distribution of the biological traits' variance. The univariate orthograms confirmed this result, with most of the traits linked to the mouth that were effectively explained by the first vectors (number of premaxillary and dentary teeth, tooth and mouth shapes, lip surface, maxillary and fringed barbels) with the addition of the number of caudal-fin rays, and the presence or absence of predorsal keels and postorbital notches, corroborating thus the findings of the first chapter using the CIA (and thus PCOs). The MSPA perfectly confirms these results and revealed strong associations between mouth features and the deepest nodes of the phylogeny, confirming that all these structures are linked. However, very few correlations were observed with the ecological and ecomorphological variables implying that the co-evolution in mouth characteristics was not related to ecological habits as hypothesized in Chapter 1. The MSPA (and univariate orthograms) highlighted in fact that such modifications were related to sexual characteristics that are the reproductive strategies and the sexual dimorphism. Reproductive strategies are diverse in Loricariinae and belong to five groups. Members of Harttiini and Farlowellina are indeed known to be open brooders (*i.e*. eggs are laid on an exposed surface

and guarded by the male), while Loricariina members display numerous alternative strategies: members of the *Pseudohemiodon-Loricaria* groups are abdomino-lip brooders (*i.e*. eggs are laid in a single layered mass, and are maintained to the surface of the lower lip and abdomen of the male); members of the *Loricariichthys* group are lip brooders (*i.e.* eggs are laid in a mass and held by the male in the fold made by its enlarged lips); and others such as *Rineloricaria* representatives are cavity brooders (*i.e.* eggs are laid attached to one another in single layer masses on the cavity floor, and are brooded by the male) (Covain and Fisch-Muller, 2007). Evers and Seidel (2005) also reported the use of a vegetal support such as a dead leaf by members of *Limatulichthys*. In this case, the eggs are laid in a mass and attached to the surface of the support. The eggs and support are then held by the male in the fold made by its lips. Sexual dimorphism displays accordingly substantial variations related to the different breeding strategies. The co-evolution among traits related to the mouth was thus shaped by behavioural constraints suggesting sexual selection. From the initial condition related to respiration and feeding, the mouth evolved in Loricariidae toward new functions related to adherence to the substrate and locomotion (see Geerinckx *et al*., 2011). In Loricariinae the mouth (and related features) evolved from this secondary function toward a third function related to reproduction. Surprisingly, these new innovations were concomitant with the loss of pronounced secondary sexual dimorphism. The hypertrophy of odontodes that is sometimes extreme in open and cavity brooders, disappeared in mouth brooders. If the appearance of a rounded tooth crown in brooding males could be explained by a higher risk for eggs and embryos to be damaged by pointed crowns, the loss of hypertrophied odontodes on the snout margin and pectoral fins does not have direct interpretation. Males of open and cavity brooders stay struck to the fry when guarding eggs and embryos (pers. obs.) and this behaviour is not more risky for the fry than having the eggs in the mouth and against the abdomen such as in abdomino-lip brooders. Moreover, the hypertrophied odontodes are seasonal and only expressed during the reproduction period. One can tentatively explain this phenomenon by the action of predation. In open and cavity brooders, the fry is often hidden in caves or exposed in fast flowing waters, and actively defended by the male. This guarding behaviour preserves the fry from predators by inaccessibility of the fry (hidden or laid in difficult to access places), and let the male free for the defence of eggs and embryos. Even in case of death of the male, the fry may thus be prevented from predation. In open and cavity brooders, the hypertrophied odontodes may play a role in the defence of the fry, but also prove to females the reproductive value of the male, larger males bearing larger odontodes accessing more easily to reproduction (pers. obs.). In mouth brooders, the situation is

inverted. Any predation activity against the male, definitely compromise the success of reproduction. In this case, bearing external attributes rendering brooding males identifiable may represent a signal for predators, and thus represents a severe disadvantage. The hypertrophy of odontodes may have been sexually selected by females, but its subsequent loss may be the result of natural selection carried out by predators. All these innovations of the Loricariinae appeared during the Oligocene period (~ 30Ma.) and evolved throughout Miocene. These results corroborate the hypothesis of Lundberg *et al.* (2010) who, based on fossil records, hypothesized a Cretaceous and tertiary diversification of Neotropical fishes favoured by the uplift of the Andes and fluctuating global sea levels. The Oligocene period is characterized by major geological events that affected the whole subcontinent (Lundberg *et al.*, 1998, 2010; Hoorn and Wesslingh, 2010). The uplift of the Andes initiated during the Middle Cretaceous about 90 Ma. ago by the low-elevation of the proto-cordillera (Lundberg *et al.*, 1998), and underwent a major orogenic phase during Oligocene responsible for the progressive establishment of modern Amazon and Orinoco.

The generalization of orthograms to any type of data, and the MSPA not only revealed co-evolution among traits but also highlighted the region of the tree that underwent these changes. This powerful analysis is thus able to detect among multiple traits of different nature, which can all be under phylogenetic dependence, those that underwent similarly evolutionary changes at different level of a phylogeny. This analysis highlights the importance of the evolutionary patterns in the comparison of multiple traits, all phylogenetically constrained traits not being necessarily linked at the same level (one can make a parallel with previous remark on the hazard of visual interpretation of co-structures and the possible existence of hidden parameters).

This thesis represents a first step in the evolutionary study of the Loricariinae, and more widely of the Neotropical ichthyofauna. I tried, as much as possible, to systematically reject all *a priori* prior to conduct any analyses, by measuring, evaluating, and controlling the data to prevent misinterpretations of the results. The multi-table methods and MSPA provide the necessary unifying frame to reach this goal, by avoiding individual interpretation of the different data sets, especially in comparisons with phylogenetic trees. These approaches were relevant and particularly powerful to correctly describe multivariate associations with the phylogenies, revealing unexpected associations as well as the importance of the evolutionary patterns in the comparison of multiple traits. These results also highlight the necessity to account for phylogenetic constraints in the data, and to develop exploratory tools to

investigate the biological questions such as the MSPA (Jombart *et al*., 2009) or the phylogenetic principal component analysis (pPCA) (Jombart *et al*., 2010). All these approaches open a plethora of new problematics in evolutionary biology and should be considered more widely to provide stronger evidences for a correct estimation of the underlying forces driving the evolution of the groups under study.

References

Abhyankar, A., Park, H.B., Tonolo, G., Luthman, H., 2009. Comparative sequence analysis of the non-protein-coding mitochondrial DNA of inbred rat strains. *PLoS One,* **4**:e8148.

Almada, V.C., Robalo, J.I., Levy, A., Freyhof, J., Bernardi, G., and Doadrio, I., 2009. Phylogenetic analysis of peri-Mediterranean blennies of the genus *Salaria*: molecular insights on the colonization of freshwaters. *Molecular Phylogenetics and Evolution,* **52(2)**: 424-31.

Armbruster, J.W., 2004. Phylogenetic relationship of the suckermouth armoured catfishes (Loricariidae) with emphasis on the Hypostominae and the Ancistrinae. *Zoological Journal of the Linnean Society*, **141**: 1-80.

Bady, P., Dolédec, S., Dumont, B., and Fruget, J-F., 2004. Multiple co-inertia analysis: a tool for assessing synchrony in the temporal variability of aquatic communities. *Comptes Rendus Biologies,* **327**: 29-36.

Bock, W.J., 2004. Species: The concept, category and taxon. *Journal of Zoological Systematics and Evolutionary Research,* **42**:178-190.

Bookstein, F.L., 1991. Morphometric Tools for Landmark Data: Geometry and Biology. 435 p., Cambridge University Press, Cambridge, UK.

Calenge, C., 2006. The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling,* **197**: 516–519.

Campo, D., Machado-Schiaffino, G., Perez, J., and Garcia-Vazquez, E., 2007. Phylogeny of the genus *Merluccius* based on mitochondrial and nuclear genes. *Gene*, **406(1-2)**: 171-9.

Cardoso, Y.P., and Montoya-Burgos, J.I., 2009. Unexpected diversity in the catfish *Pseudancistrus brevispinis* reveals dispersal routes in a Neotropical center of endemism: the Guyanas Region. *Molecular Ecology,* **18**: 947–964.

Chessel, D., and Hanafi, M., 1996. Analyses de la co-inertie de K nuages de points, *Revue de Statistique Appliquée*, **44**: 35–60.

Chiachio, M.C., Oliveira, C., and Montoya-Burgos, J.I., 2008. Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). *Molecular Phylogenetics and Evolution,* **49**: 606-617.

Claude, J., 2008. Morphometrics with R. 316 p. Springer, New York.

Colless, D.H., 1982. Review of phylogenetics: The theory and practice of phylogenetic systematics. *Systematic Zoology* **31**: 100–104.

Covain, R., Le Bail, P-Y., Sagnes, P., and Fisch-Muller, S., 2006. Les espèces du genre *Harttia* (Siluriformes : Loricariidae) en Guyane Française : morphologie, taxinomie et distribution. *Cybium*, **30**: 3–18.

Covain, R., and Fisch-Muller, S., 2007. The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: Loricariidae): a practical key and synopsis. *Zootaxa* **1462**: 1-40.

Cowman, P.F., Bellwood, D.R., and van Herwerden, L., 2009. Dating the evolutionary origins of wrasse lineages (Labridae) and the rise of trophic novelty on coral reefs. *Molecular Phylogenetics and Evolution,* **52(3)**: 621-31.

de Carvalho Paixão, A., and Toledo–Piza, M., 2009. Systematics of *Lamontichthys* Miranda-Ribeiro (Siluriformes: Loricariidae), with the description of two new species. *Neotropical Ichthyology,* **7**: 519-568.

Delsuc, F., Brinkmann, H., Chourrout, D., and Philippe, H., 2006. Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature* **439**: 965–968.

Diekmann, H., Klinger, M., Oertle, T., Heinz, D., Pogoda, H.M., Schwab, M.E., and Stuermer, C.A., 2005. Analysis of the reticulon gene family demonstrates the absence of the neurite growth inhibitor Nogo-A in fish. *Molecular Biology and Evolution*, **22(8)**:1635-48.

Dolédec, S., and Chessel, D., 1994. Co-inertia analysis: an alternative method for studying species-environment relationships. *Freshwater Biology* **31**: 277–294.

Dolédec, S., Chessel, D., ter Braak, C.J.F., and Champely, S., 1996. Matching species traits to environmental variables: a new three-table ordination method. *Environmental and Ecological Statistics*, **3**: 143–166.

Dray, S., Pettorelli, N., and Chessel, D., 2002. Matching data sets from two different spatial samplings. *Journal of Vegetation Science*, **13**: 867–874.

Dray, S., Chessel, D., and Thioulouse, J., 2003a. Procrustean co-inertia analysis for the linking of multivariate data sets. *Ecoscience*, **10(1)**:110-119.

Dray, S., Chessel, D., and Thioulouse, J., 2003b. Co-inertia analysis and the linking of ecological data tables. *Ecology*, **84**:3078-3089.

Dray, S., and Dufour, A-B., 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, **22(4)**: 1-20.

Dray, S., and Legendre, P., 2008. Testing the species traits-environment relationships: the fourth-corner problem revisited. *Ecology*, **89**: 3400–3412.

Edgar, R.C., 2004a. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32(5)**:1792-1797.

Edgar, R.C., 2004b. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, **5**: 113.

Edwards, A.W.F., and Cavalli-Sforza, L.L., 1964. Reconstruction of evolutionary trees, pp. 67–76 *in* (Heywood, V.H., and McNeill, J., Eds.) *Phenetic and Phylogenetic Classification*, Systematics Association Publication No. 6, London.

Evers, H-G., and Seidel, I., 2005. Catfish Atlas Vol. 1: South American Catfishes of the Families Loricariidae, Cetopsidae, Nematogenyidae and Trichomycteridae. 943 p. Mergus Verlag Gmbh, Melle.

Felsenstein, J., 1981. Evolutionary trees from DNA-sequences- a maximum-likelihood approach. *Journal of Molecular Evolution* **17**: 368–376.

Fernández, L., and Schaefer, S.A., 2009. Relationships among the Neotropical Candirus (Trichomycteridae, Siluriformes) and the evolution of parasitism based on analysis of mitochondrial and nuclear gene sequences. *Molecular Phylogenetics and Evolution*, **52(2)**: 416-23.

Ferraris, C.J., Jr., 2003. Loricariinae, pp. 330-350 *in* (Reis, R.E., Kullander, S.O., and Ferraris, C.J., Jr., eds.) *Check list of the freshwater fishes of South and Central America*. Edipucrs, Porto Alegre, Brazil.

Ferraris, C.J., Jr., 2007. Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. *Zootaxa*, **1418**: 1-628.

Fichberg, I., and Chamon, C.C., 2008. *Rineloricaria osvaldoi* (Siluriformes: Loricariidae): a new species of armored catfish from rio Vermelho, Araguaia basin, Brazil. *Neotropical Ichthyology,* **6**: 347-354.

Fisch-Muller, S., Mazzoni, R., and Weber, C., 2001. Genetic and morphological evidences for two new sibling species of *Ancistrus* (Siluriformes: Loricariidae) in upper rio Tocantins drainage, Brazil. *Ichthyological Exploration of Freshwaters,* **12**: 289-304.

Fisch-Muller, S., Montoya-Burgos, J.I., Le Bail, P-Y., and Covain, R., in press. Diversity of the Ancistrini (Siluriformes: Loricariidae) from the Guianas: the *Panaque* group, a molecular appraisal with descriptions of new species. *Cybium*.

Geerinckx, T., Herrel, A., and Adriaens, D., 2011. Suckermouth armored catfish resolve the paradox of simultaneous respiration and suction attachment: a kinematic study of *Pterygoplichthys disjunctivus*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology,* **315A (3)**: 1932-5231.

Ghazzi, M.S., 2005. *Sturisoma kneri*, new species, a name for an old yet poorly-known catfish (Siluriformes: Loricariidae). *Copeia*, **2005**: 559–565.

Ghazzi, M.S., 2008. Nove espécies novas do gênero *Rineloricaria* (Siluriformes,Loricariidae) do rio Uruguai, do sul do Brasil. *Iheringia, Série Zoologia,* **98**: 100-122.

Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika,* **53**: 325–338.

Hall, B.G., 2005. Comparison of the accuracies of several phylogenetic methods using protein and DNA sequences. *Molecular Biology and Evolution*, **22**:792-802.

Hall, BG., 2007. Phylogenetic trees made easy: a how-to manual. 3rd ed. 233 p., Sinauer Associates, Sunderland (MA).

Harvey, P.H., and Pagel, M.D., 1991. The Comparative Method in Evolutionary Biology. 248 p., Oxford University Press, Oxford, UK.

Hebert, P.D.N., Cywinska, A., Ball, S.L., and. De Waard, J.R., 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B*., **270**: 313–322.

Hill, M.O., and Smith, J.E., 1976. Principal component analysis of taxonomic data with multi-state discrete characters. *Taxon*, **25**: 249-255.

Hillis, D.M., and Dixon, M.T., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *The Quarterly Review of Biology*, **66**: 411–453.

Hoorn, C., and Wesselingh, F., (eds.) 2010. Amazonia, Landscape and Species Evolution: A Look into the Past. 464 p., Blackwell Publishing, London, UK.

Hrbek, T, Seckinger, J, and Meyer, A., 2007. A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. *Molecular Phylogenetics and Evolution*, **43(3)**:986-98.

Hubert, N., Duponchelle, F., Nuñez, J., Garcia-Davila, C., Paugy, D., and Renno, J-F., 2007. Phylogeography of the piranha genera *Serrasalmus* and *Pygocentrus*: implications for the diversification of the Neotropical ichthyofauna. *Molecular Ecology*, **16**: 2115-2136.

Hubert, N., Hanner, R., Holm, E., Mandrak, N.E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J., and Bernatchez, L., 2008. Identifying Canadian freshwater fishes through DNA barcodes. *Plos One*: **3**: e2490.

ICZN [International Commission on Zoological Nomenclature], 1999. International Code of Zoological Nomenclature. 4th Edition, 306 p., International Trust for Zoological Nomenclature, London.

Ingenito, L.F.S., Ghazzi, M.S., Duboc, L.F., and Abilhoa, V., 2008. Two new species of *Rineloricaria* (Siluriformes: Loricariidae) from the rio Iguaçu basin, southern Brazil. *Neotropical Ichthyology,* **6**: 355-366.

Isbrücker, I.J.H., 1979. Description préliminaire de nouveaux taxa de la famille des Loricariidae, poissons-chats cuirassés néotropicaux, avec un catalogue critique de la sous-famille nominale (Pisces, Siluriformes). *Revue Française d'Aquariologie et Herpétologie*, **5**: 86-116.

Isbrücker, I.J.H., 1981a. A treatise of the Loricariidae Bonaparte, 1831, a family of South American mailed catfishes, with emphasis on the subfamily Loricariinae (Pisces, Siluriformes). Thèse d'Université, Université de Nancy I, Nancy, i-ii; I,1-4; II,1-2; III,1-2; IV,1-17; V,1-8; VI,1-121; VII,1-6; VIII,1-7; IX,1-27; X,1-8; XI,1-22; 52 pls.

Isbrücker, I.J.H., 1981b. Revision of *Loricaria* Linnaeus, 1758 (Pisces, Siluriformes, Loricariidae). *Beaufortia*, **31(3)**: 51-96.

Isbrücker, I.J.H., and Nijssen, H., 1982. New data on *Metaloricaria paucidens* from French Guiana and Surinam (Pisces, Siluriformes, Loricariidae). *Bijdragen tot de Dierkunde*, **52**: 155–168.

Isbrücker, I.J.H., Britski, H.A., Nijssen, H., and Ortega, H., 1983. *Aposturisoma myriodon*, une espèce et un genre nouveaux de Poisson-Chat cuirassé, tribu Farlowellini Fowler,

1958 du Bassin du Rio Ucayali, Pérou (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpétologie*, **10 (2)**: 33-42.

Isbrücker, I.J.H., and Nijssen, H., 1984. *Pyxiloricaria menezesi,* a new genus and species of mailed catfish from Rio Miranda and Rio Cuiabá, Brazil (Pisces, Siluriformes, Loricariidae). *Bijdragen tot de Dierkunde*, **54 (2)**: 163-168.

Isbrücker, I.J.H., and Nijssen, H., 1986. *Apistoloricaria condei,* nouveau genre et nouvelle espèce de poisson-chat cuirassé, tribu Loricariini Bonaparte, 1831, du bassin du Rio Napo, haute Amazone, Equateur (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpetologie*, **12**: 103-108.

Isbrücker, I.J.H., Seidel, I., Michels, J.P., Schraml, E., and Werner., A., 2001. Diagnose vierzehn neuer Gatungen der Familie Loricariidae Rafinesque, 1815 (Teleostei, Ostariophysi), pp. 17-24, *in* (Stawikowski, R., ed.), *Harnischwelse 2*. Die Aquarien- und Terrarien Zeitschrift, Sonderheft, Eugen Ulmer, Stuttgart.

James Cooper, W., Smith, L.L., and Westneat, M.W., 2009. Exploring the radiation of a diverse reef fish family: phylogenetics of the damselfishes (Pomacentridae), with new classifications based on molecular analyses of all genera. *Molecular Phylogenetics and Evolution*, **52(1)**:1-16.

Javonillo, R., Malabarba, L.R., Weitzman, S.H., and Burns, J.R., 2010. Relationships among major lineages of characid fishes (Teleostei: Ostariophysi: Characiformes), based on molecular sequence data. *Molecular Phylogenetics and Evolution*, **54(2)**:498-511.

Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**: 1403-1405.

Jombart, T., Dray, S., and Dufour, A.-B., 2009. Finding essential scales of spatial variation in ecological data: a multivariate approach. *Ecography,* **32**: 161-168.

Jombart, T., Balloux, F. and Dray, S., 2010. adephylo: new tools for investigating the phylogenetic signal in biological traits. *Bioinformatics*, **26**: 1907-1909.

Jombart, T., Pavoine, S., Devillard, S., and Pontier, D., 2010. Putting phylogeny into the analysis of biological traits: a methodological approach. *Journal of Theoretical Biology*, **264**: 693-701.

Katoh, M., and Kuma, M. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research,* **30**:3059-3066.

Knaack, J., 2003. Ein neuer Prachthexenwels aus Paraguay: *Hemiloricaria aurata* n. sp. (Pisces, Siluriformes, Loricariidae). *Aquaristik aktuell*, **11**: 56–61.

Kottelat, M., 1997. European freshwater fishes. *Biologia,* **52, Suppl. 5**:1-271.

Kumar, S., and Filipski, A., 2007. Multiple sequence alignment: in pursuit of homologous DNA positions. *Genome Research*, **17(2)**:127-35.

Lake, J.A., 1991. The order of sequence alignment can bias the selection of tree topology. *Molecular Biology and Evolution*, **8**: 378-385.

Lara, A., Ponce De León, J.L., Rodríguez, R.L., Casane, D.L., Côté, G., Bernatchez, L., and Garcia-Machado, E., 2010. DNA barcoding of Cuban freshwater fishes: evidence for cryptic species and taxonomic conflicts. *Molecular Ecology Resources*, **10**: 421-430.

Legendre, P., Galzin, R., and Harmelin-Vivien, M.L., 1997. Relating behavior to habitat: solutions to the fourth-corner problem. *Ecology*, **78**: 547–562.

Legendre, P., Desdevises, Y., and Bazin, E., 2002. A statistical test for host-parasite coevolution. *Systematic Biology*, **51(2)**: 217–234.

Lévêque, C., Oberdorff, T., Paugy, D., Stiassny, M.L.J., and Tedesco, P.A., 2008. Global diversity of fish (Pisces) in freshwater. *Hydrobiologia,* **595**: 547-567.

Löytynoja, A., and Milinkovitch, M.C., 2001. SOAP, cleaning multiple alignments from unstable blocks. *Bioinformatics* **17**: 573-574.

Lundberg, J.G., Marshall, L.G., Guerrero, J., Horton, B., Malabarba, M.C.S.L., and Wesselingh, F., 1998. The stage for Neotropical fish diversification: a history of tropical South American Rivers, pp. 13–48, *In*: (Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena Z.M.S., and Lucena, C.A.S., eds.), *Phylogeny and Classification of Neotropical Fishes*. Edipucrs Porto Alegre, Porto Alegre, Brazil.

Lundberg, J.G., Sabaj Pérez, M.H., Dahdul, W.M., and Aguilera, O.A., 2010. The Amazonian Neogene fish fauna, pp. 281–301, *In* (Hoorn, C.M., and Wesselingh, F.P., eds.), *Amazonia, Landscape and Species Evolution: A Look into the Past*. Blackwell Publishing, London, UK.

Lutzoni, F., Wagner, P., Reeb, V., and Zoller, S., 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology*, **49(4)**: 628-51.

Maderbacher, M., Bauer, C., Herler, J., Postl, L., Makasa, L., and Sturmbauer, C., 2008. Assessment of traditional versus geometric morphometrics for discriminating populations of the *Tropheus moorii* species complex (Teleostei: Cichlidae), a Lake Tanganyika model for allopatric speciation. *Journal of Zoological Systematics and Evolutionary Research,* **46**: 153–161.

Martín Salazar, F.J., Isbrücker, I.J.H., and Nijssen, H., 1982. *Dentectus barbarmatus*, a new genus and species of mailed catfish from the Orinoco Basin of Venezuela (Pisces, Siluriformes, Loricariidae). *Beaufortia*, **32 (8)**: 125-137.

Mayr, E., 1963. Animal species and evolution. XVI + 797 p. Harvard University Press, Cambridge.

Mérigoux, S., Ponton, D., and Mérona, B. de, 1998. Fish richness and species-habitat relationships in two coastal streams of French Guiana, South America**.** *Environmental Biology of Fishes*, **51**: 25-39.

Mérigoux, S., Dolédec, S., and Statzner, B., 2001. Species traits in relation to habitat variability and state: Neotropical juvenile fish in floodplain creeks. *Freshwater Biology*, **46**: 1251-1267.

Mérona, B. de, Tejerina-Garro, F.L., and Vigouroux, R., in press. Fish-habitat relationships in French Guiana's rivers: a review. *Cybium*.

Mindell, D.P., and Honeycutt, R.L., 1990. Ribosomal RNA in vertebrates: evolution and phylogenetic implications. *Annual Review of Ecology, Evolution, and Systematics*, **21**: 541–566.

Montoya-Burgos, J.I., Muller, S., Weber, C., and Pawlowski, J., 1998. Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences, pp. 363-374, *in* (Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M.S., and Lucena, C.A.S., eds.), *Phylogeny and Classification of Neotropical Fishes*. Edipucrs, Porto Alegre.

Montoya-Burgos, J.I., 2003. Historical biogeography of the catfish genus *Hypostomus* (Siluriformes: Loricariidae), with implications on the diversification of Neotropical ichthyofauna. *Molecular Ecology*, **12**: 1855-1867.

Montoya-Burgos, J.I., Foulon, A., and Bahechar, I., 2010. Transcriptome screen for fast evolving genes by Inter-Specific Selective Hybridization (ISSH). *BMC Genomics*, **11**: 126.

Murphy, W.J., Thomerson, J.E., and Collier, G.E., 1999. Phylogeny of the Neotropical killifish family Rivulidae (Cyprinodontiformes, Aplocheiloidei) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, **13(2)**: 289-301.

Nelesen, S.**,** Liu, K.**,** Zhao, D.**,** Linder, C.R.**,** and Warnow, T**.,** 2008. The effect of the guide tree on multiple sequence alignments and subsequent phylogenetic analyses. *Pacific Symposium on Biocomputing*, **13**:25-36.

Nijssen, H., and Isbrücker, I.J.H., 1987. *Spectracanthicus murinus*, nouveaux genre et espèce de poisson-chat cuirassé du Rio Tapajós, Est. Pará, Brésil, avec des remarques sur d'autres genres de Loricariidés (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpétologie*, **13**: 93–98.

Nijssen, H., and Isbrücker, I.J.H., 1988. Trois nouvelles espèces du genre *Apistoloricaria* de Colombie et du Pérou, avec illustration du dimorphisme sexuel secondaire des lèvres de *A. condei* (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpétologie*, **15 (2)**: 33-38.

Oertle, T., Klinger, M., Stuermer, C.A., and Schwab, M.E., 2003. A reticular rhapsody: phylogenic evolution and nomenclature of the RTN/Nogo gene family. *FASEB Journal*, **17(10)**: 1238-47.

Ogden, T.H., and Rosenberg, M.S., 2006. Multiple sequence aligment accuracy and phylogenetic inference. *Systematic Biology*, **55(2)**:314–328.

Ollier, S., Couteron, P., and Chessel, D., 2006. Orthonormal transform to decompose the variance of a life-history trait across a phylogenetic tree. *Biometrics,* **62**: 471–477.

Ortí, G., Petry, P., Porto, J.A., Jegú, M., and Meyer, A., 1996. Patterns of nucleotide change in mitochondrial ribosomal RNA genes and the phylogeny of piranhas. *Journal of Molecular Evolution,* **42 (2)**: 169-182.

Parsons, K.J., Robinson, B.W., and Hrbek, T., 2003. Getting into shape: an empirical comparison of traditional truss-based morphometric methods with a newer geometric method applied to new world cichlids. *Environmental Biology of Fishes,* **67**: 417–431.

Pavoine, S., Ollier, S., Pontier, D., and Chessel, D., 2008. Testing for phylogenetic signal in life history variable: Abouheif's test revisited. *Theoretical Population Biology,* **73**: 79–91.

Pavoine, S., Baguette, M., and Bonsall, M.B., 2010. Decomposition of trait diversity among the nodes of a phylogenetic tree. *Ecological Monographs,* **80**: 485-507.

Pinna, M.C.C. de., 1998. Phylogenetic relationships of neotropical Siluriformes (Teleostei: Ostariophysi): historical overview and synthesis of hypotheses, pp. 279-330, *in* (Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M., and Lucena, C.A.S., eds.), *Phylogeny and classification of Neotropical fishes*. Edipucrs, Porto Alegre.

Provenzano, R.F., Machado-Allison, A., Chernoff, B., Willink, P., and Petry, P., 2005. *Harttia merevari*, a new species of catfish (Siluriformes: Loricariidae) from Venezuela. *Neotropical Ichthyology*, **3**: 519–524.

R Development Core Team, 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rannala, B., and Yang, Z., 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution*, **43(3)**: 304-11.

Rapp Py-Daniel, L.H., 1981. *Furcodontichthys novaesi* n. gen., n. sp. (Osteichthyes, Siluriformes; Loricariidae) na bacia Amazônia, Brasil. *Boletim do Museu Paraense Emilio Goeldi, Nova Serie, Zoologia*, **105**: 1-17.

Rapp Py-Daniel, L.H., 1997. Phylogeny of the Neotropical armored catfishes of the subfamilly Loricariinae (Siluriformes: Loricariidae). Unpublished Ph. D. Thesis, University of Arizona, Tucson, 280 p.

Rapp Py-Daniel, L.H., and Oliveira, E.C., 2001. Seven new species of *Harttia* from the Amazonian-Guyana region (Siluriformes: Loricariidae). *Ichthyological Exploration of Freshwaters*, **12 (1)**: 79-96.

Rapp Py-Daniel, L.H., and Fichberg, I., 2008. A new species of *Rineloricaria* (Siluriformes: Loricariidae: Loricariinae) from rio Daraá, rio Negro basin, Amazon, Brazil. *Neotropical Ichthyology,* **6**: 339-346.

Redelings, B.D., and Suchard, M.A., 2005. Joint Bayesian estimation of alignment and phylogeny. *Systematic Biology*, **54(3)**: 401-18.

Reis, R.E., Kullander, S.O., and Ferraris, C.J., Jr., (eds.), 2003. Check List of the Freshwater Fishes of South and Central America (CLOFFSCA). 728 p. Porto Alegre: EDIPUCRS.

Retzer, M.E., and Page, L.M., 1997. Systematics of the stick catfishes, *Farlowella* Eigenmann and Eigenmann (Pisces, Loricariidae). *Proceedings of the Academy of Natural Sciences, Philadelphia*, **147**: 33-88.

Retzer, M.E., 2006. A new species of *Farlowella* Eigenmann and Eigenmann (Siluriformes: Loricariidae), a stickcatfish from Bolivia. *Zootaxa*, **1282**: 59–68.

Richtsmeier J, DeLeon, V, and Lele, S., 2002. The promise of geometric morphometrics. *Yearbook of Physical Anthropology,* **45**: 63–91.

Rodiles-Hernández, R., Hendrickson, D.A., Lundberg, J.G., and J.M., Humphries, 2005. *Lacantunia enigmatica* (Teleostei: Siluriformes) a new and phylogenetically puzzling freshwater fish from Mesoamerica. *Zootaxa*, **1000**: 1-24.

Rodríguez, M.S., and Miquelarena, A.M., 2003. Una nueva especie de *Loricaria* (Siluriformes: Loricariidae) para la cuenca del río San Francisco, Jujuy, Argentina. *Anales de la Academia Nacional de Ciensas Exactas, Físicas y Naturales*, **55**: 139–149.

Rodríguez, M.S., and Miquelarena, A.M., 2005. A new species of *Rineloricaria* (Siluriformes: Loricariidae) from the Paraná and Uruguay River basins, Misiones, Argentina. *Zootaxa*, **945**: 1–15.

Rodríguez, M., and Miquelarena, A.M., 2008. *Rineloricaria isaaci* (Loricariidae: Loricariinae), a new species of loricariid catfish from the Uruguay River basin. *Journal of Fish Biology,* **73**: 1635–1647.

Rodríguez, M.S., and Reis, R.E., 2008. Taxonomic Review of *Rineloricaria* (Loricariidae: Loricariinae) from the Laguna dos Patos drainage, Southern Brazil, with the descriptions of two new species and the recognition of two species groups. *Copeia,* **2008**: 333-349.

Rodríguez, M.S., Cramer, C.A., Bonatto, S.L. and Reis, R.E., 2008. Taxonomy of *Ixinandria* Isbrücker and Nijssen (Loricariidae: Loricariinae) based on morphological and molecular data. *Neotropical Ichthyology*, **6**: 367-378.

Rosenberg, M.S., 2005. Evolutionary distance estimation and fidelity of pair wise sequence alignment. *BMC Bioinformatics,* **6**:102.

Rüber L, Britz, R, and Zardoya, R., 2006. Molecular phylogenetics and evolutionary diversification of labyrinth fishes (Perciformes: Anabantoidei). *Systematic Biology*, **55(3)**: 374-97.

Schaefer, S.A., 1986. Historical biology of the loricariid catfishes: Phylogenetics and functional morphology. Unpublished Ph. D. Thesis, University of Chicago. 198 p., 46 figs.

Schaefer, S.A., 1987. Osteology of *Hypostomus plecostomus* (Linnaeus) with a phylogenetic analysis of the loricariid subfamilies (Pisces: Siluroidei). *Contributions in Science, Natural History Museum of Los Angeles County*, **394**: 1–31.

Schaefer, S.A., 1991. Phylogenetic analysis of the loricariid subfamily Hypoptopomatinae (Pisces: Siluroidei: Loricariidae), with comments on generic diagnoses and geographic distribution. *Zoological Journal of the Linnean Society*, **102**: 1–41.

Schaefer, S.A., and Stewart, D.J., 1993. Systematics of the *Panaque dentex* species group (Siluriformes: Loricariidae), wood-eating armored catfishes from tropical South America. *Ichthyological Exploration of Freshwaters*, **4**: 309–342.

Shimabukuro-Dias, C.K., Oliveira, C., Reis, R.E., and Foresti, F., 2004. Molecular phylogeny of the armored catfish family Callichthyidae (Ostariophysi, Siluriformes). *Molecular Phylogenetics and Evolution*, **32(1)**: 152-63.

Sidlauskas, B.L., Mol, J.H., and Vari, R.P., 2011. Dealing with allometry in linear and geometric morphometrics: a taxonomic case study in the *Leporinus cylindriformis* group (Characiformes: Anostomidae) with description of a new species from Suriname. *Zoological Journal of the Linnean Society*, **162**: 103–130.

Straube, N., Iglésias, S.P., Sellos, D.Y., Kriwet, J., and Schliewen, U.K., 2010. Molecular phylogeny and node time estimation of bioluminescent Lantern Sharks (Elasmobranchii: Etmopteridae). *Molecular Phylogenetics and Evolution*, **56(3)**: 905-17.

Thioulouse, J., Chessel, D., Dolédec, S., and Olivier, J.M., 1997. ADE–4: a multivariate analysis and graphical display software. *Statistics and Computing*, **7**: 75-83.

Thioulouse, J., and Dray, S., 2007. Interactive multivariate data analysis in R with the ade4 and ade4TkGUI packages. *Journal of Statistical Software*, **22(5)**: 1-14.

Thomas, M.R., and Rapp Py-Daniel, L.H., 2008. Three new species of the armored catfish genus *Loricaria* (Siluriformes:Loricariidae) from river channels of the Amazon basin. *Neotropical Ichthyology,* **6**: 379-394.

Thomas, M.R., and Sabaj Pérez, M.H., 2010. A new species of whiptail catfish, genus *Loricaria* (Siluriformes: Loricariidae), from the Rio Curuá (Xingu Basin), Brazil. *Copeia,* **2010**: 274-283.

Thompson, J.D., Higgins, D.G., and Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22(22)**: 4673–4680.

Thompson, J.D., Gibson, TJ., Plewniak, F., Jeanmougin, F., and Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research,* **25**: 4876–4882.

Valdez-Moreno, M., Ivanova, N.V., Elías-Gutiérrez, M., Contreras-Balderas, S. and Hebert, P.D.N, 2009. Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. *Journal of Fish Biology*, **74**: 377-402.

van de Velde, H.J.K., Roebroek, A.J., Senden, N.H., Ramaekers, F.C., and Van de Ven, W.J., 1994. NSP-encoded reticulons, neuroendocrine proteins of a novel gene family associated with membranes of the endoplasmic reticulum. *Journal of Cell Science*, **107**: 2403-2416.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., and Hebert, P.D.N., 2005. Barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B*, **360**: 1847–1857.

Ward, R.D., and Holmes, B.H., 2007. An analysis of nucleotide and amino acid variability in the barcode region of cytochrome c oxidase I (cox1) in fishes. *Molecular Ecology Notes*, **7**: 899–907.

Ward, R.D., Costa, F.O., Holmes, B.H., and Steinke, D., 2008a. DNA barcoding of shared fish species from the North Atlantic and Australasia: minimal divergence for most taxa, but *Zeus faber* and *Lepidopus caudatus* each probably constitute two species. *Aquatic Biology*, **3**: 71-78.

Ward, R.D., Holmes, B.H., and Yearsley, G.K., 2008b. DNA barcoding reveals a likely second species of Asian seabass (barramundi) (*Lates calcarifer*). *Journal of Fish Biology*, **72**: 458–463.

Ward, R.D., Hanner, R., and Hebert, P.D.N., 2009. REVIEW PAPER: The campaign to DNA barcode all fishes, FISH-BOL. *Journal of Fish Biology*, **74**: 329-356.

Weber, C., and Montoya-Burgos, J.I., 2002. H*ypostomus fonchii* sp. n. (Siluriformes: Loricariidae) from Peru, a key species suggesting the synonymy of *Cochliodon* with *Hypostomus*. *Revue suisse de Zooogie,* **109**: 355-368.

Wilson, A.B., Vincent, A., Ahnesjö, I., and Meyer, A., 2001. Male pregnancy in seahorses and pipefishes (family Syngnathidae): rapid diversification of paternal brood pouch morphology inferred from a molecular phylogeny. *Journal of Heredity*, **92(2)**: 159-66.

Yan, R., Shi, Q., Hu, X., and Zhou, X., 2006. Reticulon proteins: emerging players in neurodegenerative diseases. *Cellular and Molecular Life Sciences*, **63(7-8)**: 877-89.

Zawadski, C.H., Weber, C., Pavanelli, C.S., and Renesto, E., 2002. Morphological and biochemical comparison of two allopatric populations of *Hypostomus margaritifer* (Regan, 1907) (Teleostei: Loricariidae) from the upper Paraná basin, Brazil. *Acta Scientiarum, Maringa*, **24 (2)**: 499-505.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D., and Fink, W.L., 2004. Geometric morphometrics for biologists: a primer. 443 p., Elsevier Academic Press, San Diego, CA.

Annex 1

Les espèces du genre *Harttia* (Siluriformes: Loricariidae) en Guyane française: morphologie, taxinomie et distribution.

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This is a preliminary study conducted on Harttia *in French Guiana.*

Published in: Cybium (2006), **30(1)**: 3-18.

Open acces available at: http://www.mnhn.fr/sfi/cybium/numeros/pdf/301pdf/03-Covain%20344.pdf.

Les espèces du genre *Harttia* (Siluriformes : Loricariidae) en Guyane française : morphologie, taxinomie et distribution

par

Raphaël COVAIN (1), Pierre-Yves LE BAIL (2), Pierre SAGNES (3) & Sonia FISCH-MULLER (4)

RÉSUMÉ. - Les poissons-chats du genre *Harttia* se répartissent principalement dans l'est du continent sud-américain : dans les fleuves côtiers du Sud-Est brésilien, dans la région amazonienne et sur le bouclier guyanais. Quatre espèces sont recensées en Guyane française : *H. maculata, H. fowleri, H. surinamensis* et *H. guianensis*. Cette dernière présente certaines incertitudes taxinomiques et n'est fondée que sur du matériel provenant de deux bassins (Approuague et Sinnamary). Afin de clarifier le statut taxinomique des différentes populations de l'Ouest du département et de préciser la répartition de chaque espèce, une étude morphologique de toutes les espèces et populations de *Harttia* présentes en Guyane française et de la série type de *H. surinamensis* du Surinam a été réalisée grâce à des analyses multivariées effectuées sur de nombreux caractères morphologiques. Il en résulte que *H. surinamensis* est absente de Guyane française et que sa répartition géographique pourrait être restreinte à la rivière Suriname. Les paratypes de *H. surinamensis* du haut Tapanahoni (bassin du Maroni, rive surinamienne) sont à rattacher à *H. guianensis* ainsi que toutes les autres populations du système fluvial du Maroni et de la Mana ; les paratypes de la rivière Coppename pourraient représenter une nouvelle espèce. *Harttia fowleri* est relativement proche morphologiquement de *H. guianensis* alors que *H. maculata* s'en distingue significativement. Quelques différences sont relevées entre les populations de *H. guianensis*. Les spécimens de la localité type de *H. guianensis*, l'Approuague, ont un corps plus haut que les spécimens de toutes les autres localités et les individus du Sinnamary présentent des caractéristiques méristiques particulières qui les distinguent de toutes les autres populations. Une clef de détermination pratique de toutes les espèces de *Harttia* de Guyane française et du Surinam étudiées dans ce travail est proposée.

ABSTRACT. - Species of the genus *Harttia* (Siluriformes: Loricariidae) in French Guiana: Morphology, taxonomy and distribution.

The genus *Harttia* Steindachner, 1877 belongs to the family Loricariidae, the most diversified of all catfish families with 673 valid and around 300 undescribed species. Stream water fish of the genus *Harttia* are found in the upper course of rivers on rocky and sandy bottoms. This monophyletic genus comprises at present 22 species (Rapp Py-Daniel and Oliveira, 2001) mainly distributed on the Guiana shield, the South East of Brazil in coastal rivers, and in the Amazonian region. In French Guiana, Le Bail *et al.* (2000) list 5 species of Harttiini including 1 *Harttia*, *H. surinamensis*, and 2 other species now assigned to *Harttia*: *Cteniloricaria fowleri* from the Oyapock drainage and *Cteniloricaria maculata* from the Maroni and Sinnamary drainages. Morphological differences between populations of *H. surinamensis* were noted. French Guiana specimens differ from specimens of the type locality by the absence of abdominal scutes. Later, Rapp Py-Daniel and Oliveira (2001) put *Cteniloricaria* in the synonymy of *Harttia* and describe a new species from the Approuague and Sinnamary drainages in French Guiana without considering western populations. In the light of these recent works, a revision of the genus *Harttia* in French Guiana was clearly necessary in order to clarify the taxonomical position of all populations and species and to redefine their distribution. A morphological study was carried out on all *Harttia* species and populations from French Guiana and on the type material of *H. surinamensis* from Surinam. 28 continuous quantitative variables, 20 categorical quantitative variables, and 6 qualitative variables were noted for each specimen. After selection of the suitable variables, data were analysed with multivariate analysis. According to our results, the distribution of *H. surinamensis* may be restricted to the Suriname River drainage. Paratypes of *H. surinamensis* from the Maroni drainage (upper Tapanahoni, Surinam) are now assigned to *H. guianensis*, while those from the Coppename drainage may represent a new species. As noted by Le Bail *et al.*, *H. fowleri* is morphologically close to the *H. guianensis*, whereas *H. maculata* is very different. Populations from the Maroni and Mana drainages are assigned to *H. guianensis* and differences between populations of *H. guianensis* are emphasized. Specimens from the type locality (Approuague drainage) are deeper than those of all other populations. Specimens from the Sinnamary drainage are different considering meristic data. A practical key to the species of *Harttia* from French Guiana and Surinam studied in this work is proposed.

Key words. - Loricariidae - *Harttia* - French Guiana - Surinam - Morphology - Taxonomy - Species distribution.

Les espèces du genre *Harttia* sont torrenticoles et fréquentent la partie supérieure des fleuves et de leurs affluents, dans des zones où l'eau est claire, fraîche et bien oxygénée. Elles colonisent les substrats rocheux ou sableux où la végétation aquatique est peu abondante. Le genre *Harttia* Steindachner, 1877, appartient à la famille des Lori-

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Cybium 2006, 30(1): 3-18.

cariidae ou poissons-chats cuirassés. Cette famille néotropicale, la plus diversifiée des Siluriformes, compte aujourd'hui 673 espèces valides et quelques 300 espèces non décrites (Reis *et al*., 2003). Appartenant à la sous-famille des Loricariinae, les *Harttia* ont été placés par Isbrücker (1979) dans la tribu des Harttiini. À l'intérieur de ce taxon, se retrouvent les espèces ayant en commun les caractéristiques suivantes : origine de la dorsale située approximativement à l'aplomb de l'origine des pelviennes, absence d'encoche orbitaire, peu de diversité de structure des dents et des lèvres et un dimorphisme sexuel secondaire prononcé, caractérisé par la présence d'odontodes développés sur les marges du museau et parfois sur la partie supérieure des épines pectorales du mâle mature. En accord avec les caractères donnés dans Steindachner (1877), Boeseman (1971), Isbrücker (1979, 1981), Oyakawa (1993) et Langeani *et al*. (2001), Rapp Py-Daniel et Oliveira (2001) les distinguent des autres Harttiini (*Harttiella* et *Metaloricaria* pour la Guyane française) par : une allure trapue et un corps très aplati et peu caréné, l'absence de quilles latérales, une nageoire caudale échancrée, la présence de larges plaques entourant l'anus, des orbites rondes, la présence de nombreuses dents mandibulaires (60 à 120 par dentaire), une forme de museau arrondie, des barbillons maxillaires très peu développés ou absents, l'absence de l'anneau osseux du second ptérygiophore de la dorsale et de la petite épine du mécanisme de blocage de la dorsale. En 1997, Rapp Py-Daniel établit la monophylie du genre sur la base de quatre synapomorphies déduites de caractères ostéologiques. Elle complète la description du genre en ajoutant que les *Harttia* présentent une large plaque triangulaire sur la partie inférieure de la tête, immédiatement antérieure à l'ouverture branchiale et identifiée comme étant le sous-préopercule. Isbrücker et Nijssen (*in* Isbrücker, 1979) décrivent le genre *Cteniloricaria* en le distinguant difficilement des *Harttia* par un corps beaucoup plus allongé, une caudale plus profondément échancrée et un abdomen complètement couvert de petites plaques plus saillantes. Ils placent *Parasturisoma maculata* Boeseman, 1971 et *Oxyloricaria fowleri* Pellegrin, 1908 dans le genre *Cteniloricaria*. En 2001, Rapp Py-Daniel et Oliveira mettent *Cteniloricaria* en synonymie de *Harttia* compte tenu de la faiblesse des caractères diagnostiques. Le genre *Harttia* compte à ce jour 21 espèces décrites, principalement réparties dans l'est du continent sud-américain incluant les Guyanes (5 espèces), le Sud-Est brésilien (10 espèces) et la région amazonienne (6 espèces). Toutefois, Ferraris (2003) dénombre 18 espèces de *Harttia* et maintient la validité de *Cteniloricaria* avec 3 espèces.

En Guyane française, Le Bail *et al*. (2000) recensent cinq espèces de Harttiini dont une espèce de *Harttia*, *H. surinamensis* Boeseman, 1971, et deux espèces désormais placées dans ce genre par Rapp Py-Daniel et Oliveira (2001) : *H. maculata* (Boeseman, 1971) et *H. fowleri* (Pellegrin, 1908). *Harttia maculata* possède une tête courte et une hauteur corporelle assez élevée comparée aux autres *Harttia* guyanais. L'abdomen est couvert de plaques. Il s'agit d'une espèce relativement élancée qui se retrouve de la rivière de Kaw en Guyane française à la rivière Corantijn au Surinam, sa localité type. *Harttia fowleri* est une espèce plus massive que la précédente dont la morphologie se rapproche de celle de *H. surinamensis*. Le Bail *et al*. (2000) l'en distinguent par la présence de plaques abdominales. Cette espèce est endémique de l'Oyapock. Enfin, *H. surinamensis* possède une tête longue, large et aplatie. Les populations de Guyane française ne possèdent pas de plaques abdominales alors que Boeseman (1971), dans sa description originale de l'espèce fondée sur des spécimens récoltés au Surinam, précise : "The ventral surface is wholly flattened, the lower head and the belly being naked in juveniles, with a few scattered scutes on the abdominal region in specimens with a length of about 75 mm, the number increasing with age and attaining an almost complete cover at a size of approximately 14 or 15 cm". Des différences morphologiques entre populations de Guyane française ont été soulignées par Le Bail *et al*. (2000) notamment en ce qui concerne celle du Sinnamary avec une tête plus courte, plus large et une hauteur corporelle plus élevée. *H. surinamensis* serait présente de l'Approuague à la Coppename si on se réfère à la description originale. Récemment, Rapp Py-Daniel et Oliveira (2001) ont décrit une quatrième espèce guyanaise, *H. guianensis* de l'Approuague, sa localité type, et du Sinnamary sans tenir compte des populations vivant plus à l'ouest. Cette espèce se distingue de *H. surinamensis* par une couverture abdominale incomplète limitée aux plaques latéroventrales et préanales.

Une révision des espèces de *Harttia* de Guyane française s'avère nécessaire pour répondre aux incertitudes que soulèvent ces travaux récents, parfois contradictoires. En s'appuyant sur des mesures morphométriques et méristiques, cette étude a pour but de revoir le statut taxinomique des populations de *H. surinamensis* contenues dans la série type, d'identifier toutes les populations de *H. surinamensis* et de *H. guianensis* de Guyane française et enfin de préciser l'aire de répartition des différentes espèces. Après vérification de l'homogénéité du matériel type de *H. surinamensis*, nous comparerons cette espèce à *H. maculata* et à *H. fowleri* qui ne posent pas de problème d'identification, ainsi qu'aux populations de *H. guianensis* des bassins de l'Approuague (localité type) et du Sinnamary. Les populations de l'Ouest guyanais seront considérées indépendamment afin de les rattacher à posteriori à *H. surinamensis* ou à *H. guianensis*.

MATÉRIEL ET MÉTHODES

Matériel examiné

Le matériel examiné correspond à l'ensemble des individus actuellement disponibles pour ce type d'étude. Celui provenant de Guyane française (379 individus au total) est déposé au Muséum d'histoire naturelle, Genève (MHNG), au Muséum national d'Histoire naturelle, Paris (MNHN) et à l'Institut de Recherche pour le Développement, Cayenne (IRD). La série type de *H. surinamensis* (174 individus), déposée au National Museum of Natural History - Naturalis, Leiden (RMNH), a également été examinée. Après appréciation de l'homogénéité des habitus des différentes populations et espèces, 143 individus ont été mesurés pour être soumis aux analyses statistiques. Les localités de récolte de tout le matériel examiné par les différents auteurs au cours des dernières années sont indiquées sur la figure 1.

Matériel analysé

Le matériel mesuré en vue des analyses est le suivant :

H. maculata : **Guyane française, bassin du Maroni**. - MNHN 2000-5744, 149,1 mm de LS, aval d'Antecume Pata, rivière Litani, Jégu *et al*., oct. 2000 ; MHNG 2643.15, 136,4 mm de LS, MNHN 2003-1791, 114,5 mm de LS, saut du village d'Antecume Pata, rivière Marouini, Le Bail *et al*., 10 oct. 2000 ; MHNG 2643.14, 132,8 mm de LS, saut Aweimë enï, rivière Marouini, Jégu *et al*., 8 oct. 2000 ; MNHN 2000-5810, 118,6 mm de LS ; MHNG 2643.2 (ex. MNHN 2000-5810), 81,6 mm de LS ; MHNG 2643.1 (ex. MNHN 2000-5805), 49,8 mm de LS, saut Pierkuru, rivière Tampoc, Jégu *et al*., 2000 ; MHNG 2589.22, 2, 144,4-81,9 mm de LS, saut Lobo, Maripasoula, confluence des Petit et Grand Inini, Binggeli, nov. 1993 ; MNHN 2003-1810, 99,2 mm de LS, MHNG 2643.26, 2, 117,2-79,0 mm de LS, saut Nicole, rivière Inini, 26 fév. 1997 ; MHNG 2593.93, 63,3 mm de LS, bief de la rivière Grand Inini, Le Bail *et al*., 28 sep. 1997 ; MNHN 2003-1797, 132,9 mm de LS, sans origine, probablement Maroni.

H. surinamensis : **Surinam, bassin de la Suriname**. - RMNH 26388, 188,3 mm de LS, holotype, Grandam, Gran Rio, haute rivière Suriname, Mees, 18 juil. 1965 ; RMNH 26388, 2/5, 151,9-134 mm de LS, paratypes, Grandam, Gran Rio, haute rivière Suriname, Mees, 18 juil. 1965 ; RMNH 26389, 4/20, 173,6-83,5 mm de LS, paratypes, rapides à proximité de Gran Creek, Boeseman, 31 juil. 1964 ; RMNH 26395, 4/113, 150,2-92,7 mm de LS, paratypes, Awaradam, Gran Rio, haute rivière Suriname, Mees, 17 juil. 1965 ; RMNH 26384, 2, 131,1-47,3 mm de LS, paratypes, sous les chutes principales de Mamadam, rivière Suriname, Boeseman, 16/17 jan. 1964 ; RMNH 18206, 128,7 mm de LS, paratype, Crique Coropina, près de Paramaribo, Geijskes, oct. 1946. **Bassin de la Coppename**. - RMNH 26394, 2, 142,3-139,2 mm de LS, paratypes, chutes Raleigh, rivière Coppename, Geijskes, oct. 1957 ; RMNH 26392, 96,4 mm de LS, paratype, même localité, Van Doesburg, 8-16 juil. 1962.

H. fowleri : **Guyane française, bassin de l'Oyapock**. - MNHN 2003-1798, 2, 207,9-198,8 mm de LS, MHNG 2643.24, 206,9 mm de LS, Planquette, 1986 ; MNHN 2003-1797, 5, MHNG 2643.22, 6/7, 151,0-39,5 mm de LS, crique Cabaret, saut Caïman, Le Bail *et al*., 21 oct. 1999.

H. guianensis : **Surinam, bassin du Maroni**. - MNHN 1998-4854, 2, 129,2-84,2 mm de LS, rivière Oulemani, Jégu, 7 oct. 1998 ; RMNH 26391, 2, 108,1-104,1 mm de LS, paratypes de *H. surinamensis*, environ 2 km en aval de l'aéroport de Paloemeu, haut Tapanahoni, Mees, 17 nov. 1965 ; RMNH 26393, 6, 120,2-96,9 mm de LS, paratypes de *H. surinamensis*, près de l'aéroport de Paloemeu, haut Tapanahoni, Mees, 27 nov. 1965. **Guyane française, bassin de l'Approuague**. - MHNG 2621.97, 4, 167,0-103,7 mm de LS, saut Mapaou, Weber *et al*., 5 nov. 2001 ; MHNG

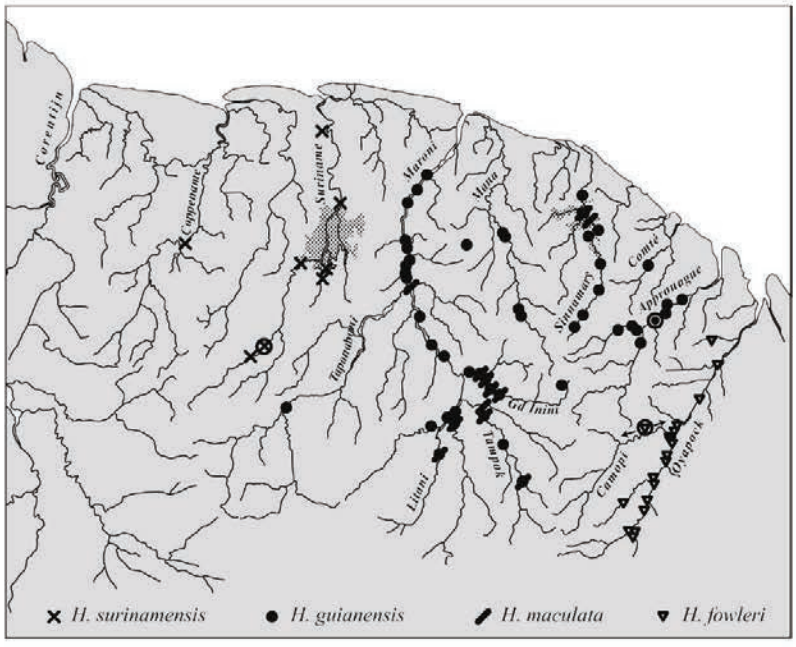

Figure 1. - Carte de répartition des espèces de *Harttia* examinées au Surinam et en Guyane française. Les symboles cerclés correspondent aux localités types des espèces pour lesquelles l'holotype a été examiné. *[Repartition map of examined* Harttia *species from Surinam and French Guiana. Circled symbols correspond to type localities of species for which holotype had been examined.]*

2621.96, 5, 122,8-76,7 mm de LS, même localité, Weber *et al*., 4 nov. 2001 ; MNHN 1994-72, 143,2 mm de LS, saut Japigny, rivière Arataye, Boujard *et al*., nov. 1988 ; MNHN 1996-922, 138,7 mm de LS, rivière Arataye, Boujard *et al*., 18 juil. 1989 ; MNHN 1996-923, 86,2 mm de LS, rivière Arataye, Boujard *et al*., 11 juil. 1989. **Bassin de la Mana**. - MNHN 1998-1712, 10, 137,5-68,5 mm de LS, saut Fracas, Planquette *et al*., 21 sep. 1994 ; MNHN 1998-1752, 26,4 mm de LS, Planquette *et al*., 27 sep. 1997 ; MNHN 1998-1744, 2, 95,8-85,7 mm de LS, MNHN 2003-1808, 1, MHNG 2643.31, 2, 98,4-34,4 mm de LS, saut Ananas, Planquette et al., 20 sep. 1995. **Bassin du Sinnamary**. - MNHN 2003-1789, 14, MHNG 2643.30, 14, 11/28, 153,2-35,2 mm de LS, crique Maroni, Petit Saut, Le Bail *et al*., 2 fév. 1983 ; IRD, Cayenne, 3, 90,0-85,4 mm de LS, saut Parasol. **Bassin du Maroni**. - MNHN 2003-1800, 116,8 mm de LS, saut Singatetei Takamalapann, rivière Litani, Planquette 15 oct. 1979 ; MNHN 2000-5766, 3, MHNG 2643.5, 3, 4/6, 129,1-44,9 mm de LS, MNHN 2000-5763, 125,5 mm de LS, MNHN 2000-5771, 103,0 mm de LS, MNHN 2000-5768, 83,1 mm

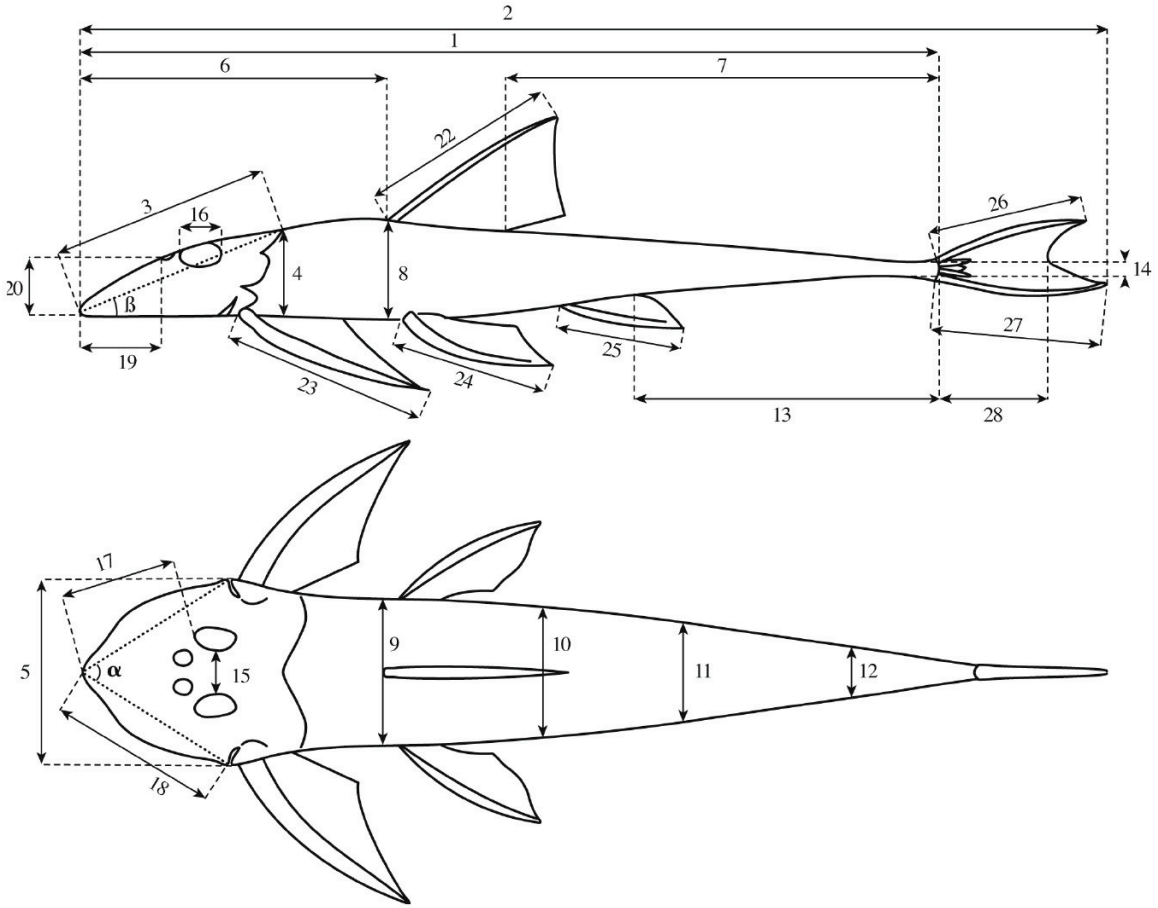

Figure 2. - Mesures et terminologie. 1 : longueur standard ; 2 : longueur totale ; 3 : longueur de la tête ; 4 : hauteur de la tête ; 5 : largeur cléithrale ; 6 : longueur prédorsale ; 7 : longueur postdorsale ; 8 : hauteur du corps à l'origine de la dorsale ; 9 : largeur du corps à l'origine de la dorsale ; 10 : largeur du corps à l'origine de l'anale ; 11 : largeur du corps à la huitième plaque postdorsale ; 12 : largeur du corps à la quatorzième plaque postdorsale ; 13 : longueur du pédoncule caudal ; 14 : hauteur du pédoncule caudal ; 15 : distance interorbitaire ; 16 : diamètre orbitaire ; 17 : longueur du museau ; 18 : distance de l'extrémité antérieure du museau à l'extrémité postérieure de l'opercule ; 19 : distance de l'extrémité antérieure du museau à l'internarine ; 20 : hauteur de la tête au niveau de l'internarine ; 21 : longueur du rameau mandibulaire (non représenté) ; 22 : longueur de l'épine dorsale ; 23 : longueur de l'épine pectorale ; 24 : longueur de l'épine pelvienne ; 25 : longueur de l'épine anale ; 26 : longueur de l'épine caudale supérieure ; 27 : longueur de l'épine caudale inférieure ; 28 : longueur minimale de l'échancrure caudale ; α, β : mesures angulaires du museau. *[Measurements and terminology. 1: standard length; 2: total length; 3: head length; 4: head depth; 5: cleithral width; 6: predorsal length; 7: postdorsal length; 8: body depth at dorsal origin; 9: body width at dorsal origin; 10: body width at anal origin; 11: body width at eighth postdorsal scute; 12: body width at fourteenth postdorsal scute; 13: caudal peduncle length; 14: caudal peduncle depth; 15: interorbital width; 16: maximum eye diameter; 17: snout length; 18: length from tip of snout to posterior extremity of operculum; 19: length from tip of snout to internostril; 20: head depth at internostril; 21: mandibular ramus length (not figured); 22: dorsal spine length; 23: pectoral spine length; 24: pelvic spine length; 25: anal spine length; 26: upper caudal spine length; 27: lower caudal spine length; 28: minimum caudal fin length; α, β: angular measures of snout.]*

de LS, saut Ouest Nord-Ouest d'Antecume Pata, rivière Litani, Jégu *et al.*, oct. 2000 ; MNHN 2000-5775, 107,4 mm de LS, Antecume Pata, rivière Litani, Jégu *et al.*, oct. 2000 ; MHNG 2593.92, 3, 157,3-100,9 mm de LS, saut Lobo, rivière Grand Inini, Le Bail *et al.*, oct. 1997 ; MHNG 2643.35, 2, MNHN 2003-1804, 2, 3/4, 126,1-108,5 mm de LS, saut Nicole, rivière Inini, 26 fév. 1997 ; MHNG 2593.36, 2, MNHN 2003-1805, 3, 50,0-38,8 mm de LS, crique 1, rivière Inini, Le Bail *et al.*, 27 sep. 1997 ; MHNG 2644.40, 25,2 mm de LS, station 4, rivière Inini, Le Bail *et al.*, 30 sep. 1997 ; MHNG 2643.11, 2, MNHN 2000-5765, 2, 143,2-117,5 mm de LS, MHNG 2643.6, 25, MNHN 2000-5747, 26, 5/51, 86,6-61,2 mm de LS, MHNG 2643.4, MNHN 2000-5811, 2, 63,2-38,1 mm de LS, MHNG 2643.3, MNHN 2000-5806, 61,0-54,7 mm de LS, saut Pierkuru, rivière Tampoc, bassin du Maroni, Jégu *et al.*, oct. 2000 ; MHNG 2643.21, 3, MNHN 2003-1796, 2, 3/5, 66,1-16,6 mm de LS, saut Kwata, rivière Tampoc, Jégu *et al.*, oct. 2000 ; MNHN 2003-1809, 55,8 mm de LS, rivière Tampoc, Le Bail *et al.*, 17 nov. 1998 ; MHNG 2643.16, MNHN 2003-1794, 152,5-144,2 mm de LS, saut du village d'Antecume Pata, rivière Marouini, Le Bail *et al.*, 10 oct. 2000 ; MHNG 2643.18, MNHN 2003-1793, 146,6-123,8 mm de LS, saut Aweimë enï, rivière Marouini, Jégu *et al.*, 8 oct. 2000 ; MHNG 2643.17, 2, MNHN 2003-1792, 122,7-74,8 mm de LS, saut Tula Lapata, rivière Marouini, Jégu *et al.*, 7 oct. 2000 ; MHNG 2643.20, 4, MNHN 2003-1795, 4, 2/8, 113,3-82,4 mm de LS, aval du village d'Antecume Pata, rivière Marouini, Jégu *et al.*, oct. 2000.

Mesures et dénombrement des données méristiques

Initialement, 28 variables quantitatives continues, 20 variables quantitatives discrètes et 6 variables qualitatives ont été relevées sur chaque spécimen. Les mesures (Fig. 2) ont été effectuées au pied à coulisse électronique et enregistrées au dixième de millimètre. Elles suivent la méthode de Boeseman (1971) et sont complétées par des mesures visant à caractériser le rétrécissement du pédoncule caudal, l'échancrure de la nageoire caudale et les angles du museau. La longueur standard (LS) est prise de l'extrémité du museau à la base des petites plaques lancéolées de la nageoire caudale, la longueur de la tête (Lt) de l'extrémité du museau à celle du supraoccipital. Les angles α et β ont été calculés par trigonométrie: $\alpha = 2 \sin^{-1}$ ((largeur cleithrale/2)/distance entre l'extrémité de l'opercule et l'extrémité du museau) et $\beta = \sin^{-1}$ (hauteur de la tête/longueur de la tête). Le calcul de ces angles permet de donner une autre appréciation de l'aplatissement dorsoventral et de l'élargissement de la tête et vise à compléter l'information fournie par les mesures usuelles.

Le dénombrement des données méristiques suit également Boeseman (1971) complété par des données visant à caractériser la couverture ventrale (liste des variables dans le tableau II). Le nombre de plaques dans la série latérale a été compté de la première plaque postérieure à la tête portant un pore de la ligne latérale à l'implantation de l'épine supérieure de la nageoire caudale. La zone de confluence des plaques inférieures et supérieures a été relevée par une différenciation entre le nombre de plaques de la série latérale situées avant cette zone et le nombre de plaques situées après cette zone. Le nombre de vertèbres inclut l'appareil de Weber. Les schémas des plaques préanales ont été réalisés à l'aide d'une loupe binoculaire munie d'une chambre claire.

Analyse des données

Les données morphométriques et méristiques ont fait l'objet d'analyses multivariées afin de mettre en évidence les différences entre espèces et populations.

Pour *H. fowleri* et *H. maculata*, qui ne présentent pas de difficultés d'identification, les individus de toutes origines ont été regroupés *a priori* par espèces dans les analyses. En revanche, les populations hétérogènes de *H. surinamensis* et de *H. guianensis* (récoltées dans différents bassins) ont été considérées de manière indépendante afin de ne pas imposer de classement *a priori*. En effet, ces deux espèces possèdent des caractéristiques morphologiques proches qui rendent leur identification délicate, le seul critère utilisable *a priori* étant la présence de la couverture de petites plaques abdominales décrite par Boeseman (1971), caractéristique observable pour une unique population. Les individus de LS inférieure à 50 mm ont été exclus des analyses, les problèmes liés aux allométries de croissance et les erreurs des mesures devenant trop forts. Cependant, ces derniers ont été pris en considération pour l'établissement des données méristiques, données généralement indépendantes de la taille. Les valeurs manquantes de certaines variables, principalement liées aux mesures des rayons des nageoires, ont été calculées selon la méthode des moindres carrés à partir de la LS. Les individus ayant plus de deux valeurs manquantes ont été exclus des analyses. Les variables comportant trop de valeurs manquantes ont été éliminées. Il s'agit des variables liées aux mensurations des nageoires, soit : la longueur de la nageoire anale, souvent abrasée du fait de sa position ventrale, la longueur de l'épine supérieure de la caudale, la longueur de l'épine inférieure de la caudale et de la longueur de l'échancrure minimum de la caudale, nageoire cassée dans la plupart des cas. La longueur totale n'a pas été considérée à cause des nombreuses nageoires caudales en mauvais état. Au final, 24 variables morphométriques et 13 variables méristiques ont été retenues pour les analyses. La liste de ces variables figure dans les tableaux I et II. L'individu RMNH 18206, 128.7 mm de LS, Crique Coropina, près de Paramaribo a été arbitrairement assigné à la population Coppename de *H. surinamensis* du fait de ses caractéristiques méristiques comparables à celles des individus du bassin de la Coppename et différant de celles des individus du bassin de la Suriname.

Initialement, la méthode de Yoccoz (1993), qui préconise de réaliser une analyse en composantes principales (ACP) doublement centrée sur les logarithmes des données brutes afin d'éliminer l'effet taille, avait été suivie. Toutefois, si cette méthode présente l'avantage de supprimer les allométries de croissance et de fortement limiter l'effet

Tableau I. - Principales données morphométriques de *Harttia maculata*, *H. fowleri* et *H. surinamensis* (numérotation selon la figure 2). *[Main morphometric data of* H. maculata, H. fowleri *and* H. surinamensis *(numbered as in figure 2).]*

	Harttia maculata		*Harttia fowleri*		*Harttia surinamensis*			
	Maroni		Oyapock		Suriname		Coppename	
Nombre d individus	14		14		13		4	
	min-max	Moy ± ET	min-max	Moy ± ET	min-max	Moy ± ET	min-max	Moy ± ET
Longueur standard [LS] (mm)	43,4 - 149,1	106,7 ± 32,5	39,5 - 207,9	121,0 ± 53,3	43,7 - 188,3	24,6 ± 38,7	96,4 - 142,3	126,6 ± 21,0
Pourcentages de LS								
Longueur de la tête [3]	19 - 23	20 ± 1	22 - 27	24 ± 1	23 - 27	24 ± 1	23 - 24	23 ± 0
Longueur prédorsale [6]	28 - 31	29 ± 1	31 - 33	32 ± 1	32 - 34	33 ± 1	32 - 33	32 ± 0
Longueur postdorsale [7]	59 - 64	62 ± 1	55 - 59	58 ± 1	55 - 58	57 ± 1	56 - 57	57 ± 1
Longueur du pédoncule caudal [13]	48 - 53	51 ± 2	46 - 50	48 ± 1	46 - 48	47 ± 1	47 - 49	47 ± 1
Longueur de l épine dorsale [22]	21 - 28	25 ± 2	20 - 27	24 ± 2	22 - 27	25 ± 1	25 - 26	25 ± 1
Longueur de l épine pectorale [23]	20 - 24	22 ± 2	19 - 34	26 ± 4	20 - 29	24 ± 2	24 - 28	26 ± 2
Longueur de l épine pelvienne [24]	17 - 20	19 ± 1	18 - 24	20 ± 2	18 - 20	19 ± 1	18 - 20	19 ± 1
Largeur du corps à l origine de la dorsale [9]	14 - 16	15 ± 1	16 - 20	18 ± 1	17 - 21	19 ± 1	19 - 22	21 ± 1
Largeur du corps à l origine de l anale [10]	11 - 14	13 ± 1	11 - 16	14 ± 1	12 - 16	15 ± 1	15 - 16	16 ± 1
Largeur du corps à la huitième plaque postdorsale [11]	9 - 11	10 ± 1	8 - 14	11 ± 1	9 - 14	13 ± 1	12 - 15	14 ± 1
Largeur du corps à la quatorzième plaque postdorsale [12]	3 - 5	4 ± 0	4 - 6	5 ± 1	4 - 7	6 ± 1	5 - 8	7 ± 1
Hauteur du corps à l origine de la dorsale [8]	9 -12	10 ± 1	8 - 10	9 ± 1	7 - 9	9 ± 1	9 - 10	9 ± 0
Hauteur du pédoncule caudal [14]	1	1 ± 0	1 - 2	1 ± 0	1 - 2	1 ± 0	1 - 2	2 ± 0
Longueur de la tête [Lt] (mm)	9,8 - 30,1	21,2 ± 6,2	10,7 - 48,9	28,2 ± 11,9	12,6 - 44,4	29,6 ± 8,8	22,7 - 32,7	29,5 ± 4,7
Pourcentages de Lt								
Longueur du museau [17]	49 - 56	53 ± 3	53 - 60	56 ± 2	56 - 60	57 ± 1	56 - 61	58 ± 2
Largeur cleithrale [5]	78 - 92	85 ± 4	80 - 101	94 ± 6	83 - 104	95 ± 5	91 - 103	98 ± 5
Hauteur de la tête [4]	37 - 52	45 ± 4	30 - 42	38 ± 3	33 - 39	37 ± 2	37 - 39	38 ± 1
Hauteur de la tête au niveau de l internarine [20]	24 - 37	32 ± 3	28 - 33	30 ± 2	26 - 32	30 ± 2	27 - 30	29 ± 1
Diamètre orbitaire [16]	20 - 26	23 ± 2	20 - 26	23 ± 2	19 - 24	22 ± 1	21 - 22	21 ± 0
Distance interorbitaire [15]	22 - 24	23 ± 1	19 - 25	21 ± 2	21 - 24	23 ± 1	24 - 25	24 ± 0
Distance de l extrémité antérieure du museau à l extrémité postérieure de l opercule [18]	71 - 76	74 ± 2	76 - 81	77 ± 2	75 - 81	78 ± 2	75 - 80	78 ± 2
Distance de l extrémité antérieure du museau à l internarine [19]	35 - 41	39 ± 2	38 - 45	41 ± 2	42 - 45	43 ± 1	42 - 43	43 ± 1
Rameau mandibulaire [21]	14 - 17	15 ± 1	15 - 21	17 ± 2	16 - 21	18 ± 2	17 - 19	18 ± 1
Alpha (degrés) [α]	64,7 - 75,9	70,6 ± 3,1	61,6 - 84,7	75,0 ± 6,5	67,2 - 80,4	74,9 ± 3,6	69,7 - 83,4	78,3 ± 6,2
Beta (degrés) [β]	21,6 - 31,2	26,5 ± 2,4	17,4 - 24,6	22,3 ± 1,6	19,0 - 22,8	21,5 ± 1,1	22,0 - 23,2	22,5 ± 0,5

taille, elle ne nous a pas permis de nous affranchir des biais liés à la forme des échantillons. En effet, les poissons étant des organismes à croissance continue et nos échantillons étant petits, il s'est avéré à l'usage que l'hétérogénéité de répartition des tailles entre les classes d'individus générait des biais majeurs et des incohérences importantes entre les résultats des analyses et ceux qui sont donnés par les tests de Wilcoxon. Ainsi, des variables présentant un poids important sur les axes, et par conséquent fortement impliquées dans la discrimination des groupes, étaient-elles non-significatives lors des comparaisons de moyennes par les tests non-paramétriques usuels, et réciproquement. Une solution alternative a été de travailler sur les proportions des différentes parties du corps afin de s'affranchir de la taille des individus. De plus, la gamme de taille dans laquelle nous avons travaillé, nous a permis de fortement limiter les problèmes liés aux allométries de croissance. Nous avons donc choisi de travailler sur les rapports à la LS pour les mesures de corps et sur les rapports à la Lt pour les mesures de tête. Certaines variables méristiques étant fortement corrélées à la taille des individus (par exemple, 13 à 83% de la variabilité enregistrée pour le nombre de dents maxillaires est expliquée par l'augmentation de la taille), elles ont été transformées par leur rapport à la LS. Les angles du museau ont été transformés par la fonction sinus. Les angles étant compris entre 0° et 90°, la fonction sinus reste strictement croissante entre 0 et 1. Le nombre de dents maxillaires et le nombre de dents mandibulaires étant deux variables fortement corrélées (r = 0,882) et leur nombre étant significativement identique (W test NS, p = 0,3018), seul le nombre de dents maxillaires rapporté à la LS a été considéré afin d'éviter toute redondance. Les données ainsi établies ont été traitées par une analyse en composantes principales (ACP) réalisée sur matrice de corrélation. Cette

Tableau II. - Principales données méristiques de *Harttia maculata*, *H. fowleri* et *H. surinamensis*. *[Main meristic data of* H. maculata, H. fowleri *and* H. surinamensis.*]*

	Harttia maculata		*Harttia fowleri*		*Harttia surinamensis*			
	Maroni		Oyapock		Suriname		Coppename	
Nombre d individus	14		14		13		4	
	min-max	Moy ± ET	min-max	Moy ± ET	min-max	Moy ± ET	min-max	Moy ± ET
Nombre de plaques dans la série latérale [a]	26 - 28	27 ± 0,5	27	-	27	-	27	-
Nombre de plaques antérieures au point de confluence dans la série latérale [b]	19 - 20	19,1 ± 0,3	18 - 20	18,4 ± 0,6	19 - 21	19,4 ± 0,6	21 - 19	20 ± 0,8
Nombre de plaques postérieures au point de confluence dans la série latérale [c]	7 - 8	8 ± 0,5	7 - 9	8,6 ± 0,6	6 - 8	7,6 ± 0,6	6 - 8	7 ± 0,8
Nombre de dents maxillaires [d]	24 - 54	39,1 ± 9,6	56 - 94	73,7 ± 10,4	53 - 105	80,7 ± 15,5	73 - 97	86,7 ± 10,4
Nombre de dents mandibulaires [e]	24 - 54	37,4 ± 9,5	51 - 81	68,5 ± 7,6	45 - 100	79,8 ± 17,4	87 - 90	88,5 ± 2,1
Nombre de rayons de la dorsale [f]	i + 7	-	i + 7	-	i + 7	-	i + 7	-
Nombre de rayons de la caudale [g]	i + 12 + i	-	i + 12 + i	-	i + 12 + i	-	i + 12 + i	-
Nombre de rayons de l anale [h]	i + 5	-	i + 5	-	i + 5	-	i + 5	-
Nombre de rayons de la pectorale [i]	i + 6	-	i + 6	-	i + 6	-	i + 6	-
Nombre de rayons de la pelvienne [j]	i + 5	-	i + 5	-	i + 5	-	i + 5	-
Nombre de plaques latéroventrales [k]	-	-	-	-	-	-	8 - 24	15,5 ± 6,8
Nombre de plaques préanales [l]	-	-	-	-	-	-	18 - 47	31,5 ± 13,8
Nombre de vertèbres [m]	32 (2 ind.)	-	32 (2 ind.)	-	32 (2 ind.)	-	31 (2 ind.)	-
Présence d une couverture abdominale (1=oui, 0=non)	1		1		1		0	
Présence d une tache caudale (1=oui, 0=non)	0		1		1		1	
Présence d une plaque nucale (1=oui, 0=non)	1		1		0		0	

méthode présente l'avantage de pouvoir traiter des données quantitatives de dimensions différentes et ne nécessite pas de classement *a priori* des individus comme le demande l'analyse discriminante. Cette dernière a été réalisée lorsque les groupes étaient morphologiquement trop proches pour être séparés par l'ACP. L'ACP interclasses a été privilégiée lorsque le nombre d'individus était trop faible en comparaison du nombre de variables. Ces analyses ont été réalisées à l'aide du logiciel ADE-4 (Thioulouse *et al*., 2001). Seules les variables informatives ont été conservées pour chaque analyse. Pour cela des tests de Kruskal-Wallis (K-W test) sur les différentes variables constituées ont été exécutés avec S-plus 2000, sous l'hypothèse H0 d'égalité des moyennes entre populations et/ou espèces. Lorsque ces tests étaient significatifs au seuil de 5% la variable était considérée comme informative car présentant au moins une différence de moyenne. Les variables non-informatives ont été exclues des analyses pour limiter le "bruit de fond". Après exploration du tableau de données ainsi établi par les analyses multivariées, des tests bilatéraux de Wilcoxon (W test) ont été exécutés afin de préciser quelle population ou espèce était différente des autres. Des tests unilatéraux ont permis, dans un second temps, de préciser comment s'exprimait cette différence.

RÉSULTATS

Les tableaux I à IV regroupent les principales données morphométriques et méristiques des différentes populations et espèces examinées.

Analyses de la série type de *Harttia surinamensis*

Cette analyse nous permet de vérifier l'homogénéité de la série type et de créer un échantillon de référence que nous pourrons comparer aux populations de l'Ouest guyanais identifiées comme *H. surinamensis* (Le Bail *et al*., 2000).

La série type de *H. surinamensis* est composée de spécimens issus des bassins de la Coppename, de la Suriname (localité type) et du Maroni (Tapanahoni). Elle apparaît hétérogène et pourrait être constituée de plusieurs entités. En effet, les spécimens de la Suriname sont les seuls à présenter une couverture abdominale qui apparaît vers une LS de 80 mm et qui se développe progressivement pour atteindre une couverture complète de l'abdomen vers 150 mm de LS (Fig. 3C), conformément à la description originale de l'espèce par Boeseman (1971). Les individus de la Coppename et du Tapanahoni ne présentent pas cette caractéristique à des tailles comparables. Pour la Coppename, cette couverture abdominale se limite, chez les individus adultes d'environ 140 mm de LS, aux plaques latéroventrales et aux plaques préanales; un chapelet de scutelles fait la jonction entre ces deux types de plaques (Fig. 3D). Chez les individus adultes (LS environ 100 mm) du Tapanahoni, seules les plaques préanales et latéroventrales sont présentes (Fig. 3E).

Seules 12 variables informatives (K-W test : $p < 0.05$) ont été conservées pour cette analyse (Fig. 4B). L'ACP normée des variables morphométriques et méristiques sépare, le long de l'axe 1, les spécimens du Tapanahoni de ceux de la Coppename et de la Suriname qui restent groupés (Fig. 4A). Les individus du Tapanahoni possèdent un diamètre orbitaire plus important [en moyenne 24% de la Lt *versus* 22% pour la Suriname (W test : $p = 0,002$) et 21% pour la Coppe-

name (W test : p = 0,0011)], un pédoncule caudal plus long [48% de la LS *versus* 47% pour la Suriname (W test : p = 0,0015)] et un nombre supérieur de dents maxillaires [84 *versus* 81 pour la Suriname (W test : p = 0,002)]. Les individus de la Coppename et de la Suriname possèdent une tête plus longue [24% de la LS pour la Suriname et 23% pour la Coppename *versus* 22% pour le Tapanahoni (W test : 0,004 > p > 0)], une distance entre l'extrémité antérieure du

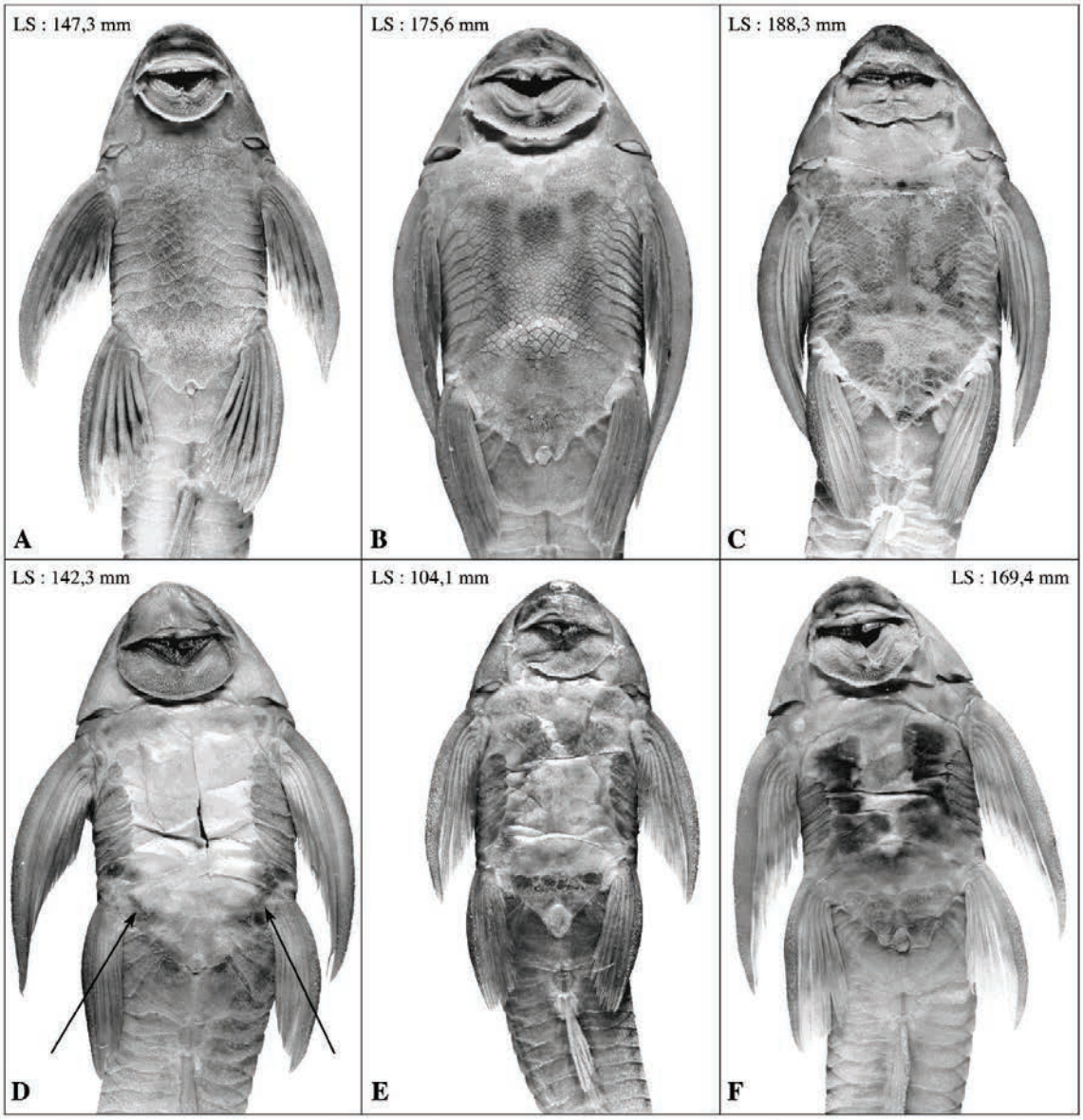

Figure 3. - Organisation de la couverture abdominale chez les espèces de *Harttia* de Guyane et du Surinam. **A** : *H. maculata* (MNHN 2000-5744) ; **B** : *H. fowleri* (MHNG 2643.23) ; **C** : *H. surinamensis* (holotype de la Suriname, RMNH 26388) ; **D** : *H. surinamensis* (paratype de la Coppename, RMNH 26394) ; **E** : *H. guianensis* (paratype *surinamensis* du Tapanahoni, RMNH 26391) ; **F** : *H. guianensis* (MHNG 2621.96). Les flèches indiquent le chapelet de petites plaques faisant jonction entre les plaques préanales et latéroventrales chez les individus de la population Coppename de *H. surinamensis*. *[Organisation of the abdominal cover of* Harttia *species from French Guiana and Surinam. Arrows indicate the string of little plates joining the preanal plates to the latero-ventral plates in the individuals from the Coppe - name population of* H. surinamensis.*]*

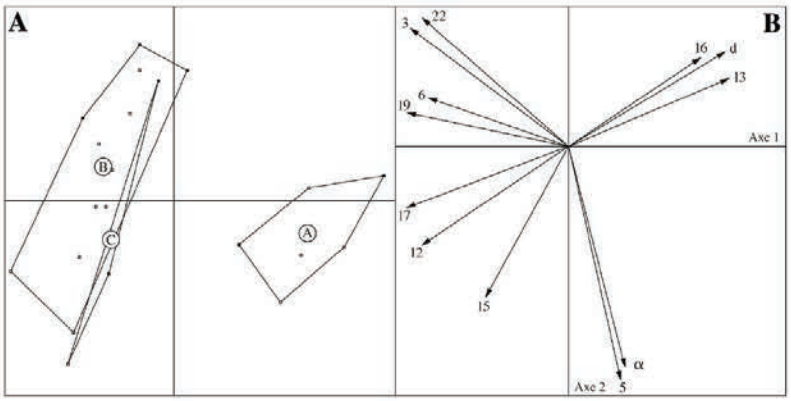

Figure 4. - ACP normée réalisée sur les variables morphologiques rapportées à la taille pour la série type de *Harttia surinamensis*, axes 1-2. **A** : Carte factorielle des individus : A = Tapanahoni (n = 8), B = Suriname (n = 12), C = Coppename (n = 4) ; **B** : Carte factorielle des variables (numérotation correspondant à celle de la figure 2 et des tableaux I et II). *[Normed PCA realised on morphological variables in ratio of the size for the type series of* H. surinamensis, *axis 1-2. **A**: Factorial map of individuals; **B**: Factorial map of variables (numbered as in figure 2 and tables I and II).]*

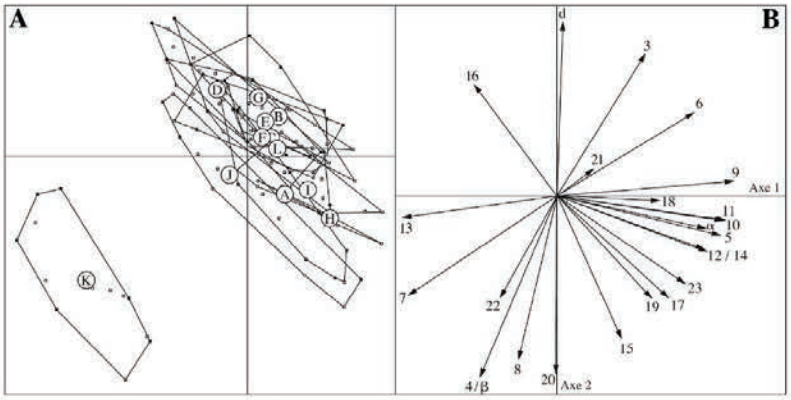

Figure 5. - ACP normée réalisée sur les variables morphologiques rapportées à la taille pour toutes les populations et espèces guyanaises de *Harttia* et pour la série type de *H. surinamensis*, axes 1-2. **A** : Carte factorielle des individus : A = Approuague (n = 12), B = Mana (n = 13), C = Marouini (n = 9), D = Tampoc (n = 14), E = Inini (n = 7), F = Litani (n = 8), G = Sinnamary (n = 13), H = Coppename (n = 4), I = Suriname (n = 12), J = *H. fowleri* (n = 13), K = *H. maculata* (n = 13), L = Tapanahoni (n = 8) ; **B** : Carte factorielle des variables (numérotation correspondant à celle de la figure 2 et des tableaux I et II). *[Normed PCA realised on morphological variables in ratio of the size for all* Harttia *populations and species from Guiana and for the type series of* H. surinamensis, *axis 1-2. **A:** Factorial map of individuals; **B**: Factorial map of variables (numbered as in figure 2 and tables I and II).]*

museau et l'internarine supérieure [43% de la Lt pour la Suriname et la Coppename *versus* 40% pour le Tapanahoni (W test : 0,004 > p > 0,0002)], une épine dorsale plus longue [25% de la LS pour la Suriname et la Coppename *versus* 22% pour le Tapanahoni (W test : 0,002 > p > 0)], un museau plus long [57% de la Lt pour la Suriname et 58% pour la Coppename *versus* 55% pour le Tapanahoni (W test : 0,002 > p > 0,0001)] et une largeur du corps à la quatorzième plaque postdorsale supérieure [6% de la LS pour la Suriname et 7% pour la Coppename *versus* 5% pour le Tapanahoni (W test : 0,0081 > p > 0,0011)].

Une analyse interclasses (non figurée) a été menée pour mieux caractériser la Coppename vis-à-vis de la Suriname, morphologiquement très proche. Elle sépare légèrement la Coppename selon une distance interorbitaire plus forte [24% de la Lt *versus* 23% pour la Suriname (W test : p = 0,0099) et le Tapanahoni (W test : p = 0,002)] et la Suriname selon une longueur prédorsale supérieure [33% de la LS *versus* 32% pour la Coppename (W test : p = 0,039) et le Tapanahoni (W test : p = 0,008)]. Le Tapanahoni est séparé de la Suriname selon une largeur cleithrale plus importante [101% de la Lt *versus* 95% pour la Suriname (W test : p = 0,002)] et un angle α différent [80,5° *versus* 74,9° pour la Suriname (W test : p = 0,0002)]. Les différences enregistrées entre les groupes ne sont pas dues au hasard, le test de permutation étant très significatif : sur 1000 permutations, aucune n'est

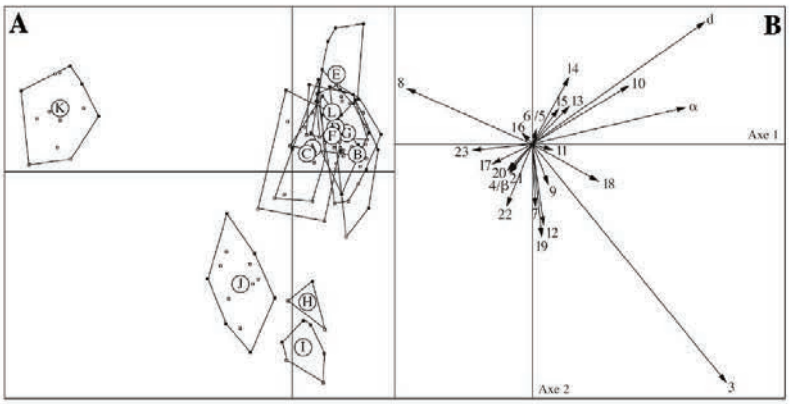

Figure 6. - Analyse discriminante réalisée sur les variables morphologiques rapportées à la taille pour toutes les populations et espèces guyanaises de *Harttia* et pour la série type de *H. surinamensis*, axes 1-2. **A** : Carte factorielle des individus : A = Approuague (n = 12), B = Mana (n = 13), C = Marouini (n = 9), D = Tampoc (n = 14), E = Inini (n = 7), F = Litani (n = 8), G = Sinnamary (n = 13), H = Coppename (n = 4), I = Suriname (n = 12), J = *H. fowleri* (n = 13), K = *H. maculata* (n = 13), L = Tapanahoni (n = 8) ; **B** : Carte factorielle des principaux facteurs de l'analyse discriminante (numérotation correspondant à celle de la figure 2 et des tableaux I et II). *[Discriminant analysis realised on morphological variables in ratio of the size for all* Harttia *populations and species from Guiana and for the type series of* H. surinamensis*, axis 1-2.* ***A****: Factorial map of individuals;* ***B****: Factorial map of discriminant analysis principal factors (numbered as in figure 2 and tables I and II).]*

supérieure à la valeur observée d'inertie interclasses. La variabilité interclasses enregistrée représente 48,94% de la variabilité totale.

Analyses de toutes les espèces et populations guyanaises de *Harttia* et de la série type de *Harttia surinamensis*

Cette analyse nous permet d'identifier à posteriori les populations de l'Ouest guyanais et de préciser comment se caractérisent les différentes espèces de *Harttia*.

Vingt-quatre variables informatives (K-W test : $p < 0,05$) ont été retenues pour cette analyse (Fig. 5B). L'ACP normée (Fig. 5) sépare clairement *H. maculata* de toutes les autres espèces et populations selon une hauteur de tête supérieure [45% de la Lt *versus* 38% pour *fowleri* (W test : $p = 0$), 33 à 38% pour *guianensis* (W test : $p = 0$), et 37 à 38% pour *surinamensis* (W test : $p = 0$)], un angle β plus important [26,5° *versus* 22,3° pour *fowleri* (W test : $p = 0$), 21,5 à 22,0° pour *surinamensis* (W test : $p = 0$) et 19,3 à 22,5° pour *guianensis* (W test : $p = 0$)], une longueur postdorsale plus grande [62% de la LS *versus* 58% pour *fowleri* (W test : $p = 0$), 57% pour *surinamensis* (W test : $p = 0$) et 56 à 58% pour *guianensis* (W test : $p = 0$)], un pédoncule caudal plus long [51% de la LS *versus* 48% pour *fowleri* (W test : $p = 0$), 48 à 49% pour *guianensis* (W test : $0,0039 > p > 0$) et 47% pour *surinamensis* (W test : $p = 0$)], une hauteur du corps à l'origine de la dorsale supérieure [en moyenne 10% de la LS *versus* 9% pour *fowleri* (W test : $p = 0,0003$) et *surinamensis* (W test : $0,005 > p > 0$) et 8 à 10% pour *guianensis* (W test : $0,0033 > p > 0$)] et une hauteur de la tête au niveau de l'internarine plus importante [32% de la Lt *versus* 30% pour *fowleri* (W test : $p = 0,003$), 29 à 30% pour *surinamensis* (W test : $0,0023 > p > 0,0017$) et 26 à 30% pour *guianensis* (W test : $0,008 > p > 0$)]. La longueur de l'épine dorsale sépare significativement *maculata* de *guianensis* [25% de la LS *versus* 22 à 23% pour *guianensis* (W test : $0,005 > p > 0$)].

D'autre part l'abdomen de *H. maculata* est couvert de plaques plus grandes que chez les autres espèces et la couverture est complète vers 60 mm (Fig. 3A), la plaque nucale située à la base de l'implantation de la nageoire dorsale est présente et bien développée, les dents sont organisées en une seule rangée, et la tache caudale est absente.

Les autres espèces et populations restant groupées et l'ACP interclasses donnant des résultats comparables, l'analyse discriminante (Fig. 6) a donc été privilégiée pour différencier les autres espèces. Cette dernière sépare les individus en au moins 4 groupes, correspondant à *H. maculata*, *H. fowleri*, *H. surinamensis* et *H. guianensis*. Nous nommerons ici le groupe *surinamensis* constitué des populations de la Suriname et de la Coppename mais excluant celle du Tapanahoni (matériel type). Les populations de l'Approuague, du Sinnamary, de la Mana et des affluents du Maroni comprenant le Tampoc, le Litani, le Marouini, l'Inini et le Tapanahoni (paratypes de *H. surinamensis*) sont regroupées au sein d'un groupe nommé *guianensis*. La carte de répartition des espèces (Fig. 1) a été réalisée en tenant compte de ces résultats.

Harttia maculata est caractérisée, comme précédemment, par une hauteur corporelle supérieure.

Le groupe *surinamensis* se distingue par une distance de l'extrémité du museau à l'internarine plus longue [43% de la Lt *versus* 39% pour *maculata* (W test : $p = 0$), 41% pour *fowleri* (W test : $0,05 > p > 0,0013$) et 38 à 41% (W test : $0,0098 > p > 0$) pour *guianensis*] et par une plus grande lar-

geur prise au niveau de la quatorzième plaque postdorsale [6 à 7% de la LS *versus* 4% pour *maculata* (W test : p = 0), 5% pour *fowleri* (W test : 0,0029 > p > 0) et 5 à 6% pour *guianensis* (W test : 0,0342 > p > 0)]. D'autre part, les spécimens de la Suriname possèdent une couverture abdominale complète alors que les spécimens de la Coppename en sont dépourvus. Ces deux populations ne possèdent pas de plaque nucale, leurs dents sont organisées selon deux rangées en quinconce et elles ont une tache caudale.

Le groupe *guianensis* est caractérisé par un nombre supérieur de dents maxillaires [64 à 88 *versus* 39 pour *maculata* (W test : p = 0), 74 pour *fowleri* (W test : NS > p > 0,0004) et 81 à 87 pour *surinamensis* (W test : NS > p > 0.0001)], un angle α plus important [73,3 à 80,7° *versus* 70,6° pour *maculata* (W test : 0,0276 > p > 0,0001), 75,0° pour *fowleri* (W test : NS > p > 0,0223) et 74,9 à 78,3° pour *surinamensis* (W test : NS > p > 0,0002)] et un corps plus large à l'origine de l'anale [13 à 16% de la LS *versus* 13% pour *maculata* (W test : 0,0127 > p > 0) et 14% pour *fowleri* (W test : NS > p > 0,0005)]. Cette dernière variable ne sépare pas *guianensis* de *surinamensis*. De plus, *H. guianensis* diffère de toutes les autres espèces par une longueur de l'épine dorsale inférieure [22 à 23% de la LS *versus* 25% pour *maculata* (W test : 0,005 > p > 0), 24% pour *fowleri* (W test : 0,0012 > p > 0) et 25% pour *surinamensis* (W test : 0,0061 > p > 0)]. Ce groupe ne possède ni couverture abdominale ni plaque nucale, ses dents sont organisées selon deux rangées en quinconce et il possède une tache caudale.

Harttia fowleri est difficilement caractérisable car cette espèce présente une morphologie intermédiaire entre *H. maculata* et *H. guianensis* et relativement proche de *H. surinamensis*. Toutes les variables prennent des valeurs moyennes comprises entre celles de ces trois espèces. La couverture ventrale est plus tardive que chez *H. maculata* et est complète vers 110 mm (Fig. 3B). *Harttia fowleri* possède aussi une plaque nucale bien visible au niveau de l'origine de la nageoire dorsale mais plus réduite que chez *H. maculata*. Cette espèce possède une tache caudale, ses dents sont organisées selon une seule rangée et les plus grands spécimens présentent de légers prolongements au niveau du premier rayon des nageoires paires.

Le test de permutation est très significatif : sur 1000 permutations, aucune n'est supérieure à la valeur observée d'inertie interclasses. Les différences observées par l'analyse discriminante ne sont pas le fait du hasard et la variabilité interclasses représente 49,59% de la variabilité totale. La fonction discriminante ainsi établie nous permet de rattacher à posteriori les deux spécimens de l'Oulemari, affluent surinamien du Maroni : le spécimen MNHN 1998-4854 de 129,2 mm de LS est rattaché à *H. guianensis* et est morphologiquement plus proche de la population Litani, et le spécimen MNHN 1998-4854 de 84,2 mm de LS est aussi rattaché à *H. guianensis* et est plus proche de la population Tampoc, deux populations du bassin du Maroni. Notons que 10,3% des individus sont mal classés dans l'analyse soit 13 individus sur 126. Toutefois, les erreurs de classement ne concernent que les populations de *H. guianensis*, très proches morphologiquement, et en particulier celles du bassin du Maroni.

Analyse des différentes populations de *Harttia* du groupe *guianensis*

Cette analyse nous permet de vérifier l'homogénéité du groupe *guianensis* précédemment établi et de confirmer, le cas échéant, les différences signalées par Le Bail *et al.* (2000). 16 variables informatives (K-W test : p < 0,05) ont été retenues pour cette analyse (Fig. 7 B). L'ACP normée

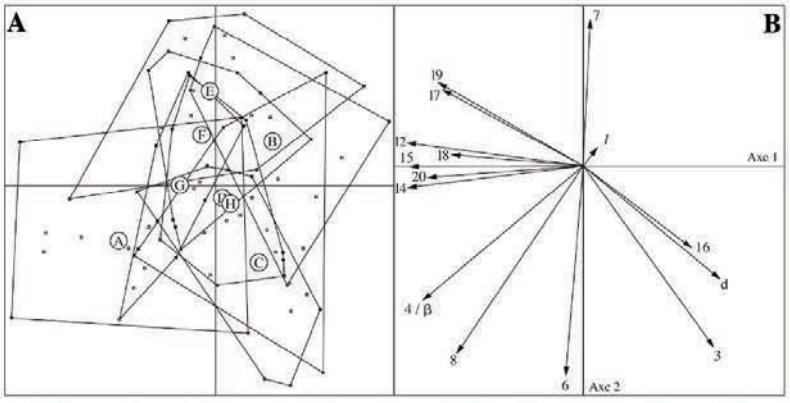

Figure 7. - ACP normée réalisée sur les variables morphologiques rapportées à la taille pour toutes les populations du groupe *guianensis*, axes 1-2. **A** : Carte factorielle des individus : A = Approuague (n = 12), B = Tampoc (n = 14), C = Sinnamary (n = 13), D = Mana (n = 13), E = Marouini (n = 9), F = Litani (n = 8), G = Tapanahoni (n = 8), H = Inini (n = 7) ; **B** : Carte factorielle des variables (numérotation correspondant à celle de la figure 2 et des tableaux I et II). *[Normed PCA realised on morphological variables in ratio of the size for all populations of the* guianensis *group. **A:** Factorial map of individuals; **B**: Factorial map of variables (numbered as in figure 2 and tables I and II).]*

(Fig. 7) sépare faiblement l'Approuague et le Sinnamary de toutes les populations des bassins du Maroni et de la Mana le long de l'axe 2.

La population de l'Approuague diffère significativement des autres populations par un corps plus haut à l'origine de la dorsale [10% de la LS *versus* 9% pour le Sinnamary (W test : p = 0,0023) et l'Inini (W test : p = 0,0049) et 8% pour la Mana, le Litani, le Tapanahoni, le Tampoc et le Marouini (W test : 0,0048 > p > 0)], une hauteur de tête plus importante [38% de la Lt *versus* 34% pour le Sinnamary, le Litani et Tampoc (W test : p = 0), 33% pour le Marouini et la Mana (W test : p = 0) et 36% pour l'Inini et le Tapanahoni (W test : 0,0287 > p > 0,0142)] et un angle β supérieur [22,5° *versus* 19,3° pour le Marouini (W test : p = 0,0002), 19,5° pour la Mana (W test : p = 0), 19,6° pour le Tampoc (W test : p = 0), 19,7° pour le Sinnamary (W test : p = 0), 19,9° pour le Litani (W test : p = 0,0004), 21° pour l'Inini (W test : p = 0,0142) et 21,2° pour le Tapanahoni (W test : p = 0,0287)]. La coloration en alcool est grisâtre avec quelques bandes brun sombre. L'abdomen est nu, la couverture ventrale se limitant aux plaques latéroventrales et préanales (Fig. 3F).

La population du Sinnamary est caractérisée par une tête plus longue [24% de la LS *versus* 22% pour le Tapanahoni et le Marouini (W test : p = 0), 23% pour le Litani et l'Approuague (W test : 0,0012 > p > 0,0007) et 24% pour le Tampoc et l'Inini (W test : 0,0188 > p > 0,0148)], un nombre supérieur de dents maxillaires [88 *versus* 69 pour l'Inini (W test : p = 0.0406), 70 pour le Marouini (W test : p = 0.0003), 82 pour le Litani (W test : p = 0.0319), 84 pour le Tapanahoni (W test : p = 0.03) et 86 pour l'Approuague (W test : p = 0,0149)] et un diamètre orbitaire plus important [23% de la Lt *versus* 22% pour le Marouini, le Litani, l'Approuague et la Mana (W test : 0,0033 > p > 0,0147) et 23% pour l'Inini (W test : p = 0,0484)]. De plus, le nombre significativement inférieur de plaques préanales [4,7 *versus* 5,2 pour l'Inini (W test : p = 0,0148), 6,2 pour le Tampoc (W test : p = 0,0072), 7,6 pour la Mana (W test : p = 0), 8 pour le Marouini (W test : p = 0,0056), 8,4 pour le Litani (W test : p = 0,0007), 8,5 pour l'Approuague (W test : p = 0,008) et 10,1 pour le Tapanahoni (W test : p = 0,0001)], ainsi que l'organisation et la forme de ces plaques distinguent clairement les individus du Sinnamary de ceux des autres populations (Fig. 8). En effet, pour toutes les populations hormis celle du Sinnamary, on observe deux plaques principales trapézoïdales situées immédiatement devant l'anus accompagnées de quatre à plus de dix plaques secondaires, qui joignent les plaques principales aux nageoires pelviennes (Figs 8A, 8C, 8D). Dans la population du Sinnamary (Figs 8B, 8E), on relève deux plaques principales arrondies accompagnées, le plus souvent, de deux à sept plaques secondaires arrondies également. Avec l'âge, ces plaques forment une sorte de bouclier autour de l'anus, ce qui n'est pas le cas chez les spécimens des autres bassins. Chez ces

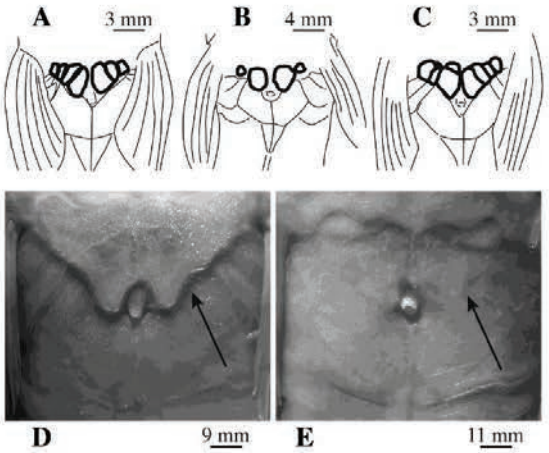

Figure 8. - Organisation des plaques préanales chez les subadultes de *Harttia guianensis*. **A** : Approuague (MHNG 2621.096, LS : 76,7 mm) ; **B** : Sinnamary, crique Maroni (MHNG 2643-30, LS : 86,2 mm) ; **C** : Maroni, Litani (MHNG 2643-8, LS : 79,2 mm), puis chez l'adulte : **D** : Approuague (MHNG 2621.097, LS : 152,0 mm); **E** : Sinnamary, crique Maroni (MHNG 2643-30, LS : 115,7 mm). Les flèches indiquent la ligne de séparation entre plaques préanales et postanales. *[Arrangement of preanal plates in sub-adults (**A**, **B**, **C**) and in adults (**D**, **E**) of* Harttia guianensis. *Arrows indicate the separation line between the preanal and postanal plates.]*

derniers, il existe une séparation nette entre les plaques préanales et les plaques postanales (Fig. 8D) alors qu'elles semblent fusionnées chez les spécimens du Sinnamary (Fig. 8E). La coloration en alcool est brune avec des bandes sombres et des marbrures.

Les différentes populations du Maroni (Tampoc, Litani, Marouini, Inini et Tapanahoni) et de la Mana présentent quelques différences entre elles mais peu sont significatives (seuls 18,89% de la variabilité totale dans le Maroni et la Mana est due à une variabilité interclasses). Leur coloration en alcool est brune avec des bandes et des marbrures sombres. L'organisation des plaques préanales est comparable à celles des spécimens de l'Approuague.

La variabilité inter-classes enregistrée à l'intérieur du groupe *guianensis* est de 29.56% et les différences enregistrées ne sont pas dues au hasard, le test de permutation étant très significatif : sur 1000 permutations, aucune n'est supérieure à la valeur observée d'inertie interclasses.

DISCUSSION

Au vu des échantillons examinés il apparaît clairement que *H. surinamensis* n'est pas présente en Guyane française. Son aire de répartition pourrait même être restreinte à sa localité type: la rivière Suriname. En effet, les paratypes du Tapanahoni, étant déjà significativement différents lors de

Tableau III. - Principales données morphométriques de *Harttia guianensis*, populations du Tapanahoni, du Tampoc, de l'Inini et du Marouini (numérotation selon la figure 2). *[Main morphometric data of* H. guianensis, *populations from Tapanahoni, Tampoc, Inini and Marouini (numbered as in figure 2).]*

	Harttia guianensis							
	Tapanahoni		Tampoc		Inini		Marouini	
Nombre d individus	8		18		12		9	
	min-max	Moy ± ET	min-max	Moy ± ET	min-max	Moy ± ET	min-max	Moy ± ET
Longueur standard [LS] (mm)	96,9 - 120,2	106,6 ± 6,5	16,6 - 143,2	72,9 ± 33,1	25,2 - 157,3	83,4 ± 46,2	74,8 - 152,5	117,6 ± 28,1
Pourcentages de LS								
Longueur de la tête [3]	22	-	22 - 30	24 ± 2	21 - 27	24 ± 2	21 - 23	22 ± 1
Longueur prédorsale [6]	31 - 33	32 ± 1	30 - 34	32 ± 1	31 - 35	33 ± 1	30 - 33	31 ± 1
Longueur postdorsale [7]	55 - 58	57 ± 1	56 - 59	58 ± 1	53 - 58	56 ± 1	56 - 59	57 ± 1
Longueur du pédoncule caudal [13]	48 - 50	48 ± 1	47 - 50	49 ± 1	46 - 50	48 ± 1	47 - 50	49 ± 1
Longueur de l épine dorsale [22]	21 - 24	22 ± 1	21 - 24	23 ± 1	20 - 25	22 ± 1	21 - 23	22 ± 1
Longueur de l épine pectorale [23]	22 - 29	24 ± 2	20 - 27	22 ± 2	19 - 25	22 ± 2	21 - 29	25 ± 3
Longueur de l épine pelvienne [24]	17 - 20	18 ± 1	16 - 19	18 ± 1	16 - 20	18 ± 1	18 - 19	19 ± 1
Largeur du corps à l origine de la dorsale [9]	18 - 21	19 ± 1	13 - 22	17 ± 2	12 - 21	18 ± 3	17 - 21	19 ± 1
Largeur du corps à l origine de l anale [10]	15 - 17	16 ± 1	8 - 17	13 ± 2	9 - 17	14 ± 2	14 - 17	15 ± 1
Largeur du corps à la huitième plaque postdorsale [11]	12 - 14	13 ± 1	8 - 14	11 ± 2	8 - 14	11 ± 2	10 - 14	12 ± 1
Largeur du corps à la quatorzième plaque postdorsale[12]	5 - 6	5 ± 0	4 - 6	5 ± 1	3 - 6	5 ± 1	4 - 6	5 ± 1
Hauteur du corps à l origine de la dorsale [8]	8 - 10	8 ± 1	7 - 8	8 ± 0	7 - 10	9 ± 1	7 - 8	8 ± 1
Hauteur du pédoncule caudal [14]	1 - 2	1 ± 0	1 - 2	1 ± 0	1 - 2	1 ± 0	1 - 1	1 ± 0
Longueur de la tête [Lt] (mm)	21,5 - 26,5	23,7 ± 1,5	4,9 - 32,5	16,9 ± 6,7	6,5 - 35,8	19,4 ± 9,7	17,4 - 32,7	26,0 ± 5,7
Pourcentages de Lt								
Longueur du museau [17]	53 - 56	55 ± 1	51 - 58	53 ± 2	45 - 56	52 ± 3	51 - 57	55 ± 2
Largeur cleithrale [5]	99 - 105	101 ± 2	76 - 111	90 ± 9	69 - 106	90 ± 12	87 - 108	99 ± 7
Hauteur de la tête [4]	34 - 39	36 ± 2	30 - 36	34 ± 2	30 - 40	36 ± 3	29 - 38	33 ± 2
Hauteur de la tête au niveau de l internarine [20]	28 - 30	29 ± 1	23 - 28	26 ± 2	24 - 29	26 ± 2	25 - 32	28 ± 2
Diamètre orbitaire [16]	22 - 25	24 ± 1	22 - 24	23 ± 1	21 - 24	23 ± 1	21 - 24	22 ± 1
Distance interorbitaire [15]	22 - 23	23 ± 1	20 - 29	22 ± 2	20 - 23	22 ± 1	20 - 23	21 ± 1
Distance de l extrémité antérieure du museau à l extrémité postérieure de l opercule [18]	76 - 80	78 ± 1	74 - 82	76 ± 2	66 - 79	74 ± 3	75 - 78	76 ± 1
Distance de l extrémité antérieure du museau à l internarine [19]	39 - 42	40 ± 1	38 - 41	39 ± 1	32 - 41	38 ± 2	39 - 42	41 ± 1
Rameau mandibulaire [21]	14 - 19	17 ± 2	14 - 22	16 ± 2	16 - 21	17 ± 2	13 - 19	17 ± 2
Alpha (degrés) [α]	78,8 - 82,0	80,5 ± 1,1	60,0 - 89,4	73,3 ± 7,3	62,7 - 88,9	75,3 ± 9,8	71,0 - 89,3	80,7 ± 6,7
Beta (degrés) [β]	19,8 - 22,7	21,2 ± 1,1	17,5 - 21,2	19,6 ± 1,1	17,6 - 23,8	21,0 ± 1,7	17,0 - 22,0	19,3 ± 1,4

Tableau IV. - Principales données méristiques de *Harttia guianensis*, populations du Tapanahoni, du Tampoc, de l'Inini et du Marouini. *[Main meristic data of* H. guianensis, *populations from Tapanahoni, Tampoc, Inini and Marouini.]*

	Harttia guianensis							
	Tapanahoni		Tampoc		Inini		Marouini	
Nombre d individus	8		18		12		9	
	min-max	Moy ± ET	min-max	Moy ± ET	min-max	Moy ± ET	min-max	Moy ± ET
Nombre de plaques dans la série latérale [a]	26 - 27	26,9 ± 0,3	25 - 28	26,8 ± 0,7	26 - 28	27 ± 0,4	27 - 28	27,1 ± 0,3
Nombre de plaques antérieures au point de confluence dans la série latérale [b]	19 - 20	19,7 ± 0,5	18 - 20	19,3 ± 0,6	18 - 20	19,4 ± 0,8	17 - 20	18,7 ± 1,1
Nombre de plaques postérieures au point de confluence dans la série latérale [c]	6 - 8	7,1 ± 0,6	6 - 10	7,4 ± 0,9	6 - 9	7,5 ± 0,9	7 - 10	8,4 ± 1,2
Nombre de dents maxillaires [d]	78 - 89	83,9 ± 3,1	18 - 81	64,3 ± 15,9	15 - 100	68,7 ± 23,6	44 - 96	70,3 ± 18,3
Nombre de dents mandibulaires [e]	80 - 85	82,1 ± 1,7	20 - 82	63,0 ± 14,8	18 - 104	70,3 ± 25,5	41 - 80	62,5 ± 16,2
Nombre de rayons de la dorsale [f]	i + 7	-	i + 7	-	i + 7	-	i + 7	-
Nombre de rayons de la caudale [g]	i + 12 + i	-	i + 12 + i	-	i + 12 + i	-	i + 12 + i	-
Nombre de rayons de l anale [h]	i + 5	-	i + 5	-	i + 5	-	i + 5	-
Nombre de rayons de la pectorale [i]	i + 6	-	i + 6	-	i + 6	-	i + 6	-
Nombre de rayons de la pelvienne [j]	i + 5	-	i + 5	-	i + 5	-	i + 5	-
Nombre de plaques latéroventrales [k]	37108	7,2 ± 0,7	0 - 9	6,5 ± 2,0	0 - 10	6,4 ± 2,9	6 - 8	7,1 ± 0,9
Nombre de plaques préanales [l]	41455	10,1 ± 3,1	0 - 17	6,2 ± 4,1	0 - 9	5,2 ± 3,1	5 - 11	8,0 ± 1,9
Nombre de vertèbres [m]	32 (2 ind.)	-	32 (2 ind.)	-	32 (2 ind.)	-	32 (2 ind.)	-
Présence d une couverture abdominale (1=oui, 0=non)	0		0		0		0	
Présence d une tâche caudale (1=oui, 0=non)	1		1		1		1	
Présence d une plaque nucale (1=oui, 0=non)	0		0		0		0	

Tableau V. - Principales données morphométriques de *Harttia guianensis*, populations du Litani, de la Mana, du Sinnamary et de l'Approuague (numérotation selon la figure 2). *[Main morphometric data of* H. guianensis, *populations from Litani, Mana, Sinnamary and Approuague (numbered as in figure 2).]*

	Harttia guianensis							
	Litani		Mana		Sinnamary		Approuague	
Nombre d individus	9		16		14		12	
	min-max	Moy ± ET	min-max	Moy ± ET	min-max	Moy ± ET	min-max	Moy ± ET
Longueur standard [LS] (mm)	44,9 - 129,1	99,6 ± 26,4	26,4 - 137,5	86,2 ± 32,3	35,2 - 153,2	94,0 ± 26,5	76,7 - 167,0	118,5 ± 27,1
Pourcentages de LS								
Longueur de la tête [3]	22 - 25	23 ± 1	22 - 30	24 ± 2	23 - 29	24 ± 2	21 - 23	23 ± 1
Longueur prédorsale [6]	31 - 34	32 ± 1	31 - 35	33 ± 1	32 - 36	33 ± 1	31 - 33	32 ± 1
Longueur postdorsale [7]	56 - 60	58 ± 1	52 - 58	56 ± 2	55 - 58	56 ± 1	56 - 59	57 ± 1
Longueur du pédoncule caudal [13]	47 - 52	49 ± 1	43 - 50	48 ± 2	46 - 50	48 ± 1	48 - 50	48 ± 1
Longueur de l épine dorsale [22]	22 - 24	23 ± 1	18 - 26	22 ± 2	20 - 24	22 ± 1	22 - 24	23 ± 1
Longueur de l épine pectorale [23]	21 - 28	24 ± 3	20 - 29	24 ± 3	20 - 29	24 ± 3	21 - 29	25 ± 3
Longueur de l épine pelvienne [24]	18 - 19	18 ± 0	17 - 20	19 ± 1	17 - 20	19 ± 1	19 - 20	19 ± 1
Largeur du corps à l origine de la dorsale [9]	16 - 22	19 ± 2	13 - 21	18 ± 3	16 - 21	19 ± 1	17 - 21	19 ± 1
Largeur du corps à l origine de l anale [10]	12 - 18	15 ± 2	9 - 17	14 ± 2	10 - 16	15 ± 2	14 - 18	16 ± 1
Largeur du corps à la huitième plaque postdorsale [11]	10 - 13	12 ± 1	8 - 14	12 ± 2	8 - 14	12 ± 1	11 - 15	13 ± 1
Largeur du corps à la quatorzième plaque postdorsale[12]	4 - 6	5 ± 1	3 - 6	5 ± 1	4 - 6	5 ± 1	5 - 6	6 ± 0
Hauteur du corps à l origine de la dorsale [8]	7 - 9	8 ± 1	7 - 9	8 ± 0	7 - 10	9 ± 1	9 - 10	10 ± 1
Hauteur du pédoncule caudal [14]	1 - 2	1 ± 0	1 - 2	1 ± 0	1	-	1 - 2	1 ± 0
Longueur de la tête [Lt] (mm)	11,2 - 29,2	22,5 ± 5,7	7,8 - 30,9	20,3 ± 6,8	10,3 - 35,1	22,3 ± 5,7	17,8 - 37,7	26,8 ± 5,7
Pourcentages de Lt								
Longueur du museau [17]	52 - 58	56 ± 2	51 - 56	54 ± 2	50 - 58	54 ± 2	51 - 58	56 ± 2
Largeur cleithrale [5]	82 - 106	96 ± 8	68 - 105	92 ± 11	73 - 110	95 ± 9	91 - 108	100 ± 6
Hauteur de la tête [4]	31 - 36	34 ± 1	26 - 36	33 ± 3	30 - 36	34 ± 1	33 - 42	38 ± 3
Hauteur de la tête au niveau de l internarine [20]	26 - 32	28 ± 2	20 - 29	26 ± 3	23 - 32	29 ± 2	26 - 34	30 ± 3
Diamètre orbitaire [16]	21 - 24	22 ± 1	19 - 24	22 ± 1	20 - 27	23 ± 2	20 - 25	22 ± 1
Distance interorbitaire [15]	21 - 23	22 ± 1	19 - 24	22 ± 1	20 - 25	21 ± 2	21 - 26	24 ± 2
Distance de l extrémité antérieure du museau à l extrémité postérieure de l opercule [18]	73 - 80	77 ± 2	72 - 80	76 ± 2	70 - 78	75 ± 2	73 - 80	78 ± 2
Distance de l extrémité antérieure du museau à l internarine [19]	38 - 42	40 ± 1	37 - 43	40 ± 2	35 - 41	39 ± 2	39 - 42	41 ± 1
Rameau mandibulaire [21]	15 - 19	17 ± 1	13 - 23	16 ± 2	15 - 18	17 ± 1	15 - 18	16 ± 1
Alpha (degrés) [α]	68,2 - 84,5	76,8 ± 5,7	53,4 - 86,5	73,9 ± 8,5	62,8 - 91,6	77,9 ± 7,2	71,3 - 90,3	80,2 ± 6,1
Beta (degrés) [β]	18,1 - 21,0	19,9 ± 0,8	14,9 - 21,2	19,5 ± 1,6	17,5 - 21,0	19,7 ± 0,9	19,4 - 25,0	22,5 ± 1,6

Tableau VI. - Principales données méristiques de *H. guianensis*, populations du Litani, de la Mana, du Sinnamary et de l'Approuague. *[Main meristic data of* H. guianensis, *populations from Litani, Mana, Sinnamary and Approuague.]*

	Harttia guianensis							
	Litani		Mana		Sinnamary		Approuague	
Nombre d individus	9		16		14		12	
	Variation	Moy ± ET	Variation	Moy ± ET	Variation	Moy ± ET	Variation	Moy ± ET
Nombre de plaques dans la série latérale [a]	27	-	25 - 27	26,7 ± 0,7	26 - 28	27 ± 0,5	27 - 28	27,1 ± 0,3
Nombre de plaques antérieures au point de confluence dans la série latérale [b]	18 - 20	19,1 ± 0,6	16 - 20	18,9 ± 1,2	19 - 20	19,1 ± 0,4	19 - 21	19,7 ± 0,6
Nombre de plaques postérieures au point de confluence dans la série latérale [c]	7 - 9	7,9 ± 0,6	7 - 9	7,8 ± 0,7	7 - 9	7,8 ± 0,5	6 - 9	7,4 ± 0,8
Nombre de dents maxillaires [d]	50 - 102	81,6 ± 17,3	46 - 95	75,7 ± 14,7	49 - 107	88,1 ± 15,1	73 - 100	85,5 ± 8,1
Nombre de dents mandibulaires [e]	51 - 99	78,4 ± 13,2	42 - 98	76,1 ± 19	55 - 101	87,2 ± 13,1	68 - 89	80,3 ± 7,1
Nombre de rayons de la dorsale [f]	i + 7	-	i + 7	-	i + 7	-	i + 7	-
Nombre de rayons de la caudale [g]	i + 12 + i	-	i + 12 + i	-	i + 12 + i	-	i + 12 + i	-
Nombre de rayons de l anale [h]	i + 5	-	i + 5	-	i + 5	-	i + 5	-
Nombre de rayons de la pectorale [i]	i + 6	-	i + 6	-	i + 6	-	i + 6	-
Nombre de rayons de la pelvienne [j]	i + 5	-	i + 5	-	i + 5	-	i + 5	-
Nombre de plaques latéroventrales [k]	5 - 8	7,1 ± 1,0	0 - 10	6,4 ± 2,2	3 - 8	6,3 ± 1,2	7 - 9	8,2 ± 0,9
Nombre de plaques préanales [l]	3 - 14	8,4 ± 3,3	0 - 15	7,6 ± 4,7	0 - 8	4,7 ± 1,9	6 - 13	8,5 ± 2,5
Nombre de vertèbres [m]	32 (2 ind.)	-	32 (2 ind.)	-	32 (2 ind.)	-	32 (2 ind.)	-
Présence d une couverture abdominale (1=oui, 0=non)	0		0		0		0	
Présence d une tâche caudale (1=oui, 0=non)	1		1		1		1	
Présence d une plaque nucale (1=oui, 0=non)	0		0		0		0	

l'analyse de la série type de *H. surinamensis* (Fig. 4), sont rattachés à posteriori au groupe *guianensis* par l'analyse discriminante (Fig. 6). Ils sont donc ici réidentifiés en tant que *H. guianensis*. Les paratypes de la rivière Coppename pourraient aussi représenter une espèce distincte, différant principalement des individus de la localité type par l'absence de plaques abdominales. L'examen d'un plus grand nombre d'individus de la Coppename s'avère nécessaire afin d'analyser cette différence. Les individus des deux populations étant très proches morphologiquement, seul le critère présence ou absence de plaques ventrales est utilisé comme caractère de distinction. La présence de plaques osseuses entourant l'anus est un des caractères qui a permis l'établissement du genre *Harttia* par Steindachner en 1877. En ce qui concerne les autres types de plaques latéroventrales, préanales et abdominales, il semblerait que leur présence, leur nombre et leur disposition soient assez variables d'une espèce à l'autre. Oyakawa (1993) utilise pour ses diagnoses et ses clefs de détermination de neuf espèces du Sud-Est brésilien le critère présence ou absence des plaques latéroventrales (absentes chez *H. leiopleura*), du sous-préopercule (absent chez *H. novalimensis* et *H. leiopleura*), des plaques préanales (présentes chez *H. loricariformis*, *H. gracilis* et *H. torrenticola*) et des plaques abdominales (présentes chez *H. rhombocephala*). Le critère présence de plaques abdominales est également utilisé dans la diagnose de *H. longipinna* Langeani *et al*., 2001. Rapp Py-Daniel et Oliveira (2001) soulignent que: "Examination of all *Harttia* species has shown that features such as presence of lateral keels and abdominal scutes can be highly variable within the group and are not suitable as diagnostic features". Malgré cela, ces auteurs utilisent dans les diagnoses de *H. punctata*, *H. duriventris*, *H. dissidens*, *H. uatumensis* et *H. depressa* le critère présence ou absence de plaques abdominales. Dans les diagnoses de *H. guianensis* et *H. trombetensis*, ils caractérisent la couverture abdominale comme étant incomplète. La pertinence de l'utilisation de ce seul critère dans la distinction des populations de la Coppename et de la Suriname, à l'image de nombre d'espèces, est donc à confirmer. Une analyse génétique pourrait permettre de vérifier que ce caractère n'est pas soumis à des contraintes environnementales.

Si la plupart des espèces de *Harttia* en Guyane française possèdent une morphologie proche (Figs 5, 6) et difficile à caractériser, *H. maculata* se singularise significativement par 20 variables quantitatives sur 24 et par 3 variables qualitatives. De telles différences devraient être confrontées à d'autres données, moléculaires et ostéologiques, afin de statuer sur son attribution au genre *Harttia*. Cette espèce devra être comparée à *H. platystosma* (Günther, 1868) (espèce type du genre *Cteniloricaria* Isbrücker & Nijssen, 1979) du système de l'Essequibo au Guyana afin d'éprouver la validité de *Cteniloricaria* Isbrücker & Nijssen, 1979.

Les différences morphologiques soulignées entre les différentes populations de *H. guianensis* (Fig. 7) sont trop ténues pour permettre de conclure à la présence de plusieurs espèces distinctes. Une autre approche s'avère indispensable pour lever cette interrogation. En effet, si la population de l'Approuague diffère par sa stature plus élevée et sa coloration et si la population du Sinnamary, sur des bases méristiques, est différente, les données morphologiques restent insuffisantes pour conclure sur leur statut taxinomique. L'analyse d'autres caractères notamment moléculaires devrait permettre de répondre à cette question. L'isolement d'une espèce propre au Sinnamary pourrait corroborer les observations de Le Bail *et al*. (2000) pour d'autres espèces de Siluriformes dont les répartitions allant de L'Approuague au Maroni présentent un hiatus au niveau du Sinnamary (*Glanidium leopardus, Pseudoplatystoma fasciatum, Heptapterus bleekeri, Rhamdella leptosoma, Corydoras guianensis, Ancistrus* cf. *leucostictus* ou *Farlowella reticulata*).

Au vu de ces résultats, l'aire de répartition de *H. guianensis* est plus importante que celle qui est donnée dans la description originale (Rapp Py-Daniel et Oliveira, 2001) et inclut la Mana et le Maroni ainsi que ses affluents Oulemani et Tapanahoni, situés sur le versant surinamien. Cette espèce se répartit donc de l'Approuague, sa localité type, jusqu'au Maroni et ses affluents.

Au Surinam, *H. maculata* est sympatrique de *H. surinamensis* et en Guyane française, elle fréquente les mêmes bassins que *H. guianensis* à l'exception de l'Approuague et de la Mana. Cette espèce est toutefois moins abondante que la précédente, les taux de capture étant de l'ordre d'un spécimen *maculata* pour dix *guianensis*. Sa répartition s'étend du Sinnamary jusqu'à la Corantijn au Surinam. Sa présence sur la rivière Kaw reste à confirmer. Un unique spécimen desséché a été trouvé sur les berges de la crique Wapou, mais aucun individu vivant n'y a été collecté jusqu'à présent.

Harttia fowleri est endémique du bassin de l'Oyapock.

Si l'on excepte *H. maculata*, à la répartition géographique très étendue, les *Harttia* se répartissent ainsi en trois, voire quatre, grandes entités allopatriques dans les Guyanes française et anciennement néerlandaise. Selon un gradient d'est en ouest, on trouvera *H. fowleri*, suivi de *H. guianensis*, puis de *H. surinamensis*. La population de la Coppename, si des travaux complémentaires devaient l'élever au rang spécifique, constituerait la quatrième entité, la plus occidentale. Au Guyana, ces quatre espèces seraient remplacées par *H. platystoma*.

CLÉ DE DÉTERMINATION PRATIQUE DES ESPÈCES DU GENRE *HARTTIA* EN GUYANE FRANÇAISE ET AU SURINAM

1a. - Absence d'une couverture abdominale constituée de petites plaques 2

1b. - Présence d'une couverture abdominale constituée de petites plaques se développant progressivement avec l'âge 3

2a. - Absence d'un chapelet de petites plaques faisant jonction entre les plaques préanales et latéroventales ..... *H. guianensis*

2b. - Présence d'un chapelet de petites plaques faisant jonction entre les plaques préanales et latéroventales ..... *H.* sp. cf. *surinamensis* (Coppename)

3a. - Absence d'une plaque nucale ..... *H. surinamensis* (Suriname)

3b. - Présence d'une plaque nucale 4

4a. - Absence d'une tache caudale ..... *H. maculata*

4b. - Présence d'une tache caudale ..... *H. fowleri* (Oyapock)

Remerciements. - Nous tenons à remercier François Meunier et Guy Duhamel, MNHN, Paris, et Martien van Oijen, RMNH, Leiden, pour les prêts de matériel ; Volker Mahnert, MHNG, Genève, pour son accueil au sein de son établissement ; la Fondation Georges et Antoine Claraz pour sa participation financière à la mission Surinam 2001 ; Claude Weber, MHNG, Genève pour ses conseils et relectures avisés ; Emmanuel Castella de l'Université de Genève et Stéphane Dray de l'Université Lyon 1 pour leur aide éclairée en analyses multivariées, ainsi que Sylvie Mérigoux de l'université Lyon 1 pour son aide judicieuse. Les photographies ont été prises par Claude Ratton du MHNG et les figures ont été réalisées par Florence Marteau et Gilles Roth du MHNG. Les résultats préliminaires de cette étude ont été présentés lors des RIF 2003 grâce au soutien financier du Département des affaires culturelles de la Ville de Genève.

RÉFÉRENCES

BOESEMAN M., 1971. - The "comb-toothed" Loricariinae of Surinam, with reflections on the phylogenetic tendencies within the family Loricariidae (Siluriformes, Siluroidei). *Zool. Verh.*, 116: 1-56.

FERRARIS C.J., 2003. - Subfamily Loricariinae (Armored catfishes). *In* : Check List of the Freshwater Fishes of South and Central America (CLOFFSCA) (Reis R.E., Kullander S.O. & C. J. Ferraris, eds), pp. 330-350. Porto Alegre: EDIPUCRS.

ISBRÜCKER I.J.H., 1979. - Descriptions préliminaires de nouveaux taxa de la famille des Loricariidae, poissons-chats cuirassés néotropicaux, avec un catalogue critique de la sous-famille nominale (Pisces, Siluriformes). *Rev. Fr. Aquariol.*, 5 (4, for 1978) : 86-116.

ISBRÜCKER I.J.H., 1981. - A treatise of the Loricariidae Bonaparte, 1831, a family of South American mailed catfishes, with emphasis on the subfamily Loricariinae (Pisces, Siluriformes). Thèse de doctorat, 224 p. Univ. Nancy I.

LANGEANI F., OYAKAWA O.T. & J.I. MONTOYA-BURGOS, 2001. - New species of *Harttia* (Loricariidae, Loricariinae) from the Rio São Francisco Basin. *Copeia*, 2001(1): 136-142.

LE BAIL P.-Y., KEITH P. & P. PLANQUETTE, 2000. - Atlas des Poissons d'Eau douce de Guyane. Tome 2, fasc. II : Siluriformes. 307 p. Paris: Patrimoines naturels (MNHN/SPN).

OYAKAWA O.T., 1993. - Cinco espécies novas de *Harttia* Steindachner, 1876 da região sudeste do Brasil, e comentários sobre o gênero (Teleostei, Siluriformes, Loricariidae). *Comun. Mus. Ciênc. PUCRS (Sér. Zool.)*, 6: 3-27.

RAPP PY-DANIEL L.H., 1997. - Phylogeny of the Neotropical armored catfishes of the subfamilly Loricariinae (Siluriformes: Loricariidae). Unpubl. Ph.D. thesis, 280 p. Univ. of Arizona, Tucson.

RAPP PY-DANIEL L.H. & E.C. OLIVEIRA, 2001. - Seven new species of *Harttia* from the Amazonian-Guyana region (Siluriformes: Loricariidae). *Ichthyol. Explor. Freshw.*, 12: 79-96.

REIS R.E., KULLANDER S.O. & C.J. FERRARIS (eds), 2003. - Check List of the Freshwater Fishes of South and Central America (CLOFFSCA). 742 p. Porto Alegre: EDIPUCRS.

STEINDACHNER F., 1877. - Die Süsswasserfische des Südöstlichen Brasilien (III). *Sitzungsber. Akad. Wiss. Wien*, 74: 559-694.

THIOULOUSE J., CHESSEL D., DOLEDEC S., OLIVIER J.M., GOREAUD F. & R. PELISSIER, 2001. - ADE-4, Ecological Data Analysis: Exploratory and Euclidian methods in environmental sciences. Version 2001-CNRS 1995-2000.

YOCCOZ N.G., 1993. - Morphométrie et analyses multidimentionnelles. Une revue des méthodes séparant taille et forme. *In*: Biométrie et Environnement (Lebreton J.D. & Asselain B., eds), pp. 73-99. Paris: Masson.

Reçu le 3 mars 2005.
Accepté pour publication le 23 août 2005.

Annex 2

The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: Loricariidae): a practical key and synopsis.

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This is a preliminary study conducted on the Loricariinae. Morphological diagnostic characters were used for the construction of an identification key of all the genera of the subfamily. The HSA was used to unify quantitative and qualitative data in order to organize the information.

Published in: Zootaxa (2007), **1462**: 1-40.

Open acces financed by the All Catfish Species Inventory (NSF-DEB 0315963) and available at: http://www.mapress.com/zootaxa/2007f/zt01462p040.pdf.

Zootaxa 1462: 1–40 (2007)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

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Table of contents

Abstract

The subfamily Loricariinae belongs to the Neotropical mailed catfish family Loricariidae. Members of Loricariinae are recognized by their long and flattened caudal peduncle and absence of an adipose fin. Despite important studies conducted on this group, no comprehensive generic key is presently available. A Hill & Smith (1976) analysis and cluster analysis were performed on external morphological characters taken from specimens or borrowed from the literature. The two main groups recognized correspond to the tribes Harttiini and Loricariini. Within the Loricariini, four morphological groups were found: the *Rineloricaria* group, the *Loricariichthys* group, the *Loricaria* group, and the *Pseudohemiodon* group. Results of these analyses were used to construct a practical key to thirty genera, followed by a synopsis for each genus.

Key words: Loricariinae, genus, morphology, multivariate analyses, identification key

Introduction

The Neotropics contain one of the most diverse freshwater ichthyofaunas in the world with around 6,000 of the 13,000 known species (Reis *et al.* 2003). In Central and South America, the Ostariophysi are undoubtedly the largest represented group and among them, the Siluriformes exhibit the greatest diversity with around 1,647 described species (Reis *et al.* 2003) distributed in 16 families, one of which was discovered and described only recently (Rodiles-Hernández *et al.* 2005). Among the Siluriformes, the Loricariidae, or armored catfish, is the most speciose family in the world comprising 673 valid species and around 300 recognized as undescribed (Reis *et al.* 2003). Loricariids are characterized by a depressed body covered by bony

Accepted by L. Page: 16 Mar. 2007; published: 30 Apr. 2007

plates, a unique pair of maxillary barbels, and above all, by an important modification of the mouth structure into a sucker disk. This structural transformation enables these fishes to adhere to the substrate, even in particularly fast flowing waters. The mouth and teeth show strong adaptations to feeding by scraping submerged substrates to eat algae, small invertebrates, detritus, and even wood. Loricariids have undergone an evolutionary radiation on a subcontinental scale, from Costa Rica to Argentina, both on the Pacific and Atlantic slopes of the Andes. They have colonized nearly all freshwater habitats from the torrential waters flowing from the Andes to quiet brackish waters of the estuaries, black and acidic waters of the Guiana Shield, and subterranean systems. Schaefer & Stewart (1993) compare this radiation to that of the Cichlidae of the Great Lakes of the Rift Valley in Africa. Extremely variable color patterns and body shapes among loricariid taxa reflect their high degree of ecological specialization. Because of their highly specialized morphology loricariids have been recognized as a monophyletic assemblage in the earliest classifications of the Siluriformes (de Pinna 1998). The family comprises five or six subfamilies, depending on different authors' classifications. Isbrücker (1980), and Ferraris in Reis *et al.* (2003) divide Loricariidae into six subfamilies, the Ancistrinae, the Hypoptopomatinae, the Hypostominae, the Lithogeneinae, the Loricariinae, and the Neoplecostominae. Armbruster (2004) recognized five subfamilies placing Ancistrinae as a tribe within Hypostominae, even though this statement does not resolve paraphylies highlighted by Montoya-Burgos *et al.* (1998) within both subfamilies. Reis *et al.* (2006) followed Armbruster's (2004) classification, and described the new subfamily Delturinae according to the phylogenetic results of Montoya-Burgos *et al.* (1998), and Armbruster (2004).

Members of the subfamily Loricariinae are characterized by a long and depressed caudal peduncle and by the absence of an adipose fin. They also show dramatic variation in body shape, lip morphology and dentition. The sexual dimorphism is often pronounced and is expressed through the hypertrophy of odontodes on the pectoral-fin rays, on the snout margin, and sometimes on the predorsal area of mature males. Certain genera also show sexual differences in lip and tooth structures.

Isbrücker (1979) listed twenty-seven genera of Loricariinae, described eight as new, and classified them into four tribes and eight subtribes on the basis of morphology, without phylogenetic inferences. These include the Loricariini, including six subtribes (Loricariina, Planiloricariina, Reganellina, Rineloricariina, Loricariichthyina and Hemiodontichthyina), the Harttiini, including two subtribes (Harttiina and Metaloricariina), the Farlowellini, and the Acestridiini. The same author (1981a: p. VI, 71) voiced doubts concerning the placement of Acestridiini among Loricariinae, noting that: "The exposed cleithrum and coracoid, together with the peculiar odontodes on the unbranched pelvic fin ray ('spine') are characters otherwise occurring typically only in various members of the subfamily Hypoptopomatinae."; nevertheless, he maintained them as members of Loricariinae. In the same work he also described two new subtribes, Ricolina and Pseudoloricariina, developed the main characteristics of each rank: subfamily, tribe, subtribe, and genera, and provided a provisional key to the genera of Loricariidae. Rapp Py-Daniel (1981) described a new genus, *Furcodontichthys*, and placed it in the Loricariini, subtribe Loricariina. Martín Salazar *et al.* (1982) described *Dentectus* as a representative of the tribe Loricariini, subtribe Planiloricariina. In this paper, he completed the diagnosis of Planiloricariina, in which he transferred the genera *Rhadinoloricaria*, *Crossoloricaria*, and *Pseudohemiodon*. Isbrücker *et al.* (1983) described *Aposturisoma* as a representative of the Farlowellini. Isbrücker & Nijssen (1984, 1986a) described *Pyxiloricaria* and *Apistoloricaria,* respectively, and placed them in the Loricariini, subtribe Planiloricariina. Using phylogenetic methods, Schaefer (1986, 1987) established the monophyly of the Loricariinae on the basis of morphological data. Finally, Nijssen & Isbrücker (1987) suggested, refering to a Ferraris personal communication, that the Acestridiini were representatives of the subfamily Hypoptopomatinae. Schaefer (1991) confirmed this status and diagnosed the tribe Hypoptopomatini including, among others, the Acestridiini. Rapp Py-Daniel (1997) proposed a phylogeny of the Loricariinae based on a phylogenetic analysis of morphological characters. She confirmed the monophyly of the subfamily, and of two of the three remaining tribes *sensu* Isbrücker (1979), Harttiini and Loricariini; members of Farlowellini were placed within Harttiini. Montoya-Burgos *et al.* (1998) proposed the first molecular phylogeny of the

family Loricariidae with emphasis on the subfamily Hypostominae. Although, their analysis included only nine representatives of the subfamily Loricariinae, they partially confirmed their subdivision into two main groups, with *Farlowella*, a representative of the Farlowellini, being the sister genus of *Sturisoma*, a representative of the Harttiini, and *Harttia* located at the base of the subfamily. Outside of *Harttia*, the two main groups supported were *Farlowella* and *Sturisoma* sister group of the remaining six genera corresponding to Loricariini. Isbrücker and Isbrücker & Michels (in Isbrücker *et al*. 2001) described four new genera: *Fonchiichthys*, *Leliella*, *Quiritixys* and *Proloricaria*, and revalidated the genus *Hemiloricaria* Bleeker, 1862 on the basis of a very restricted number of characters of questionable validity because they focus mainly on sexual dimorphism. Rapp Py-Daniel & Oliveira (2001) put *Cteniloricaria* in the synonymy of *Harttia*. Ferraris (2003) maintained the validity of *Cteniloricaria*, put in synonymy all the genera described by Isbrücker and Isbrücker & Michels (in Isbrücker *et al*. 2001) and listed 197 species of Loricariinae distributed in 31 genera: *Apistoloricaria* (4 species), *Aposturisoma* (1 species), *Brochiloricaria* (2 species), *Crossoloricaria* (5 species), *Cteniloricaria* (3 species), *Dasyloricaria* (5 species), *Dentectus* (1 species), *Farlowella* (25 species), *Furcodontichthys* (1 species), *Harttia* (18 species), *Harttiella* (1 species), *Hemiodontichthys* (1 species), *Ixinandria* (2 species), *Lamontichthys* (4 species), *Limatulichthys* (1 species), *Loricaria* (11 species), *Loricariichthys* (17 species), *Metaloricaria* (2 species), *Paraloricaria* (3 species), *Planiloricaria* (1 species), *Pseudohemiodon* (7 species), *Pseudoloricaria* (1 species), *Pterosturisoma* (1 species), *Pyxiloricaria* (1 species), *Reganella* (1 species), *Rhadinoloricaria* (1 species), *Ricola* (1 species), *Rineloricaria* (47 species), *Spatuloricaria* (11 species), *Sturisoma* (14 species), and *Sturisomatichthys* (4 species). Among all these genera, 13 are monotypic and very few of the most speciose have been revised. *Loricaria* was revised by Isbrücker (1981b), *Metaloricaria* by Isbrücker & Nijssen (1982), *Apistoloricaria* by Nijssen & Isbrücker (1988), and *Farlowella* by Retzer & Page (1997).

In light of all these works, which are sometimes contradictory, a taxonomic synthesis of Loricariinae is needed to provide a foundation for more detailed studies of its members. Furthermore, despite the large number of studies conducted on this group, a complete key to the genera of the subfamily Loricariinae is presently unavailable; partial keys are available in Isbrücker & Nijssen (1974a; 1986b), Isbrücker (1981b), Rapp Py-Daniel (1981), and Burgess (1989). To rectify this situation, a key to all the genera of the subfamily is proposed herein on the basis of external morphological data, and a synopsis is given for each genus. Multivariate and hierarchical analyses were conducted to classify and organize the information used to construct the key. Our study follows the classification of Ferraris (2003), except for maintaining *Cteniloricaria* in synonymy with *Harttia* (Rapp Py-Daniel & Oliveira 2001). As a result, we recognize herein 30 genera of Loricariinae.

Material and methods

Examined material. A total of 1691 specimens, representing about 115 species in 26 of the 30 genera were examined. All material examined was deposited in the Museum of Natural History, Geneva (MHNG). Data were taken from publications of original descriptions of the four genera unavailable from MHNG. After evaluating for the homogeneity of the different genera and species samples, that is to say to verify that all the specimens of each batch share the same features, and that all these features were in agreement with those of the literature, a part of this material was used in multivariate analyses. The abbreviations of the institutions follow Leviton *et al.* (1985).

Analyzed material. Data of a single specimen representing each genus were subjected to multivariate analyses. The selected specimens exhibited all the characteristics of their respective genera given in the literature. Data extracted from the literature were those from the holotype of the type species. Material included in the analyses is as follows: ***Aposturisoma***: *A. myriodon*:—MHNG 2087.1–2, 153.9 mm of SL, paratype, Peru, Ucayali Department, Province Coronel Portillo, Rio Aguaytia drainage, Rio Huacamayo (09°00'S, 75°29'W),

near the road from Pucallpa to Tingo Maria, around 8 km N.W. from the village of Aguaytia, de Rham *et al*., 24 August 1981. ***Brochiloricaria***: *B. macrodon*:—MHNG 2583.93, 305.4 mm of SL, Paraguay, Rio Pirapo, Caazapa, zoological expedition of the Museum of Geneva, camp 7, 28–31 March 1985. ***Crossoloricaria***: *C. rhami*:—MHNG 2108.16, 69.8 mm of SL, paratype, Peru, Ucayali Department, Province Coronel Portillo, Rio Aguaytia drainage, Rio Huacamayo (09°00'S, 75°29'W), near the road from Pucallpa to Tingo Maria, around 8 km N.W. from the village of Aguaytia, de Rham *et al*., 18 September 1981. ***Dasyloricaria***: *D. cf. filamentosa*:—MHNG 2674.052, 275.8 mm of SL, Panama, Panama Department, Rio Ipeti, de Rham, 11 March 2000. ***Farlowella***: *F. platoryncha*:—MHNG 2588.93 (72), 185.3 mm of SL, Peru, Ucayali Department, Yarinacocha, Isla del Amor in the vicinity of Pucallpa, de Rham *et al*., 29 May 1996. ***Harttia***: *H. guianensis*:—MHNG 2621.97, 167.0 mm of SL, French Guiana, Approuague drainage, saut Mapaou, Weber *et al*., 5 November 2001. ***Harttiella***: *H. crassicauda*:—MHNG 2674.051 (MUS 221), 39.4 mm of SL, Suriname, Nassau mountains, Ijskreek, Mol, 2 November 2005. ***Hemiodontichthys***: *H. acipenserinus*:—MHNG 2550.28, 129.8 mm of SL, Brazil, State of Pará, Rio Guamá at 12 km below Ourem, Stawikowski, 24 September 1990. ***Ixinandria***: *I. montebelloi*:—MHNG 2676.31, 59.1 mm of SL, Bolivia, Province Tarija at Narvaez, Vaucher, 9 November 1993. ***Lamontichthys***: *L. filamentosus*:—MHNG 2639.30, 127.8 mm of SL, Peru, aquarist import, donation Goldblatt, 29 October 2002. ***Limatulichthys***: *L. griseus*:—MHNG 2090.25, Peru, Ucayali Department, Rio Neshuya at 60 km S.W. of Pucallpa, de Rham *et al*., 21 August 1981. ***Loricaria***: *L. sp*.:—MHNG 2583.85, 171.1 mm of SL, Paraguay, Rio Pirapo, Caazapa at 3 km E. of Yegros, zoological expedition of the Museum of Geneva, 28–31 March 1985. ***Loricariichthys***: *L. platymetopon*:—MHNG 2583.100, 185.6 mm of SL, Paraguay, Arroyo Passo Ybucu at 35 km S.E. of Paraguari, zoological expedition of the Museum of Geneva, 24–26 March 1985. ***Metaloricaria***: *M. paucidens*:—MHNG 2676.09, 220.8 mm of SL, French Guiana, Maroni drainage, Marouini River, surroundings of Antecume Pata, fishermen donation, 19 October 2000. ***Paraloricaria***: *P. agastor*:—MHNG 2407.81, 279.2 mm of SL, Paraguay, Rio Alto Paraná, facing Cardelaria, Dlouhy, 25 September 1986. ***Planiloricaria***: *P. cryptodon*:—MHNG 2625.86, 147.7 mm of SL, Peru, Loreto, Rio Amazonas, Mayoras, de Rham, 26 February 1998. ***Pseudohemiodon***: *P. laticeps*:—MHNG 2584.58, 216.7 mm of SL, Paraguay, Rio Pirapo, Caazapa at 3 km E. of Yegros, zoological expedition of the Museum of Geneva, 31 March 1985. ***Pseudoloricaria***: *P. laeviuscula*:—MHNG 2538.78, 205 mm of SL, Brazil, State of Pará, Rio Tapajos, temporary pond between Vila Nova and Urua, Stawikowski *et al*., 26–28 September 1992. ***Pterosturisoma***: *P. microps*:—MHNG 2574.13, 127.3 mm of SL, Peru, Rio Nanay, Caazapa, Bleher, 1993. ***Reganella***: *R. depressa*:—MHNG 2676.05 (732068), 162.8 mm of SL, Brazil, Pará, Rio Trombetas, Lago Batata, Porto Trombetas locality, Oriximiná municipality (1° 25' to 1° 35' S, 56° 15' to 56° 25' W), D. A. Halboth, November 1991. ***Rhadinoloricaria***: *R. macromystax*:—MHNG 2551.45, 2/5, (CMK 7514), 65.3 mm of SL, Brazil, Rio Tocantins facing São Felix, Stawikowski, 17 September 1990. ***Ricola***: *R. macrops*:—MHNG PY 100025, 262.8 mm of SL, Paraguay, Rio Paraguay at Ita Enramada, Dlouhy, 24 July 1984. ***Rineloricaria***: *R. steindachneri*:—MHNG 2583.65, 132.1 mm of SL, Brazil, Rio Paraiba do Sul W. of Sapucaia, Mazzoni *et al*., 10 December 1990. ***Spatuloricaria***: *S. sp*.:—MHNG 2676.10, 197.8 mm of SL, Brazil, Rio Tocantins, Serra da Mesa, Caramaschi *et al*., October 1996. ***Sturisoma***: *S. robustum*:—MHNG 2584.74, 244.5 mm of SL, Paraguay, Rio Pirapo, Caazapa at 3 km E. of Yegros, zoological expedition of the Museum of Geneva, 28 March 1985. ***Sturisomatichthys***: *S. citurensis*:—MHNG 2676.04, 152.3 mm of SL, Panama, Darien, Rio Chucunaque near the village La Alba, de Rham, 13 March 1997.

The data extracted from the literature are: ***Apistoloricaria*** (in Isbrücker & Nijssen 1986a): *A. condei*:—FMNH 94683, 126.2 mm of SL, holotype, Ecuador, Province Napo, Rio Napo drainage, mouth of Rio Tiputini in the Rio Napo at the confluence of the main tributary, in deep water (00° 48.9' S, 75° 32.5' W), Stewart *et al*., 28 October 1981. ***Dentectus*** (in Martín Salazar, Isbrücker & Nijssen 1982): *D. barbarmatus*:—MBUCV–v–12780, 136.5 mm of SL, holotype, Venezuela, State of Cojedes, Rio Salinas, tributary of the Rio Pao Viejo N.E. of El Baul (9° 13'N, 68° 07' W), Fernandez Yépez, 25 February 1950. ***Furcodontichthys*** (in Rapp Py-Daniel 1981): *F. novaesi*:—INPA T. 79–014, 102 mm of SL, holotype, Brazil, State of Amazonas,

Rio Solimões, Lake Téfé at Caititu, Best *et al*., 25 May 1979. ***Pyxiloricaria*** (in Isbrücker & Nijssen 1984): *P. menezesi*:—MZUSP 26800, 136 mm of SL, holotype, Brazil, State of Mato Grosso do Sul, in ponds located along the road Transpantaneira, at Miranda, Garavello *et al*., 8–22 November 1981.

Data analyses. Six discrete quantitative variables, representing fin-ray and tooth counts, and eleven qualitative variables totalizing thirty character states, mainly representing the characteristics of abdominal cover, lips and dentition, were recorded from each specimen (for details, see Table 1). These variables were selected from the literature and on the basis of their diagnostic value at the genus level following examination of a large number of specimens. Data were first subjected to a Hill & Smith analysis (1976) which allows the mixing of quantitative and qualitative data in the same analysis. Quantitative data were first subjected to a Principal Components Analysis (PCA) using a correlation matrix. The normed PCA proceeds initially to the centering and reduction of the data (null mean and unitary variance) that permit the comparison of data expressed in different units. Invariable data such as the number of rays in the anal fin (n = i–4) were excluded to allow this transformation. The weighting of the rows (individuals) is uniform (1/30) whereas that of the columns (variables) is unitary (1). A Multiple Correspondence Analysis (MCA) was used to analyse qualitative data. Each variable of the qualitative data set comprises a certain number of character states. Before the execution of the analysis, this first table was converted into a full disjunctive table where each column represents a character state. The sum of the weighting of the character states (frequencies) for each variable equals 1 and the weighting of each variable is uniform (1/11) in the analysis. The weighting of the rows is also uniform (1/30). In the Hill & Smith analysis, the two types of data are made compatible by reweighting the columns. A new statistical triplet [data table X with 30 rows and 36 columns, table Q containing row weights (30 rows and 1 column), and table D containing column weights (36 rows and 1 column)] was created by coupling the two tables of the two initial analyses. Uniform weighting of the rows in the two separate analyses (1/30) is maintained while one of the columns is modified. Weighting of the quantitative and qualitative variables becomes uniform, with each assigned a weight of 1 across the total number of variables (1/17). This gives the same value to each variable in the analysis, even though qualitative variables possess several character states (sum of frequencies of the different character states for each qualitative variable equals 1). Thus, PCA looks for axes that maximize square of correlations of the quantitative variables, MCA looks for axes that maximize the sum of ratios of correlations between modalities (character states) of the qualitative variables, and Hill & Smith analysis establishes a compromise between these two analyses by looking for axes that maximize the mean between the square of correlations (quantitative variables) and the ratios of correlations (qualitative variables). Secondly, this new statistical triplet was converted into a morphological distances matrix calculated from the coordinates of the individuals (rows) projected onto the factorial axes. This transformation allows a hierarchical sorting of the information represented in a dendrogram. Euclidian distances were measured and analyzed using the Unweighted Pair-Group Method using Arithmetic Averages algorithm (UPGMA) (Sneath & Sokal 1973). This analysis was used to classify the different genera based on morphological similarity calculated from shared characters and combination of characters. These analyses were conducted using the ADE–4 software (Thioulouse *et al*. 2001).

Results

Morphological data used in the analyses are given in Table 1. The Hill & Smith analysis revealed structuring of the data on the two first axes (Fig. 1c) that explained 52% of the total inertia of the scatter. The projection of the individuals onto the two first factorial axes (Fig. 1a) showed a partition of the 30 genera into two groups along the first axis. These two groups corresponded to the two tribes, Loricariini and Harttiini, the former comprising *Planiloricaria*, *Dentectus*, *Crossoloricaria*, *Apistoloricaria*, *Rhadinoloricaria*, *Pseudohemiodon*, *Pyxiloricaria*, *Spatuloricaria*, *Paraloricaria*, *Ricola*, *Brochiloricaria*, *Reganella*, *Loricaria*, *Dasyloricaria*,

Rineloricaria, *Pseudoloricaria*, *Limatulichthys*, *Furcodontichthys*, *Loricariichthys*, and *Hemiodontichthys*, and the latter comprising *Harttia*, *Harttiella*, *Lamontichthys*, *Pterosturisoma*, *Sturisomatichthys*, *Aposturisoma*, and *Sturisoma*. *Metaloricaria* and *Ixinandria* appeared intermediate between these two groups. The second axis organized the genera at an infra-tribal level according to their morphological resemblance. For example, *Planiloricaria*, *Dentectus*, *Crossoloricaria*, *Apistoloricaria*, *Rhadinoloricaria*, *Pseudohemiodon*, and *Pyxiloricaria* appeared morphologically more closely related to each other than to *Pseudoloricaria*, *Limatulichthys*, *Furcodontichthys*, *Loricariichthys*, and *Hemiodontichthys*. The projection onto the first factorial plane of the variables (Fig. 1b) defined the primary morphological tendencies of each tribe along the first axis. The Harttiini were characterized by numerous and pedunculated teeth, a caudal fin with more branched rays, the absence of postorbital notches and predorsal keels, a rounded mouth, papillose lips weakly or not fringed, and short maxillary barbels. The Loricariini were characterized by a more important variation in lips and teeth shape, the frequent presence of postorbital notches and predorsal keels, longer maxillary barbels, and less numerous teeth and branched rays in the caudal fin. The second axis defined morphological groups in each tribe mainly on the basis of dentition and lip structure. Among the Loricariini, *Planiloricaria*, *Dentectus*, *Crossoloricaria*, *Apistoloricaria*, *Rhadinoloricaria*, *Pseudohemiodon*, and *Pyxiloricaria* shared filamentous lips, a trapezoidal mouth opening, and teeth often spoon-shaped and smaller. *Pseudoloricaria*, *Limatulichthys*, *Furcodontichthys*, *Loricariichthys*, and *Hemiodontichthys* shared bilobate lips and bicuspid teeth often reduced in size. Among the Harttiini, *Metaloricaria* showed a horse-shoe like mouth shape and smaller pedunculated teeth. Other genera were difficult to characterize and another approach was clearly necessary. A cluster analysis (Fig. 2) grouped genera based on their degree of morphological resemblance. *Metaloricaria* and *Farlowella* were the most divergent genera and formed the base of the tree. Two groups were then partitioned that corresponded to the two tribes, Harttiini and Loricariini. The Harttiini was comprised of *Aposturisoma*, the most morphologically divergent, followed by *Lamontichthys*, and then *Ixinandria*. This tribe was then divided into two other groups, one formed by *Harttia* and *Harttiella*, and another by *Sturisoma* joined with *Pterosturisoma* and *Sturisomatichthys*. The Loricariini divided into two principal groups. The *Loricariichthys* group was formed by *Furcodontichthys*, *Loricariichthys*, *Hemiodontichthys*, *Pseudoloricaria*, and *Limatulichthys*. The second group was subdivided into three groups: the *Pseudohemiodon* group consisting of *Reganella*, *Pseudohemiodon*, *Pyxiloricaria*, *Planiloricaria*, *Dentectus*, *Rhadinoloricaria*, *Crossoloricaria*, and *Apistoloricaria*; the *Rineloricaria* group formed by *Spatuloricaria*, *Rineloricaria*, and *Dasyloricaria*; and the *Loricaria* group formed by *Loricaria*, *Paraloricaria*, *Ricola*, and *Brochiloricaria*. Our analyses resulted in the placement of two taxa that was inconsistent with previous classifications: *Ixinandria* appeared among Harttiini although its dentition and presence of postorbital notches align it with Loricariini; and *Spatuloricaria* appeared at the base of the *Rineloricaria* and *Loricaria* groups. Because these genera share similar lip structures, we followed Isbrücker (1979) by assigning them to the *Rineloricaria* group of the Loricariini. *Metaloricaria* and *Farlowella*, located at the base of the tree because of their particular morphology, were assigned to the tribe Harttiini, following Isbrücker (1979) and Rapp Py-Daniel (1997). To extract the main characteristics of each genus, shared as well as unique, new Hill & Smith analyses were performed on the above named groups. The projection of the individuals and qualitative variables onto the first factorial plane (Fig. 1c) summarized all this information by connecting the individuals to the center of gravity of the different modalities of the different variables possessed. For example, *Metaloricaria* (species 17) possesses a complete abdominal cover (A3) without particular organization (B2), neither postorbital notches nor predorsal keels (C1, D1), papillose lips (E1), no fringed barbels (F1), a horse-shoe like mouth shape (G2, unique character), teeth pedunculated and reduced in size (H3, unique character), short maxillary barbels (I2), no rostrum (J2), and a pointed snout (K2). Some variables with modalities close to the axes and to the center appear weakly informative on the first plane, such as the presence or absence of secondary organization in the abdominal cover (B) or the presence or absence of a rostrum (J).

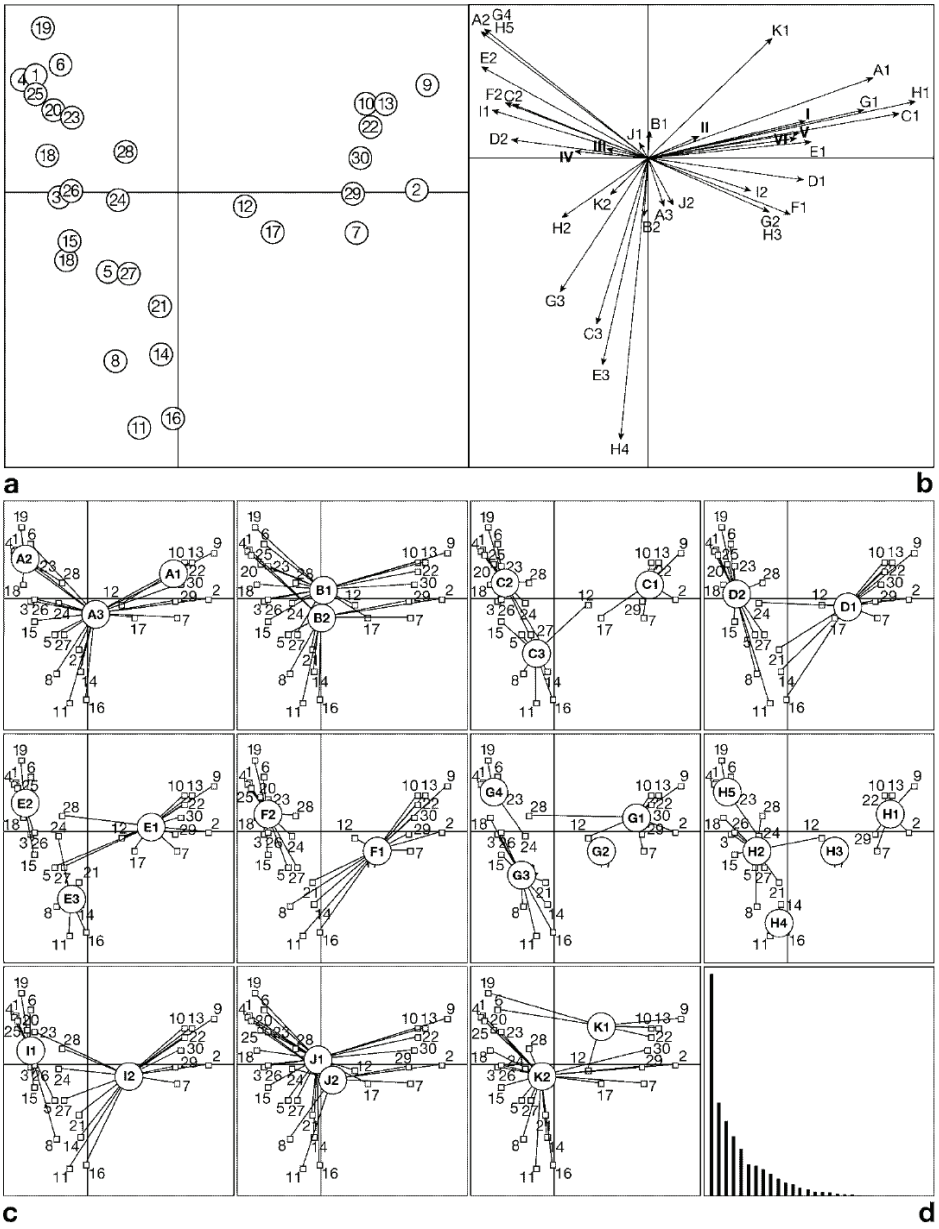


FIGURE 1. Hill & Smith analysis (1976) of the subfamily Loricariinae. **a**: projection of 30 individuals representing the 30 genera numbered as in Table 1 onto the first factorial plane of the Hill & Smith analysis (axis 1 horizontal and axis 2 vertical); **b**: projection of the 17 variables onto the first factorial plane of the Hill & Smith analysis (title and modalities in Table 1); **c**: projection of the individuals and of the qualitative variables onto the first factorial plane of the Hill & Smith analysis, one plane representing one qualitative variable (title and modalities in Table 1); **d**: eigenvalues.

TABLE 1. Main morphological data recorded on the selected specimen for each genus of the subfamily Loricariinae. A star (*) indicates data taken from the literature. **I** to **VI**: Quantitative data. **I**: number of caudal-fin rays (including spines); **II**: number of pectoral-fin rays (including spine); **III**: number of pelvic-fin rays (including spine); **IV**: number of dorsal-fin rays (including spine); **V**: number of premaxillary teeth; **VI**: number of dentary teeth. **A** to **K**: qualitative data. **A**: abdominal cover with three modalities: 1 = absent, 2 = present incomplete, 3 = present complete; **B**: secondary organization in the abdominal cover with two modalities: 1 = absent, 2 = present; **C**: postorbital notches with three modalities: 1 = absent, 2 = present weak, 3 = present deep; **D**: predorsal keels with two modalities: 1 = absent, 2 = present; **E**: lips structure with three modalities: 1 = papillose, 2 = filamentous, 3 = rather smooth; **F**: fringed barbels with two modalities: 1 = absent, 2 = present; **G**: mouth shape with four modalities: 1 = elliptical, 2 = horse shoe like, 3 = bilobate, 4 = bilobate with trapezoidal opening; **H**: tooth shape with five modalities: 1 = pedunculated, 2 = straight bicuspid, 3 = pedunculated size reduced, 4 = straight bicuspid size reduced, 5 = spoon shaped size reduced; **I**: maxillary barbels with two modalities: 1 = conspicuous, 2 = inconspicuous; **J**: rostrum with two modalities: 1 = absent, 2 = present; **K**: snout shape with two modalities: 1 = rounded, 2 = pointed.

Genus	Species	I	II	III	IV	V	VI	A	B	C	D	E	F	G	H	I	J	K
Apistoloricaria	*A. condei** [1]	12	7	6	7	4	7	2	2	2	2	2	2	4	5	1	1	2
Aposturisoma	*A. myriodon* [2]	13	7	6	6	85	85	3	2	1	1	1	1	1	1	2	2	2
Brochiloricaria	*B. macrodon* [3]	12	7	6	7	5	6	3	1	2	2	2	2	3	2	1	1	2
Crossoloricaria	*C. rhami* [4]	12	7	6	7	3	4	2	2	2	2	2	2	4	5	1	1	2
Dasyloricaria	*D. cf. filamentosa* [5]	12	7	6	7	13	13	3	2	3	2	1	2	3	2	1	1	2
Dentectus	*D. barbarmatus** [6]	12	7	6	7	3	3	3	1	2	2	2	2	4	5	1	1	1
Farlowella	*F. platoryncha* [7]	13	7	5	6	23	17	3	2	1	1	1	1	1	1	2	2	2
Furcodontichthys	*F. novaesi** [8]	12	7	6	7	10	6	3	2	3	2	3	1	3	2	1	2	2
Harttia	*H. guianensis* [9]	14	7	6	7	85	68	1	1	1	1	1	1	1	1	2	1	1
Harttiella	*H. crassicauda* [10]	14	7	6	7	33	31	1	1	1	1	1	1	1	1	2	1	1
Hemiodontichthys	*H. acipenserinus* [11]	12	7	6	7	0	8	3	2	3	2	3	1	3	4	2	2	2
Ixinandria	*I. montebelloi* [12]	12	7	6	7	10	8	1	1	3	1	1	1	1	2	2	1	1
Lamontichthys	*L. filamentosus* [13]	14	8	6	7	38	36	3	1	1	1	1	1	1	1	2	1	1
Limatulichthys	*L. griseus* [14]	12	7	6	7	7	10	3	1	2	1	3	1	3	4	2	1	2
Loricaria	*L. sp.* [15]	12	7	6	7	3	6	3	1	3	2	2	2	3	2	1	1	2
Loricariichthys	*L. platymetopon* [16]	12	7	6	7	8	15	3	2	3	1	3	1	3	4	2	1	2
Metaloricaria	*M. paucidens* [17]	13	7	6	7	11	13	3	1	1	1	1	1	2	3	2	1	2
Paraloricaria	*P. agastor* [18]	12	7	6	7	4	7	2	1	2	2	2	2	3	2	1	1	2
Planiloricaria	*P. cryptodon* [19]	12	7	6	7	0	3	2	1	2	2	2	2	4	5	1	1	1
Pseudohemiodon	*P. laticeps* [20]	12	7	6	7	5	7	3	1	2	2	2	2	4	5	1	2	2
Pseudoloricaria	*P. laeviuscula* [21]	12	7	6	7	10	12	3	1	2	1	3	1	3	2	2	1	2
Pterosturisoma	*P. microps* [22]	14	7	6	7	46*	46*	3	1	1	1	1	1	1	1	2	1	1
Pyxiloricaria	*P. menezesi** [23]	12	7	6	7	3	3	3	1	2	2	2	2	4	5	2	1	2
Reganella	*R. depressa* [24]	12	7	6	7	0	15	3	1	2	1	3	2	4	5	2	2	2
Rhadinoloricaria	*R. macromystax* [25]	12	7	6	7	4	8	2	2	2	2	2	2	4	5	1	2	2
Ricola	*R. macrops* [26]	12	7	6	7	10	10	3	1	2	2	2	2	3	2	1	1	2
Rineloricaria	*R. steindachneri* [27]	12	7	6	7	7	9	3	1	3	2	1	2	3	2	2	1	2
Spatuloricaria	*S. sp.* [28]	12	7	6	7	3	4	2	1	2	2	1	2	1	2	2	1	2
Sturisoma	*S. robustum* [29]	14	7	6	7	45	38	3	2	1	1	1	1	1	1	2	2	2
Sturisomatichthys	*S. citurensis* [30]	14	7	6	7	56	46	3	1	1	1	1	1	1	1	2	1	2

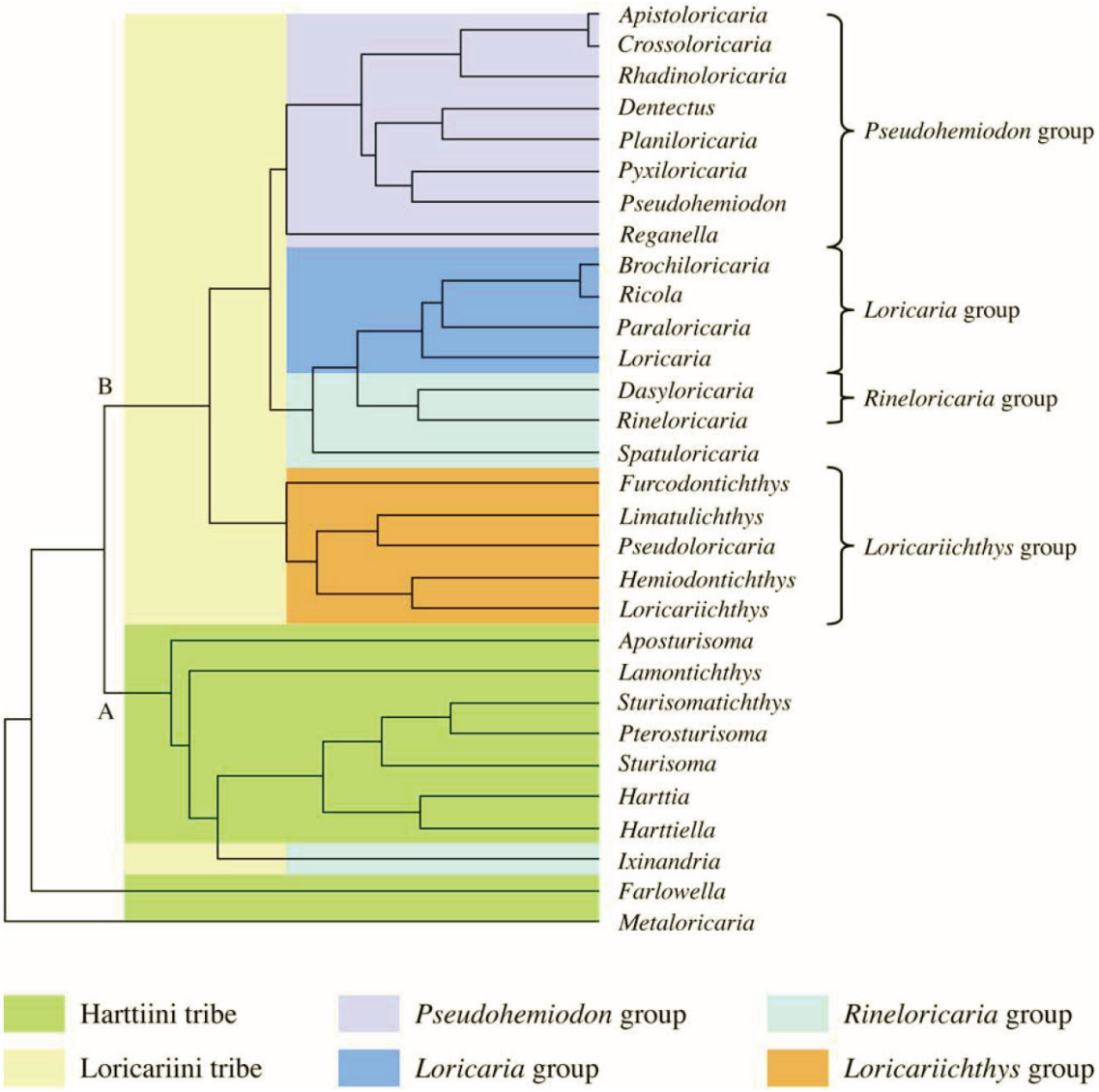


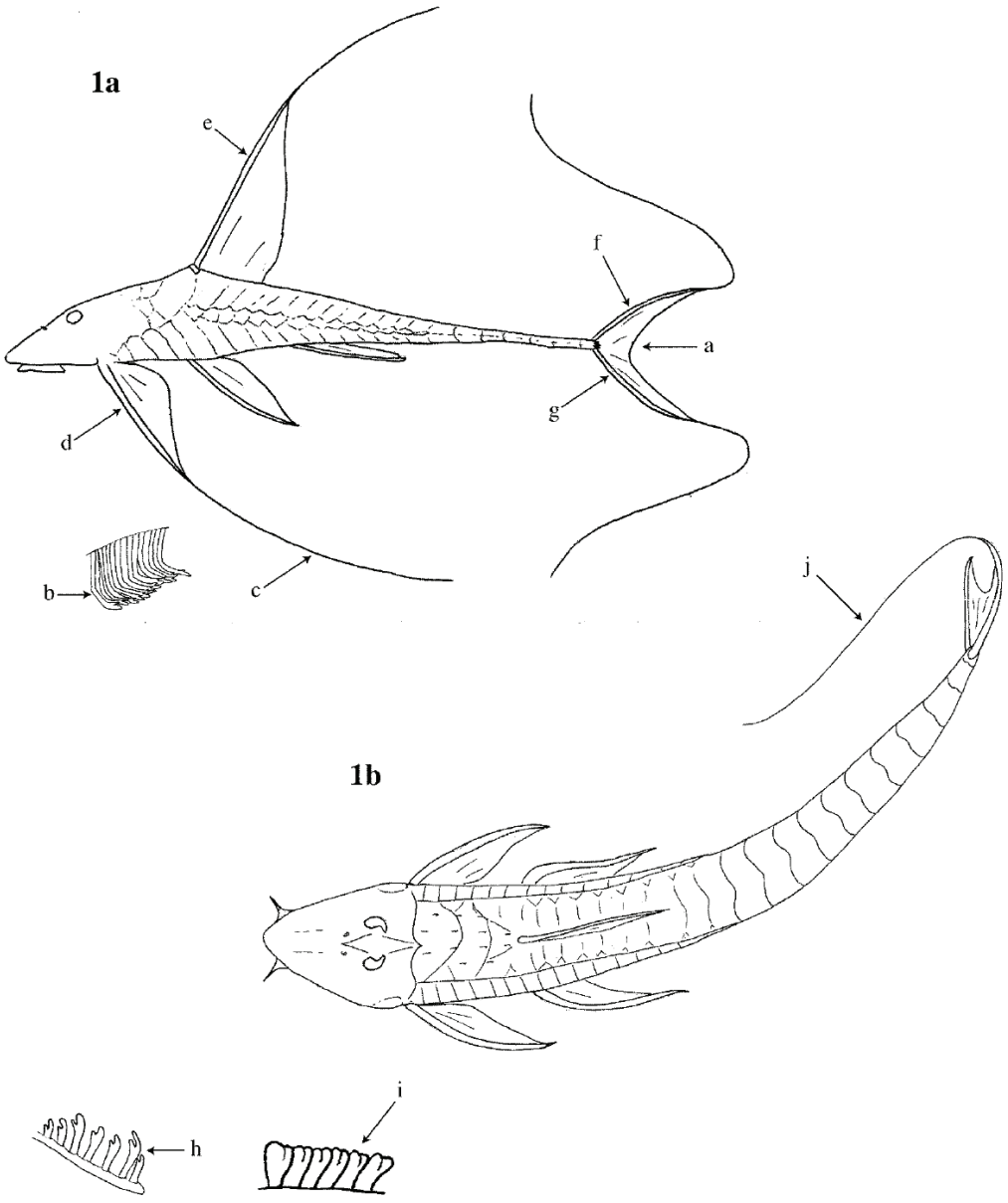
FIGURE 2. Cluster analysis of the subfamily Loricariinae. Dendrogram based on morphological distances matrix using UPGMA. **A**: Harttiini tribe; **B**: Loricariini tribe. Colors represent the different ranks established: : Harttiini tribe; : Loricariini tribe; : *Loricariichthys* group; : *Rineloricaria* group; : *Loricaria* group; : *Pseudohemiodon* group. Braces indicate the genera confirmed inside their own group.

Key to the genera

The methodology used in this study enabled us to sort the valuable information (grouping of individuals according to combinations of variables) into a hierarchy and create morphologically coherent groups by summarizing the characteristics of each genus. These results are intended to rectify problems with the identification of taxa and should not be interpreted as a phylogeny. We do not use the subtribal rank defined by Isbrücker (1979, 1981a) because the divisions are not well-defined. The results of our analyses support the recognition of two tribes, Harttiini and Loricariini, the latter of which contains four morphological groups. Characters that are underlined in the key couplets are also illustrated on the same page and identified by letters between brackets [a, b, c… aq, ar]. These letters are presented in alphabetical order in the couplets and in the illustrations, with two exceptions: character [ab] (first proposed in lead 13a, is illustrated in Figure 20b), and character [af] (first proposed in lead 15b, is illustrated in Figure 23a). Arrows indicate the most important characters for identification purposes. The numbers of the figures correspond to the numbers of the key couplet. Additional features are given at the level of identification to confirm this identification, certain specimens being sometimes poorly preserved or poorly characterized like juveniles, and certain genera being relatively variable.

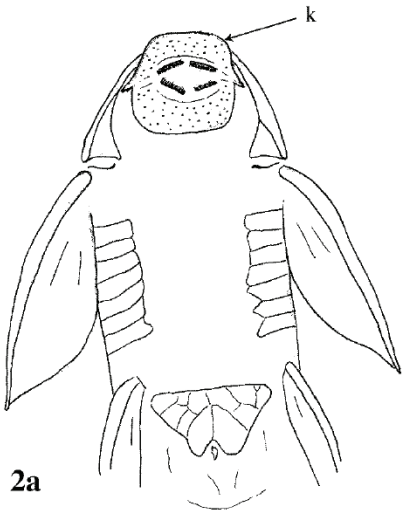
1a. – Caudal fin [a] with **i-12-i** or **i-11-i** rays; teeth pedunculated [b], bicuspid, numerous (≥10 per premaxillae), organized in comb and weakly differentiated; sometimes with filamentous extensions [c] on pectoral [d], dorsal [e], upper [f] and/or lower caudal [g] spines: **Harttiini**.....**2**

1b. – Caudal fin [a] with **i-10-i** rays; teeth straight [h] bicuspid, spoon-shaped [i], not numerous (≤20 per premaxillae), strongly differentiated, sometimes reduced in size or absent; often with a more or less strong whip [j] on upper caudal spine [f]: **Loricariini**.....**10**

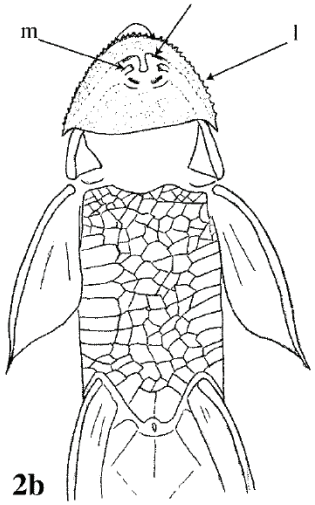


2a. – Mouth shape elliptical [k] .....…..... **3**

2b. – Mouth shape horseshoe like [l]; with three buccal papillae [m], lateral ones trilobate; teeth small and not numerous (≈ 10 per premaxillae): ..... *Metaloricaria*



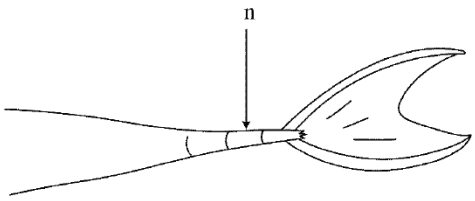
2a



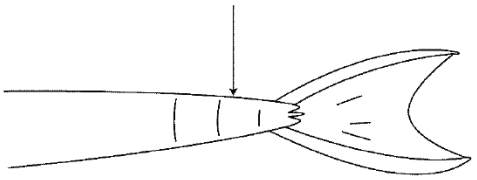
2b

3a. – Caudal peduncle [n] strongly depressed, elliptical in transverse section (in average, the minimal depth of the caudal peduncle represents 1 to 3 % of the SL) ….....**4**

3b. – Caudal peduncle [n] weakly depressed, more or less circular in transverse section (in average, the minimal depth of the caudal peduncle represents 5 % of the SL); abdomen naked; body covered by numerous, short and dense odontodes giving a velvety aspect; species of small size (≈ 50 mm): .....*Harttiella*



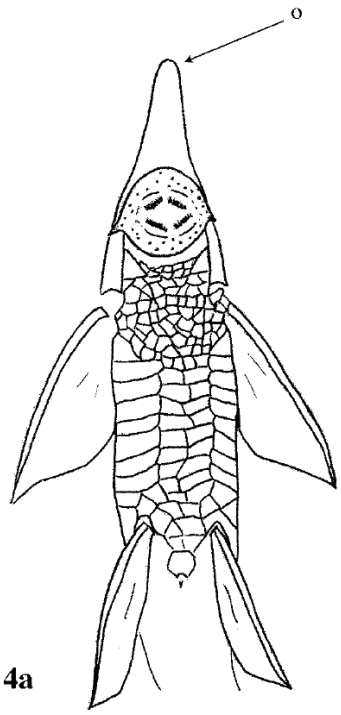
3a



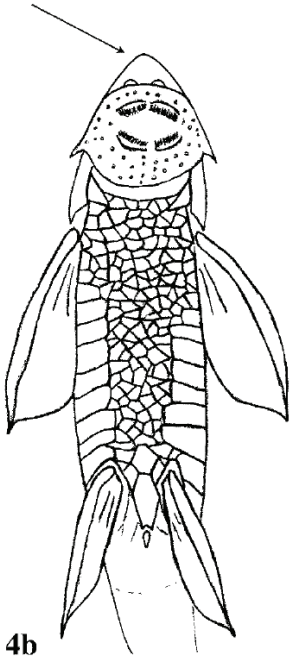
3b

4a. – Rostrum [o] present **5**

4b. – Rostrum [o] absent **7**



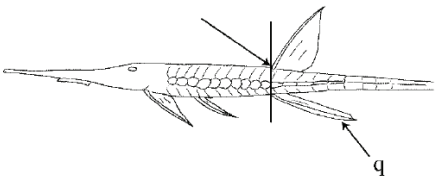
4a



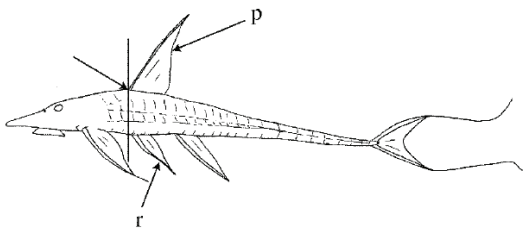
4b

5a. – Dorsal fin [p] originating more or less in front of the anal-fin [q] origin **6**

5b. – Dorsal fin [p] originating more or less in front of the pelvic-fin [r] origin; abdominal cover complete and weakly structured in two to three rows: ...*Sturisoma*



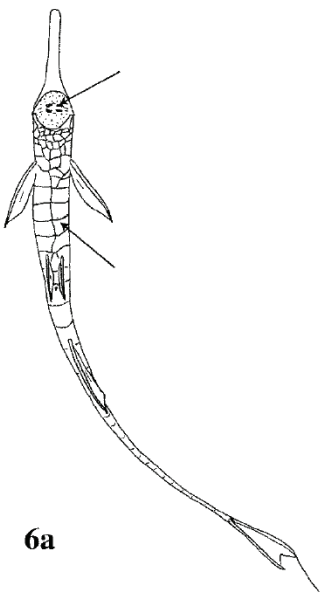
5a



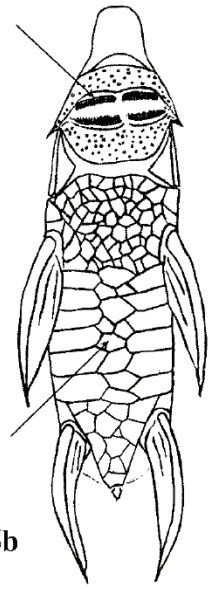
5b

6a. – Teeth not numerous (≈ 20 per premaxillae); two to three rows of abdominal plates; general aspect slender, reminiscent of a stick: .....*Farlowella*

6b. – Teeth numerous (≈ 100 per premaxillae); three rows of abdominal plates: .....*Aposturisoma*

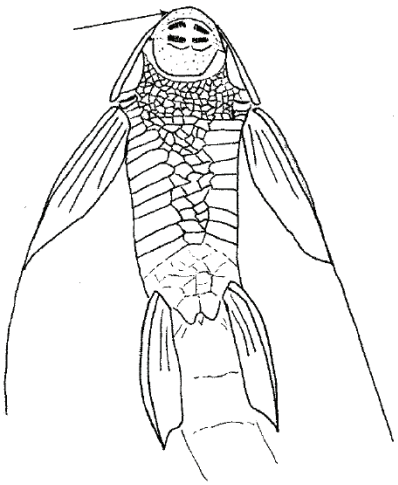


6a

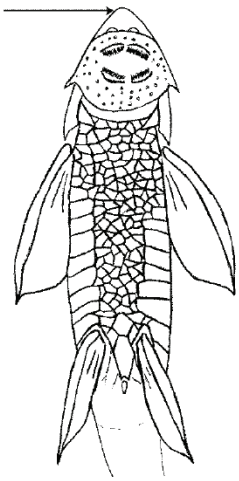


6b

7a. – Snout rounded **8**
7b. – Snout pointed; abdominal cover complete without particular organization, or weakly structured in two to three rows .....*Sturisomatichthys*

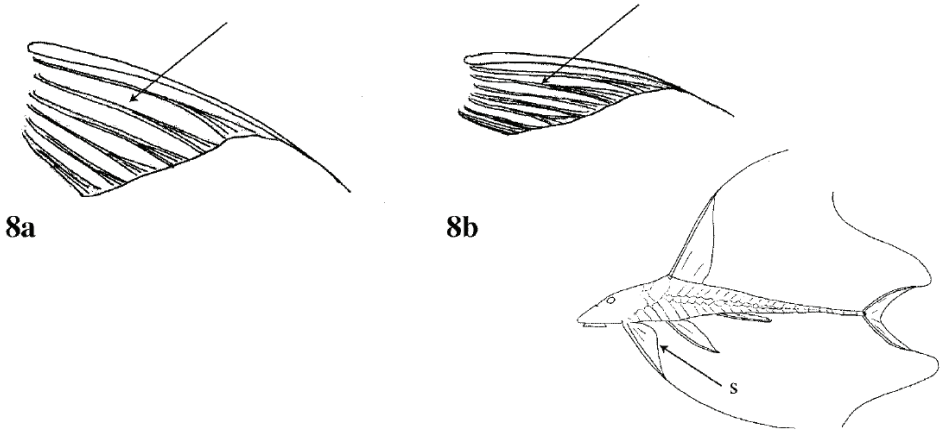


7a



7b

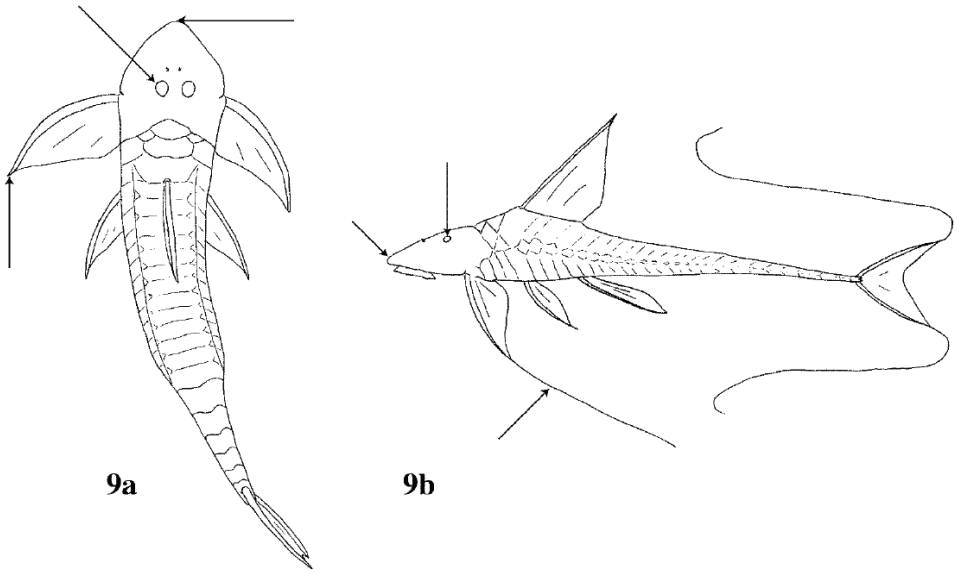
8a. – Pectoral fins [s] with **i-6** rays **9**
8b. – Pectoral fins [s] with **i-7** rays; pectoral spine [d] sometimes with filamentous extensions [c]: ..... *Lamontichthys*



8a **8b**

9a. – Eye diameter large (on average ≈ 20% of head length); tip of snout naked; without filamentous extensions [c] on pectoral [d], upper [f] and lower caudal [g] spines: .....*Harttia*

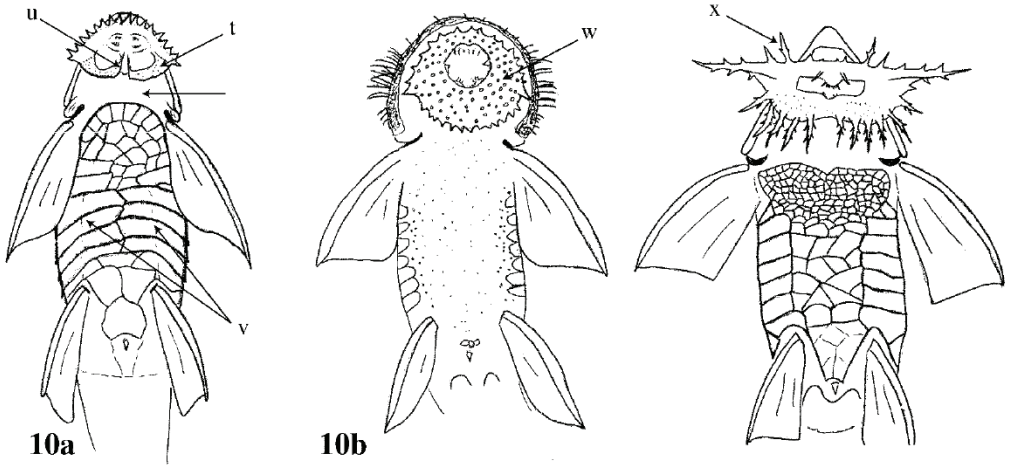
9b. – Eye diameter small (on average ≈ 10% of head length); tip of snout covered by plates; with filamentous extensions [c] on pectoral [d], upper [f] and lower caudal [g] spines: .....*Pterosturisoma*



9a **9b**

10a. – Lower lip bilobate [t] with a median furrow [u]; surface of this lip more or less smooth or weakly papillose; presence of a double abdominal keel [v]; throat never covered; whip [j] on upper caudal spine [f] weak or absent:
***Loricariichthys* group** **11**

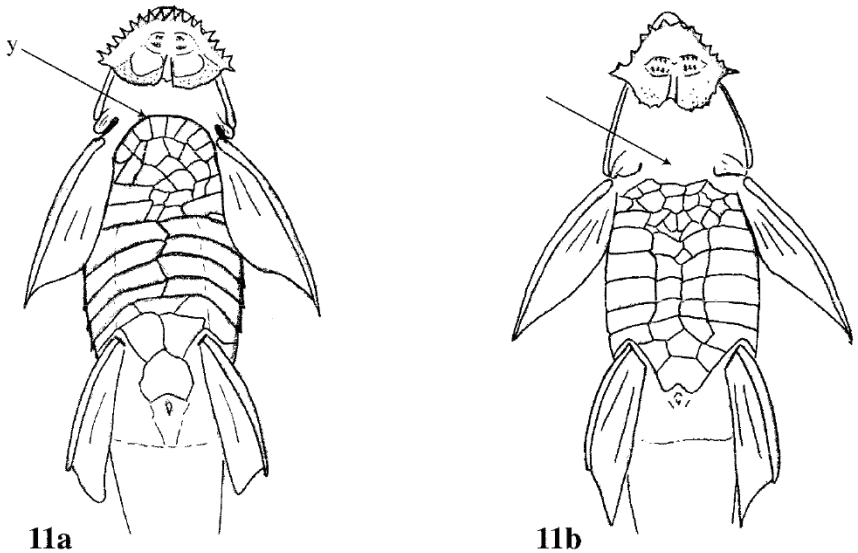
10b. – Absence of such a combination of characters; lower lip more often strongly papillose [w] or filamentous [x].....**15**



10a **10b**

11a. – Presence of a secondary structure in the organization of the abdominal cover forming a perfect elliptical area [y] at the level of the pectoral girdle:
.....*Loricariichthys*

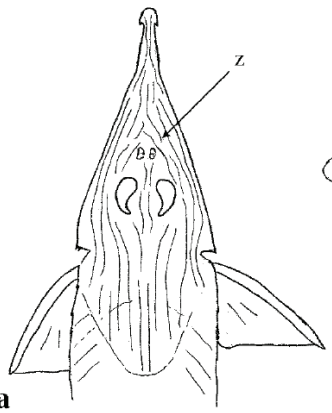
11b. – Without such structure **12**



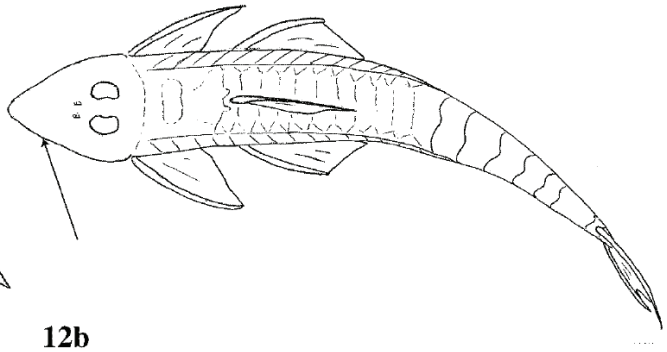
11a **11b**

12a. – With conspicuous lines of odontodes [z] on head and snout **13**

12b. – Without lines of odontodes [z] on head and snout **14**



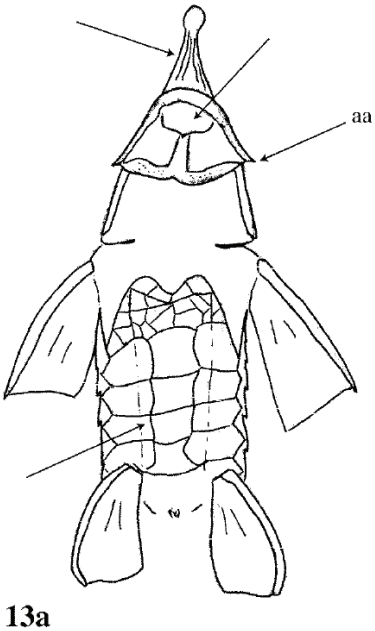
12a



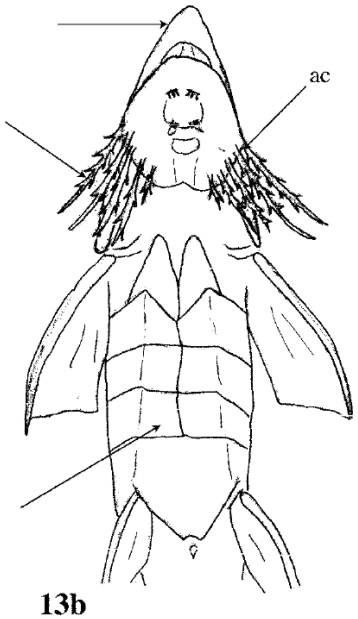
12b

13a. – Rostrum [o] strongly pronounced; maxillary barbels [aa] short; premaxillary teeth [ab] absent; abdomen covered by large rectangular plates organized in three rows: .....*Hemiodontichthys*

13b. – Rostrum [o] weakly pronounced; maxillary [aa] and fringed [ac] barbels conspicuous and gathered in series at the lip corners; premaxillary teeth [ab] present; abdomen covered by large plates organized in two rows: .....*Furcodontichthys*



13a

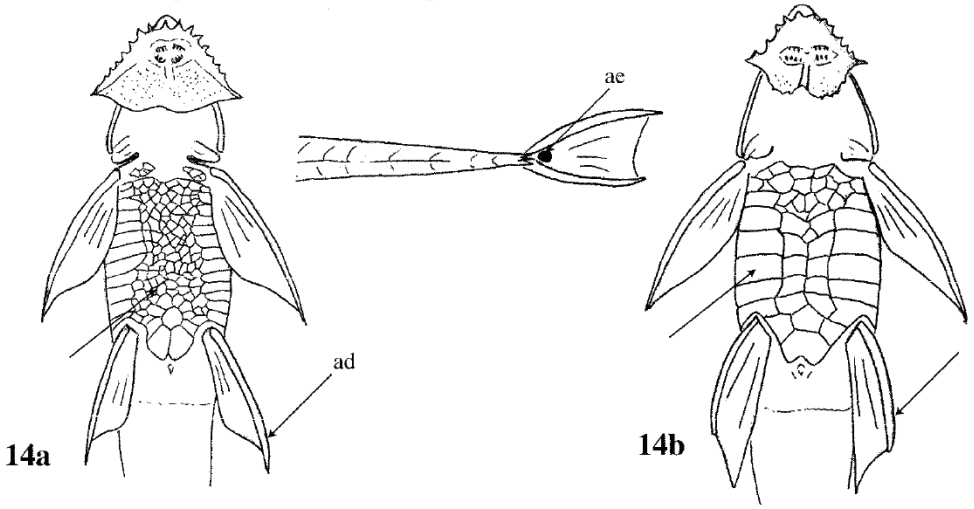


13b

14a. – Abdomen covered by small plates without particular organization; in adults pelvic-fin spine [ad] longer than last pelvic-fin [r] ray; in juveniles presence of a conspicuous basicaudal spot [ae]: .....*Pseudoloricaria*

14b. – Abdomen covered by medium-sized plates weakly structured in two to three rows; in adults last pelvic-fin [r] ray longer than pelvic-fin spine [ad]; in juveniles absence of a conspicuous basicaudal spot [ae]: .....*Limatulichthys*

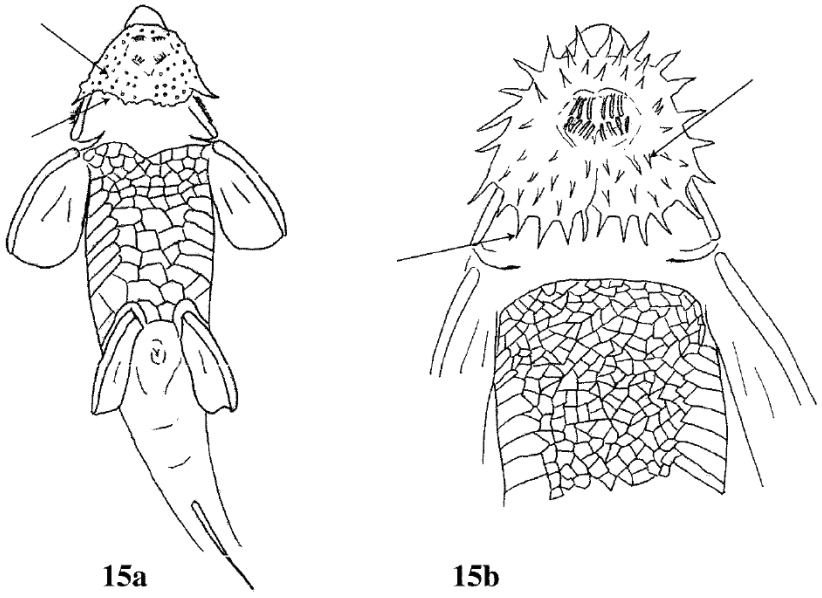
.



14a **14b**

15a. – Lips papillose [w]; fringed barbels [ac] of lower lip absent or inconspicuous: ***Rineloricaria* group** **16**

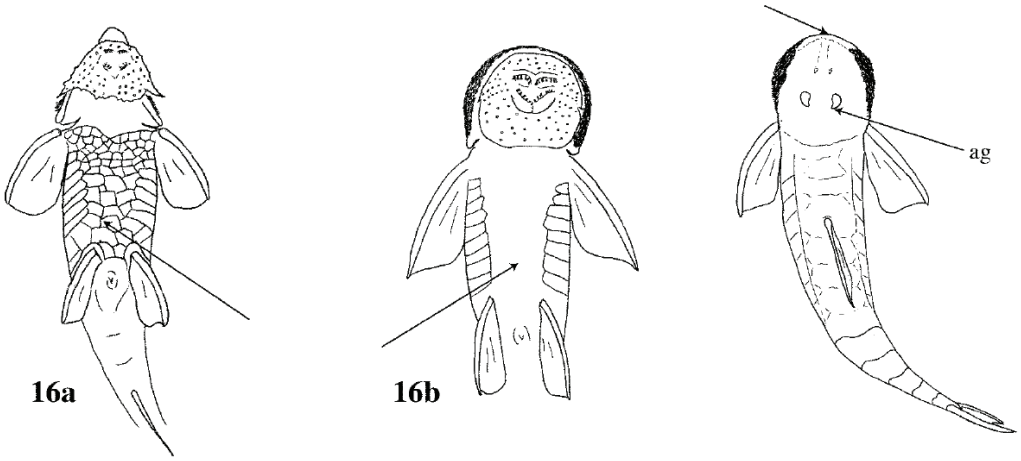
15b. – Lips generally filamentous [x] or smooth [af]; fringed barbels [ac] of lower lip generally conspicuous **19**



15a **15b**

16a. – Abdomen partially to completely covered by plates **17**

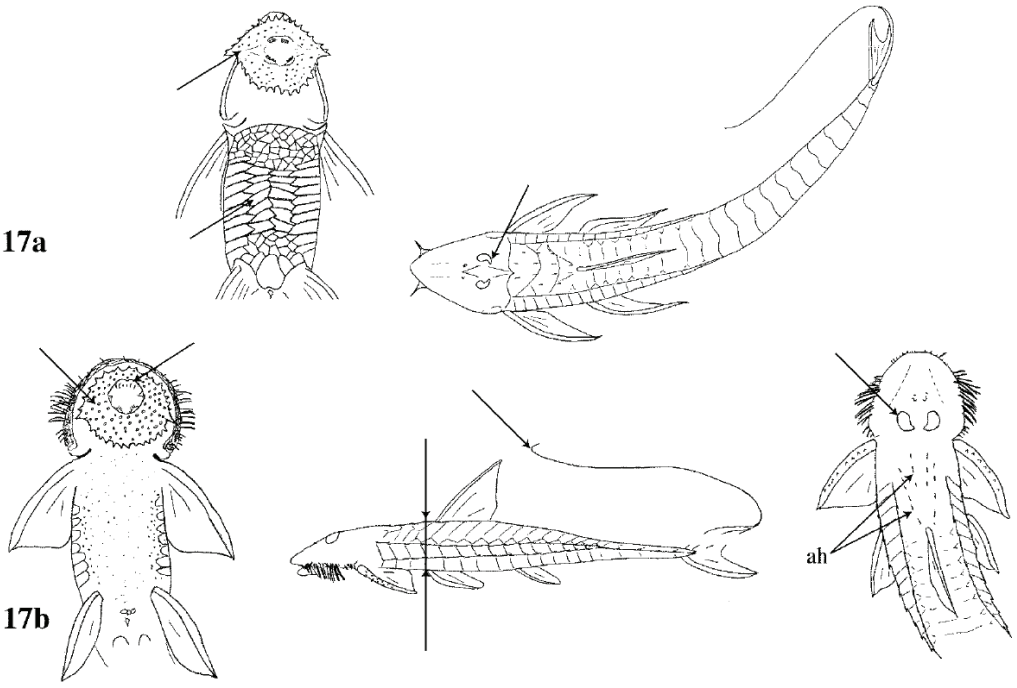
16b. – Abdomen naked; snout rounded; mouth circular; postorbital notches [ag] deep; strongly depressed body covered by numerous, short, and dense odontodes giving a velvety aspect; species of small size (≈ 90 mm): .....*Ixinandria*



16a **16b**

17a. – Abdomen partially to completely covered by medium-sized plates; mouth not circular; postorbital notches [ag] deep **18**

17b. – Abdomen covered by very small plates not contiguous; mouth circular and thick; postorbital notches [ag] weak; teeth few (≈ 4 per premaxillae); body depth strong (≈ 12% of SL); presence of a long whip [j] on upper caudal spine [f]; predorsal keels [ah] strong; .....*Spatuloricaria*

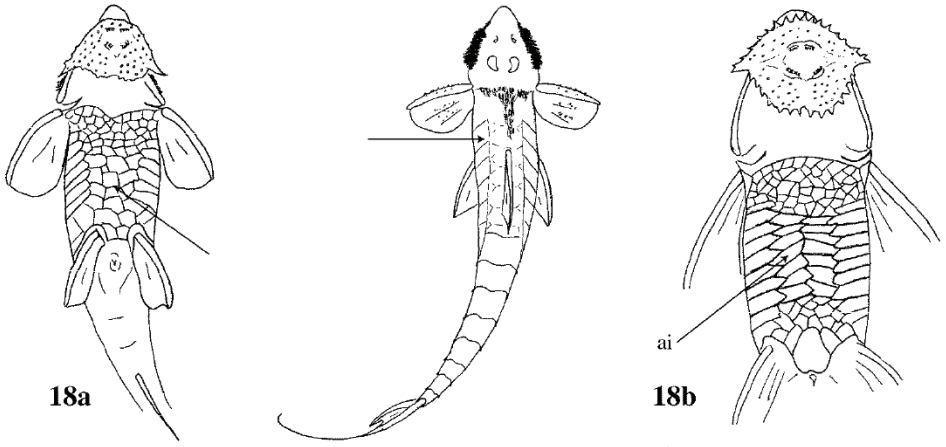


17a

17b

18a. – Without a secondary structure in abdominal cover; abdominal cover weakly organized in rows; predorsal keels [ah] more or less pronounced; species of medium size (generally ≤ 20 cm): .....*Rineloricaria*

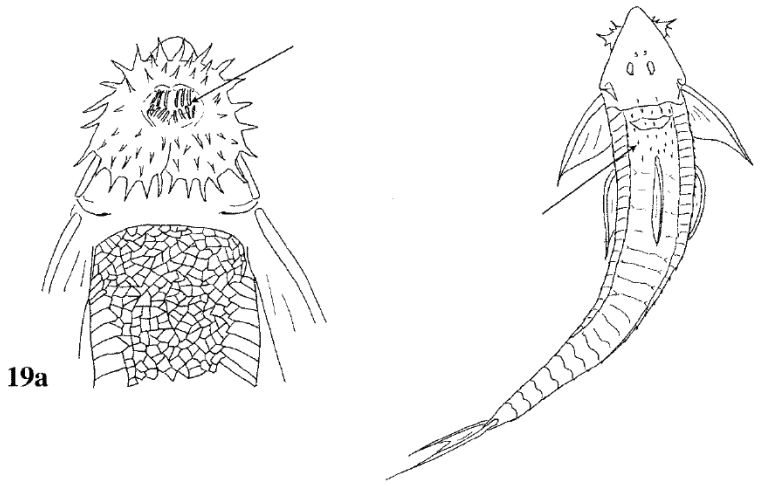
18b. – With a secondary structure on abdominal cover consisting in double median row of plates organized in chevrons [ai]; predorsal keels [ah] strong; species of large size (generally ≥ 25 cm): .....*Dasyloricaria*



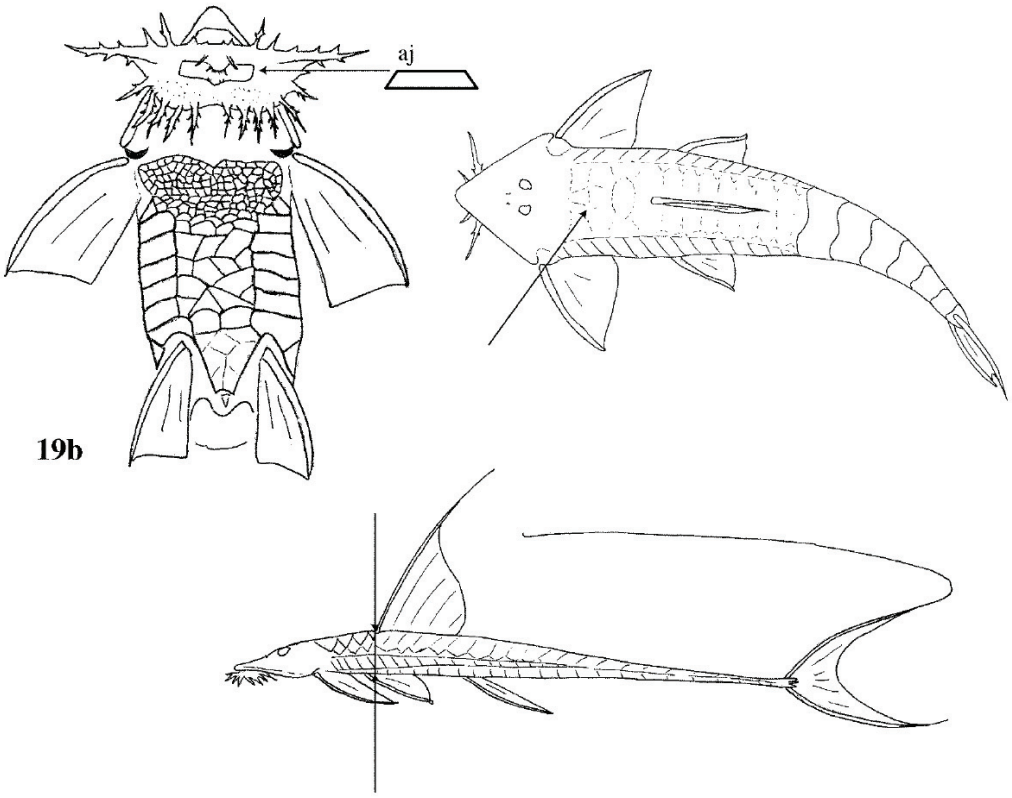
18a **18b**

19a. – Mouth opening without particular shape; the most often predorsal keels [ah] strong; body generally weakly depressed: ***Loricaria*** **group** **20**

19b. – Mouth opening trapezoidal [aj]; predorsal keels [ah] weak; body strongly depressed: ***Pseudohemiodon*** **group** **23**

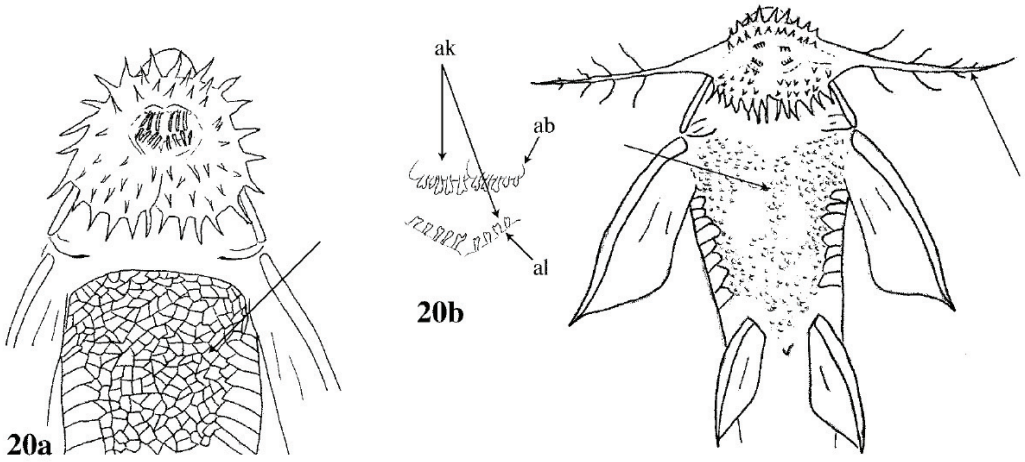


19a



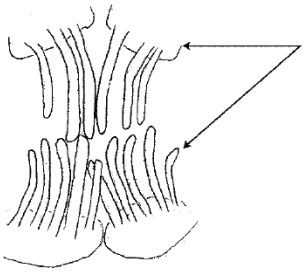
20a. – Abdominal cover the most often complete made of medium to small plates **21**

20b. – Abdominal cover incomplete, made of very small plates not contiguous; equal size [ak] of dentary [al] and premaxillary teeth [ab]; tooth size-reduced; maxillary barbels [aa] long, branched and reaching pectoral-fin [s] origin: .....*Paraloricaria*

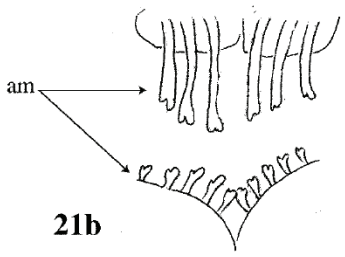


21a. – Equal size [ak] of dentary [al] and premaxillary teeth [ab]; tooth very long: .....*Brochiloricaria*

21b. – Different size [am] of dentary [al] and premaxillary teeth [ab], the latter almost two times longer than the former **22**



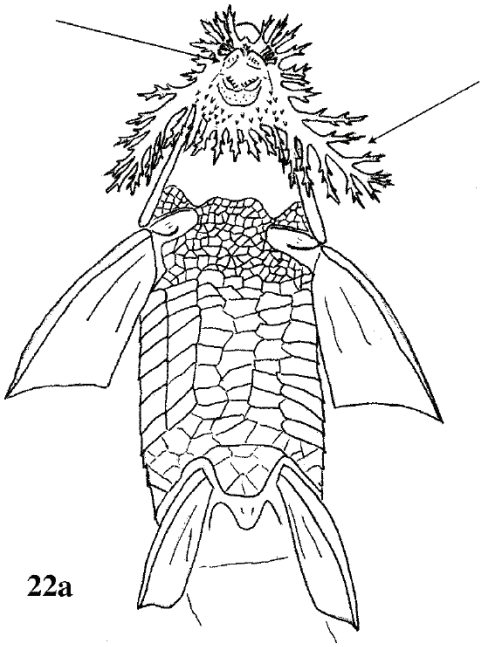
21a



21b

22a. – 10 to 15 premaxillary teeth [ab]; lips extremely filamentous [x]; maxillary barbels [aa] long, strongly branched, and reaching pectoral-fin [s] origin: .....*Ricola*

22b. – 3 to 5 premaxillary teeth [ab]; lips filamentous [x]; maxillary barbels [aa] not reaching pectoral-fin [s] origin: .....*Loricaria*

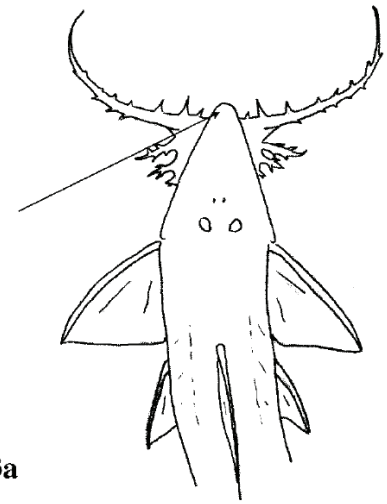


22a

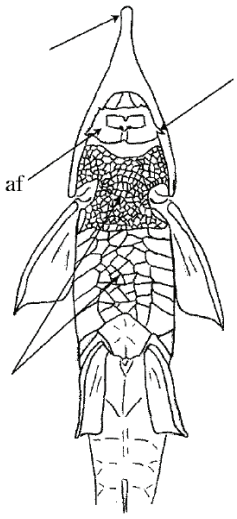
22b

23a. – Rostrum [o] weakly pronounced or absent: **24**

23b. – Rostrum [o] strongly pronounced; premaxillary teeth [ab] absent; dentary teeth [al] numerous (≈ 15 per dentary) and reduced in size; lips smooth [af]; maxillary barbels [aa] short; abdominal cover complete, made of little plates without particular organization; throat covered: .....*Reganella*



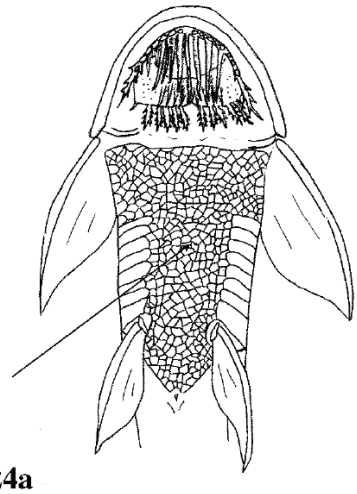
23a



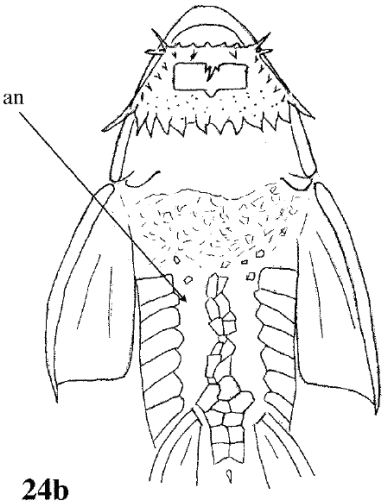
23b

24a. – Abdominal cover complete: **25**

24b. – Abdominal cover incomplete, most often consisting in a double median row [an] of plates: **27**



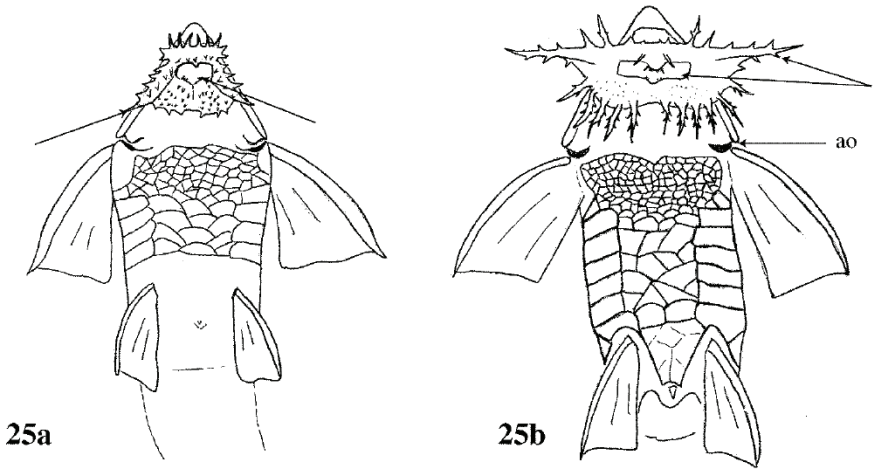
24a



24b

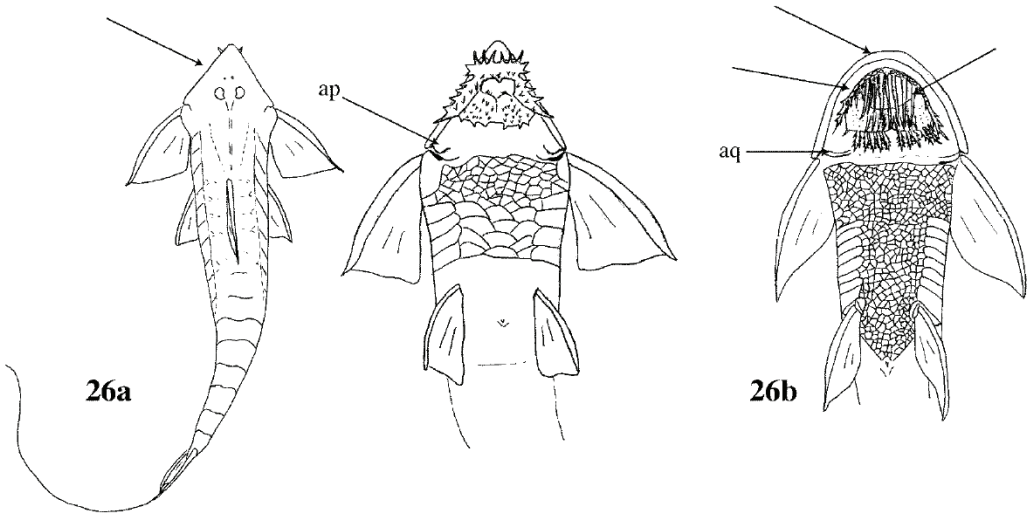
25a. – Maxillary barbels [aa] inconspicuous, not reaching gill opening [ao]; teeth very difficult to observe, invisible in normally preserved specimens **26**

25b. – Maxillary barbels [aa] conspicuous, reaching gill opening [ao]; teeth visible; head large; body strongly depressed: .....*Pseudohemiodon*
.



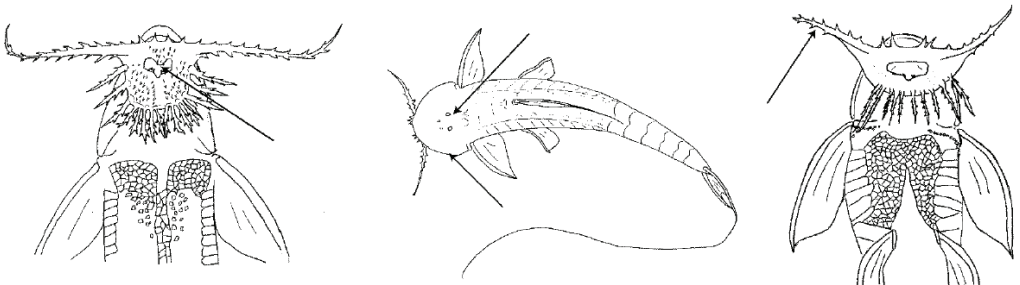
26a. – Head triangular and little; body large; trapezoidal in transverse section; with a fleshy flap [ap] partially covering the branchiostegal membrane [aq]: .....*Pyxiloricaria*

26b. – Head rounded; upper lip with numerous filaments reaching the lower lip margin; with plates on the external margin of the maxillary barbels [aa]: .....*Dentectus*



27a. – Premaxillary teeth [ab] present **28**

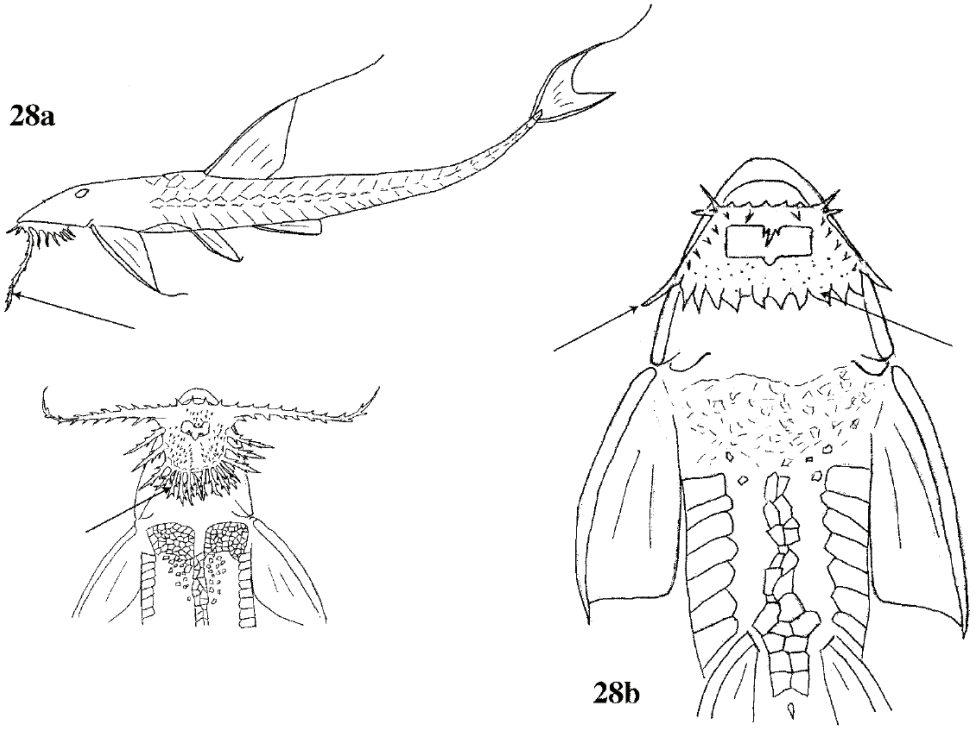
27b. – Premaxillary teeth [ab] absent; head rounded; eyes small; maxillary barbels [ac] conspicuous, reaching beyond pectoral-fin [s] origin: .....*Planiloricaria*



27a **27b**

28a. – maxillary barbels [aa] conspicuous, reaching beyond pectoral-fin [s] origin; lips stongly filamentous [x] **29**

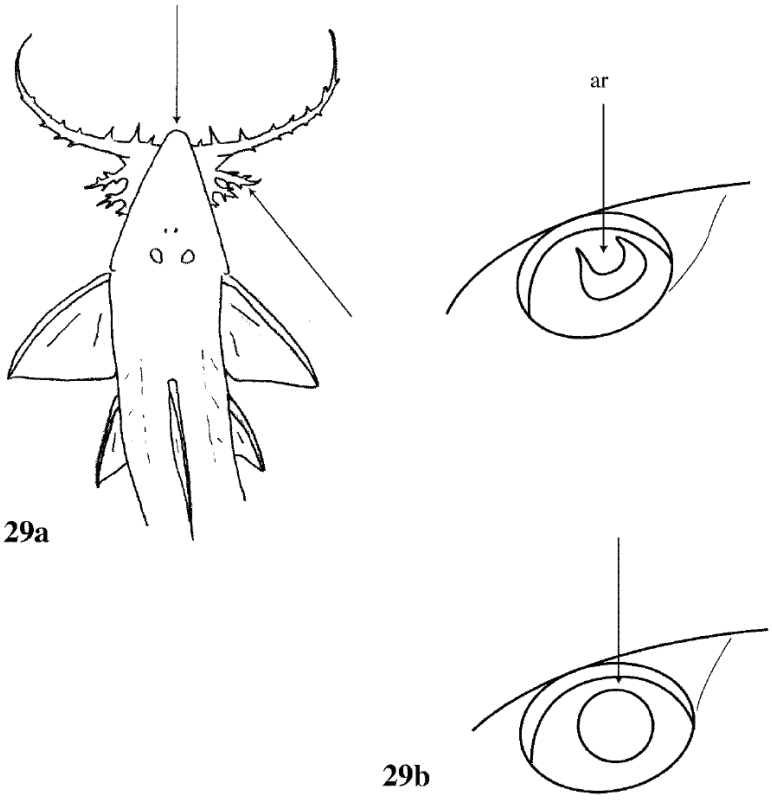
28b. – maxillary barbels [aa] reaching gill opening [ao]; lips fairly filamentous [x] .....*Crossoloricaria*



28a **28b**

29a. – Rostrum [o] pointed; 12 fringed barbels [ac]; iris operculum [ar] generally present: .....*Rhadinoloricaria*

29b. – Rostrum [o] generally rounded; 14 fringed barbels [ac]; iris operculum [ar] absent or vestigial: .....*Apistoloricaria*



Synopsis of the genera

***Apistoloricaria* Isbrücker & Nijssen, 1986.** Type species: *Apistoloricaria condei* Isbrücker & Nijssen, 1986. Holotype: FMNH 94683, Ecuador, Rio Napo drainage, mouth of Rio Tiputini. Gender: feminine. Representatives of this genus are distributed in the upper Amazon and Orinoco drainages, along the Atlantic slope of the Andes. They inhabit sand substrates and are morphologically adapted to this habitat. As with other representatives of the *Pseudohemiodon* group, their body is strongly depressed and the pelvic fins are used for locomotion, enabling these fish to appear to "walk" on the substrate. Sexual dimorphism is apparent through differentiated lip structure. The lip surfaces of the male are rather papillose while those of the female are filamentous (Nijssen & Isbrücker 1988). These taxa are abdomino-lip brooders. Eggs are laid in a single layered mass, and are attached to the surface of the lower lip and abdomen of the male. Isbrücker & Nijssen (1986a) described the biotope of *A. condei* according to D. J. Stewart's field notes. This species was collected in turbid and dark waters, in moderately fast flowing streams, between 2 to 10 meters deep. No submerged vegetation was noted, and the bottom was made of sand, mud, dead leaves, twigs, branches, and trunks. *Apistoloricaria*

is not well diagnosed and upon further examination, may prove to be a synonym of *Rhadinoloricaria*. These two genera are distinguished primarily by the presence or absence of the iris operculum (absent or vestigial in *Apistoloricaria* versus present in *Rhadinoloricaria*), a more conspicuous rostrum in *Rhadinoloricaria*, and by the number of fringed barbels (14 in *Apistoloricaria* versus 12 in *Rhadinoloricaria*). Four valid species are recognized (Ferraris 2003) and a key to the species is available in Nijssen & Isbrücker (1988).

***Aposturisoma* Isbrücker, Britski, Nijssen & Ortega, 1983.** Type species: *Aposturisoma myriodon* Isbrücker, Britski, Nijssen & Ortega, 1983. Holotype: MZUSP 15328, Peru, Ucayali Department, Coronel Portillo Province, Rio Aguaytia drainage, Rio Huacamayo at Tingo Maria. Gender: neuter. This monotypic genus is only known from its type locality and few data are presently available. This is a rheophilic species that has been collected with representatives of the genus *Chaetostoma* (Isbrücker *et al*. 1983). Evers & Seidel (2005) described the habitat of *Aposturisoma myriodon* as clear, with swift current and shallow waters. They noticed that *Aposturisoma* frequentes areas with rubble substrates in this biotope. Its morphology is similar to that of *Farlowella*, to which it is closely related because of the numerous predorsal plates and the origin of the dorsal fin in front of the origin of the anal fin. Nevertheless it has a larger mouth, a much deeper and wider body and a much thicker caudal peduncle which can be interpreted as an adaptation to stream habitat, as this kind of adaptations is also found in rheophilic fishes of the genus *Chaetostoma*, rather than an intermediary between *Farlowella* and *Sturisoma* as supposed by Isbrücker *et al*. (1983), these two genera not being particularly rheophilic.

***Brochiloricaria* Isbrücker & Nijssen, 1979.** Type species: *Brochiloricaria chauliodon* Isbrücker, 1979. Holotype: ZSM 23342, Argentina, Entre Rios Province, Isla El Dorado, Paraná, Guaza (locality not stated). Gender: feminine. The species of this genus are distributed in the Paraná system, including the Paraguay River. *Brochiloricaria* is an abdomino-lip brooder. The species reproduce like those of *Loricaria* (Evers & Seidel 2005). Kavalco *et al.* (2005) made a cytogenetic analysis of four species of Loricariidae and provided a synthesis of the karyotypic diversity of the family. These authors hypothesized that the diploid number of 2n = 54 chromosomes was a plesiomorphic character within Loricariidae. This number was found in all characterized species and populations of Neoplecostominae and Delturinae, and in twelve of the fourteen characterized species of Hypoptopomatinae. For this reason, they considered these three subfamilies basal. Considering Loricariinae, they argued that according to the sampling and to the great observed diversity in diploid numbers (from 2n = 36 to 2n = 74), evolutionary trends were difficult to establish. Nevertheless, they underlined that a dozen of species possessed 2n = 54 ± 2 chromosomes as found in Hypoptopomatinae, Neoplecostominae and Delturinae. Kavalco *et al.* (2005) gave karyotypic characteristics of *B. macrodon*, with 2n = 58 chromosomes. *Brochiloricaria* is morphologicaly very similar to *Loricaria* and can be distinguished from the latter only by its teeth characteristics (teeth very long of equal size on both jaws *versus* premaxillary teeth almost two times longer than dentary ones). On the basis of molecular data and tooth shape of *Hypostomus fonchii* Montoya-Burgos *et al.* (2002) and Weber & Montoya-Burgos (2002) demonstrated that dentition is not always a reliable character to define a genus. The genus *Cochliodon*, placed in Hypostominae, was erected on the basis of its spoon-shaped dentition; it is now considered as a junior synonym of *Hypostomus*. Consequently, *Brochiloricaria* may be a synonym of *Loricaria*, but complementary studies based on molecular data need to be conducted. Two valid species are assigned to the genus (Ferraris 2003).

***Crossoloricaria* Isbrücker, 1979.** Type species: *Loricaria variegata* Steindachner, 1879. Holotype: NMW 45138, Panama, Rio Mamoni drainage at Chepo. Gender: feminine. The species of this genus are distributed in the northwestern part of the South-American continent along the Pacific slope (in Panama and Columbia), Lake Maracaibo region (Venezuela), and the upper Amazon system (Peru). Like other representatives of the *Pseudohemiodon* group, *Crossoloricaria* species occur over sandy substrates of larger rivers and their tributaries. Sexual dimorphism is unknown but males are abdomino-lip brooders. *Crossoloricaria* is poorly diagnosed and is in need of revision. Its only diagnostic character (incomplete abdominal cover consisting of a double median row of plates) is shared by two other representatives of the *Pseudohemiodon* group,

Apistoloricaria and *Rhadinoloricaria*. Rapp Py-Daniel (1997) suggested the synonymy of *Apistoloricaria* and *Crossololoricaria* with *Rhadinoloricaria*, but the structure of the lips and the length of the maxillary barbels tend to place *Crossoloricaria* closer to *Pseudohemiodon*. Moreover, *Crossoloricaria rhami*, described by Isbrücker & Nijssen (1983), like *Pseudohemiodon* possesses complete abdominal plate development, thus rendering the diagnostic feature of *Crossoloricaria* invalid. Five valid species are currently recognized in this genus (Ferraris 2003).

***Dasyloricaria* Isbrücker & Nijssen, 1979.** Type species: *Loricaria filamentosa* Steindachner, 1878. Holotype: NMW 44874, Colombia, Rio Magdalena drainage. Gender: feminine. Distribution includes the northwestern South America on the Pacific slope of Colombia and Panama. *Dasyloricaria* is poorly known and no ecological data are available. Sexual dimorphism is similar to that of *Rineloricaria*, including hypertrophied odontodes forming brushes on the lateral surfaces of the head in mature males. Steindachner (1878) reported hypertrophied development of the lower lip, a characteristic of representatives of the *Loricariichthys* group, suggesting that *Dasyloricaria* is also a lip brooder. This genus is unusual in several respects. Its distribution is restricted to the Pacific slope of the Andes, unique pattern of distribution within the subfamily, distinguishing it from the rest of the Loricariinae, although it shares morphological characteristics with representatives of other groups mainly distributed on the Atlantic slope. Along with other members of the *Rineloricaria* group, it shares papillose lips and hypertrophied odontodes along the sides of the head in breeding males. With some representatives of the *Loricariichthys* group, it shares deep postorbital notches, an abdominal cover strongly structured, and a similar mouth shape, including the hypertrophied lower lip of breeding males. Finally, with some representatives of the *Loricaria* group, it shares a triangular head, strong predorsal keels, and the upper caudal fin ray produced into a long whip. *Dasyloricaria* could represent a link between all other morphological groups. Based on sexually dimorphic characters and lip structure it is placed within the *Rineloricaria* group following Isbrücker (1979). Currently, five valid species are recognized (Ferraris 2003).

***Dentectus* Martín Salazar, Isbrücker & Nijssen, 1982.** Type species: *Dentectus barbarmatus* Martín Salazar, Isbrücker & Nijssen, 1982. Holotype: MBUCV V–12780, Venezuela, State of Cojedes, Rio Salinas drainage, tributary of the Rio Pao Viejo, northeast of El Baul. Gender: masculine. This monotypic genus occurs in the upper Orinoco drainage. Ecological and behavioral data are unavailable. Although it has been placed within the *Pseudohemiodon* group based on its strongly depressed body, its filamentous lips with long fringed barbels, and its spoon shaped and size reduced dentition, *Dentectus* also shows unique derived features such as the presence of plates along the outer margin of its maxillary barbels, and a unique mouth structure that distinguish it from all other genera.

***Farlowella* Eigenmann & Eigenmann, 1889.** Type species: *Acestra acus* Kner, 1853. Holotype: NMW 47795, Venezuela, Caracas. Gender: feminine. The genus *Farlowella* is broadly distributed in Amazon, Orinoco, Paraná, and coastal rivers of the Guyana Shield. Curiously, it seems to be absent from the Pacific slope of the Andes and from the coastal rivers of the Brazilian shield. *Farlowella* has a very unique body shape that resembles of a thin stick of wood. The body is slender and elongate, often with a pronounced rostrum and a brownish color with two lateral dark stripes beginning at the tip of the rostrum, passing over the eyes and ending at the tail, which are periodically interrupted on the caudal peduncle. Species inhabit areas of gently flowing water in submerged dead leaves and sticks, among which it blends remarkably (pers. obs.). Some specimens can sometimes be found in swift current over rocks and submerged wood. These species appear scarce (Le Bail *et al.* 2000, Evers & Seidel 2005) but their mimicry can explain in part this apparent scarcity (Le Bail *et al.* 2000). Sexual dimorphism includes hypertrophied odontodes along the sides of the rostrum or the head in species with a short rostrum. These species are open water brooders (pers. obs.). The eggs are laid on open vertical surfaces such as submerged vegetation or rocks, in a single layer and are guarded by the male. Morphological (Rapp Py-Daniel 1997) and molecular (Montoya-Burgos *et al.* 1998) phylogenetic studies have placed *Farlowella* as sister to *Sturisoma*. This relationship is supported by their sexual dimor-

phism and reproduction strategy, which are identical in all respects. For these reasons, the tribe Farlowellini described by Isbrücker (1979) herein is considered a synonym of Harttiini. The character used to define Farlowellini (i. e. the relative position of the dorsal and anal fins), is subjective and could be interpreted as a generic feature following the example of European cyprinids like *Scardinius erythrophthalmus* and *Rutilus rutilus* which are also distinguished by this criterion. A revision and a key to twenty five valid species of *Farlowella* were made available by Retzer & Page (1997), and an additional species from the Rio Beni drainage in Bolivia has been described recently by Retzer (2006).

***Furcodontichthys* Rapp Py-Daniel, 1981.** Type species: *Furcodontichthys novaesi* Rapp Py-Daniel, 1981. Holotype: INPA T.79–014, Brazil, State of Amazonas, Rio Solimões, Tefé Lake at Caititu. Gender: masculine. This monotypic genus is known from the middle Amazon at Lake Tefé and from the upper Jurua River drainage in the Solimões River basin. *Furcodontichthys* inhabits sandy substrates. Evers & Seidel (2005) captured *Furcodontichthys novaesi* at night, on a sand bank, in black waters of the Rio Tefé. As with representatives of the *Loricariichthys* group, males of *Furcodontichthys* show a hypertrophied development of the lips suggesting that this genus is a lip brooder. The presence of conspicuous fringed barbels at the lip corners is unique among the Loricariinae. These barbels have branching patterns comparable to those of the *Pseudohemiodon* group.

***Harttia* Steindachner, 1877.** Type species: *Harttia loricariformis* Steindachner, 1877. Lectotype: NMW 46346, Brazil, Rio Paraiba do Sul. Gender: feminine. Distribution primarily includes rivers draining the Guyana Shield, coastal rivers in northeastern Brazil, and the Amazon basin. These rheophilic fishes are found in the upper courses of rivers over rocky and sandy bottoms. Casatti & Castro (2006) characterized ecomorphological trends in fishes living in riffles of the Rio São Francisco. They split the fish communities into three groups comprising nektonic, nektobenthic, and benthic species. Among the latter, *Harttia sp.* is supposed to be able to exploit areas with the strongest current, because of its extremely depressed body and long caudal peduncle, comparing to other species. This fact was also empirically noted by Le Bail *et al.* (2000). Sexual dimorphism includes hypertrophied odontodes on the pectoral spines and along the margins of the snout in mature males. Representatives of this genus seem to be open brooders (Dotzer & Weidner 2003). Recent evidence has suggested that Harttiini could represent a paraphyletic assemblage. Using molecular data, Montoya-Burgos *et al.* (1998) demonstrated *Harttia* to be sister to two sister clades, one consisting of *Farlowella* and *Sturisoma*, two representatives of the Harttiini, and the second including the representatives of the Loricariini. Moreover, *Harttia* is in need of revision. For example, the synonymy of *Cteniloricaria* with *Harttia* (Rapp Py-Daniel & Oliveira 2001) is questionable (Covain *et al*. 2006) because it rests solely on the characteristics of *Harttia fowleri* (Pellegrin, 1908) without considering the type species of *Cteniloricaria*. Likewise, the genus *Quiritixys* Isbrücker, 2001, also placed in synonymy by Ferraris (2003) is possibly valid. The description of *Quiritixys* is based on the unusual sexual dimorphism of *Harttia leiopleura* Oyakawa, 1993. This feature alone is insufficient to define a genus because it only concerns mature males and is most of the time seasonal in Loricariinae. This means that the majority of individuals of the species (juveniles, females, and non breeding males) cannot be diagnosed by this single criterion because they do not possess this feature. Nevertheless, the addition of some other features by Oyakawa (1993), such as the absence of the subpreopercle, supports the validity of *Quiritixys*. Other species such as *H. novalimensis* could also belong to *Quiritixys* because this species also lacks the subpreopercle, but its sexual dimorphism is undescribed. Nevertheless, Langeani *et al.* (2001) noted well developed odontodes on the posterior body of this species. *Harttia* also exhibits considerable karyotypic diversity with chromosome numbers between 2n = 52 and 2n = 58 in the four species characterized (Kavalco *et al.* 2005, Centofante *et al.* 2006). Kavalco *et al.* (2005) also reported differences in karyotypic formula, symmetry, nucleolar organizing regions (NOR), and diploid number (2n = 52 *versus* 2n = 56) between two different populations (respectively Grande Stream and Paraitinga River) of *H. loricariformis* from the Paraíba do Sul basin. These authors hypothesized the sedentary habits of some species to explain these differences. Centofante *et al.* (2006) characterized a heteromorphic XX/XY_1Y_2 sex chromo-

some system in *H. carvalhoi*. Currently, *Harttia* comprises 22 species (Provenzano *et al*. 2005). Partial keys are available for species occurring in Atlantic coastal drainages (Oyakawa 1993, Langeani *et al.* 2001), Amazon and Guianas drainages (Rapp Py-Daniel & Oliveira 2001), and Guianas drainages (Covain *et al.* 2006).

***Harttiella* Boeseman, 1971.** Type species: *Harttia crassicauda* Boeseman, 1953. Holotype: RMNH 19418, Surinam, in a creek of the Nassau Mountains, Marowijne River drainage. Gender: feminine. This monotypic genus is only known from its type locality. The single species was collected in a little forest creek over sandy and rocky bottoms (Boeseman 1971). Sexual dimorphism is similar to that of *Harttia*, in which mature males develop hypertrophied odontodes on the pectoral spines, along the margin of the snout, and on the entire body except for the abdominal region. The phylogenetic position of *Harttiella* remains uncertain. Boeseman (1971), and Isbrücker (1981a) gave it a rather basal position within Loricariinae but its geographic isolation in the Marowijne drainage could also correspond to a more derived state within Harttiini. The morphology of *Harttiella* suggests it is a dwarf form closely related to *Harttia*. Rapp Py-Daniel (1997) tentatively placed *Harttiella* within Harttiina because of its close resemblance with *Harttia*. Unfortunately, this question may remain unresolved as Mol & Ouboter (2004) mentioned the possible disappearance of this species because of mining activities in the Nassau Mountains. This species is also interesting because of its morphological convergence with *Ixinandria*, a genus within Loricariini found in the southwestern part of the continent. The morphological likeness between *Ixinandria* and *Harttiella* may have misled Boeseman (1971) when he moved *Canthopomus montebelloi* Fowler, 1940 into *Harttiella*.

***Hemiodontichthys* Bleeker, 1862.** Type species: *Hemiodon acipenserinus* Kner, 1853. Lectotype: NMW 46139, Brazil, State of Mato Grosso, Rio Guaporé drainage. Gender: masculine. This monotypic genus is widely distributed in the Amazon basin and the Essequibo, Oyapock, and Paraguay River drainages. *Hemiodontichthys* is a sand dweller that lives partially buried in the substrate, its cryptic coloration providing efficient protection. As with other representatives of the *Loricariichthys* group, mature males develop hypertrophied lips for brooding eggs. Eggs are laid in a mass and held by the male in the fold made by its lips (pers. obs.). This taxon is often compared with morphologically similar *Reganella depressa* (Kner, 1853). Isbrücker & Nijssen (1974b) and Isbrücker (1979, 1981a) characterized these two genera without discussing any relationship between them or to other taxa. Isbrücker (1979) created the subtribes Reganellina and Hemiodontichthyina to accomodate these two genera. However a comparison is made in Isbrücker (1979) between Hemiodontichthyina and Loricariichthyina with reference to abdominal cover and sexual dimorphism, comparable in both subtribes. Rapp Py-Daniel (1997) considers *Hemiodontichthys* to be the sister genus of *Reganella* on the basis of osteological data. However, the similar external morphology of these two taxa could be interpreted as an evolutionary convergence, as they occupy the same ecological niche. Considering morphological data given in the key, particularly the mouth structure and abdominal cover, *Hemiodontichthys* is assigned herein to the *Loricariichthys* group. Molecular data (Montoya-Burgos *et al*. 1998) tend to support this relationship by placing *Hemiodontichthys* as sister to *Loricariichthys*. We assign *Reganella* to the *Pseudohemiodon* group on the basis of its mouth shape, the presence of vestigial fringed barbels, its strongly depressed body, and the characteristics of its abdominal cover made of little plates without particular organization and extending toward the lower lip margin. This kind of organization in the abdominal plating is never observed within the *Loricariichthys* group. Thus, the appearance of a rostrum and the loss of maxillary teeth could have evolved independently in different lineages subjected to similar environmental constraints. Given its broad geographic range and variation in morphometric features, *Hemiodontichthys acipenserinus* could comprise a species complex. Isbrücker & Nijssen (1974b) reported that populations from the Amazonian region tend to be more slender than those from the Paraguay and Guaporé Rivers.

***Ixinandria* Isbrücker & Nijssen, 1979.** Type species: *Loricaria steinbachi* Regan, 1906. Lectotype: BMNH 1906.5.31: 37, Argentina, Rio Salado drainage at Salta. Gender: feminine. Distribution includes rivers of the Atlantic slope of the Andes in Bolivia and Argentina. According to Gladys Monasterio de Gonzo (pers. comm.), *Ixinandria steinbachi* occurs in Salta province in mountainous areas at high altitudes ranging from

around 1000 to 2900 meters above sea level. This rheophilic species lives in fast flowing and very oxygenated waters. Its color pattern reflects mimicry with stoned bottoms. Fertilized eggs have been found on the hidden surface of a stone, suggesting that *I. steinbachi* could be a cavity spawner. Sexual dimorphism includes hypertrophied odontodes around the head margin and on the pectoral spines of mature males. The phylogenetic position of *Ixinandria* within Loricariini remains uncertain. Reis & Cardoso (2001) suggested that *Ixinandria* could be synonym of *Rineloricaria*. This hypothesis seems plausible considering the weakness of the diagnostic feature given for *Ixinandria*, its naked belly, and several species of *Rineloricaria* from South-East Brasil or Argentina with the belly partly covered such as *R. maquinensis*, *R. aequalicuspis* or *R. misionera*. Nevertheless, the typology of *Rineloricaria lima* needs clarification prior to further investigations on the taxonomy of *Ixinandria*. Two valid species are currently recognized (Ferraris 2003).

***Lamontichthys* Miranda Ribeiro, 1939.** Type species: *Harttia filamentosa* La Monte, 1935. Holotype: AMNH 12616, Brazil, Rio Jurua drainage, Rio Embira. Gender: masculine. *Lamontichthys* is distributed in the northwestern part of South America in the upper Amazon and Orinoco River drainages, and in the Lake Maracaibo region. Species in this genus occupy the same ecological niche as those of *Harttia*. They mainly live in the mainstream of rivers, on rocky and sandy bottoms (Taphorn & Lilyestrom 1984). Sexual dimorphism includes hypertrophied odontodes on the pectoral spines in mature males. *Lamontichthys* is an open brooder. Eggs are laid on an open surface such as rocks, submerged wood or plants, and are generally exposed to the current. Females lay a few large-sized (1.4 to 1.8 mm in diameter) yellowish eggs during each spawning event (Taphorn & Lilyestrom 1984). These authors expressed doubts concerning the value of the character given by Isbrücker & Nijssen (1978) to diagnose *Lamontichthys* (i. e. the number of pectoral fin rays i–7 versus i–6 for all other Loricariinae). However, this feature is present in all species assigned to this genus, suggesting a common origin. Isbrücker & Nijssen (1976a) demonstrated that fin ray numbers, with few exceptions, were particularly conservative among members of Loricariinae. The phylogenetic position of *Lamontichthys* remains uncertain. Rapp Py-Daniel (1997) considers it to be sister to *Harttia*, whereas *Lamontichthys* shows much more similarities with *Pterosturisoma*, a monotypic genus which only differs from *Lamontichthys* by the number of pectoral fin rays. Nevertheless, Rapp Py-Daniel (1997) tentavely placed *Pterosturisoma* within Harttiina because of its similarity with *Lamontichthys*, but did not provide any hypotheses on the phylogenetic relationships between these genera. *Lamontichthys* includes four valid species, and a key to their identification is available in Taphorn & Lilyestrom (1984).

***Limatulichthys* Isbrücker & Nijssen, 1979.** Type species: *Loricaria punctata* Regan, 1904. Lectotype: BMNH 1893.4.24: 18, Brazil, Rio Negro at Manaos. Gender: masculine. This monotypic genus is widely distributed in the Amazon basin and in the Essequibo River drainage. *Limatulichthys* is a sand dweller. As suggested by the hypertrophied development of lower lip in males, *Limatulichthys* may be a lip brooder like many representatives of the *Loricariichthys* group. Isbrücker & Nijssen (1976b) revised *Pseudoloricaria* Bleeker, 1862, in which they assigned two species: *P. laeviuscula* (Valenciennes, 1840) and *P. punctata* (Regan, 1904), and designated a lectotype for the latter from BMNH 1893.4.24: 18, described by Regan (1904) as *Loricaria punctata*. Isbrücker & Nijssen (1976b) also put *Loricaria griseus* Eigenmann, 1909, *Rhineloricaria petleyi* Fowler, 1940, and *Loricariichthys parnahybae* Fowler, 1941 into the synonymy of *Pseudoloricaria punctata* (Regan, 1904). Isbrücker & Nijssen (1976b: p. 125) wrote: "Kner (1854b: 281) published a manuscript name "*Loricaria punctata* Natterer" in the synonymy of "*Ancistrus duodecimalis*?" (Valenciennes). Since this name fails to satisfy the provisions in article 11(d) of the International Code of Zoological Nomenclature (1964), therefore *Loricaria* (*Pseudoloricaria*) *punctata* Regan, 1904 is not subject to the Law of Homonymy". According to Ferraris (2003) *Loricaria punctata* Regan, 1904 constitutes a primary homonymy of *Loricaria punctata* Kner (ex Natterer), 1854, a representative of the genus *Glyptoperichthys*. However, it appears that Kner (1854) does not refer to Natterer's manuscript for a description of *Loricaria punctata*, but only to a specimen (number 87) recorded in Natterer's field notes and identified as a representative of the genus *Ancistrus*. Consequently, according to articles 11.5, 11.6, and 12.3 of the International Code of Zoological Nomenclature

(ICZN, 1999), the name *Loricaria punctata* Kner is not made available. Later, Günther (1864) refers to Kner's work using the name *Loricaria punctata* as an available name in a new combination *Pterygoplichthys punctatus*. Acording to ICZN article 11.5.2, referring to an unavailable name does not make it available by its mere citation. Consequently, this species name is hereby new and has to be considered as *Pterygoplichthys punctatus* Günther, 1864. Steindachner (1881) used the name "*Chaetostomus punctatus* sp. Gthr." and redescribed the species without taking into account the name *Loricaria punctata*. Consequently, the new species described as *Loricaria punctata* by Regan (1904) is not a homonym of *Loricaria punctata* Kner (1854). Regan (1904) also confirmed the status of *Pterygoplichthys punctatus* Günther, 1864. Conversely, Isbrücker (1980) considered that Günther (1864) validated the name *Loricaria punctata* Natterer, 1854 by authorship to Natterer. However, the name *Loricaria punctata* Natterer is not available as seen previously. Moreover, Günther (1864) published the description of *Pterygoplichthys punctatus* with *Loricaria punctata* Natterer listed as junior synonym. Considering ICZN article 11.6, the name *Loricaria punctata* Natterer is not available. Isbrücker *et al*. (2001) accepted the primary homonymy between *L. punctata* Natterer and *L. punctata* Regan, considered *Limatulichthys griseus* (Eigenmann, 1909) as a distinct species, and therefore established *Limatulichthys petleyi* (Fowler, 1940) as a substitution name for *Limatulichthys punctatus* (Regan, 1904). Ferraris (2003) considered *L. petleyi* to be a synonym of *L. griseus* and also established the latter as a substitution name for *L. punctata*. The nomenclatural status of *Limatulichthys* remains unclear and this case should be submitted to the International Commission on Zoological Nomenclature in order to rule on the validity of *Loricaria punctata* Regan, 1904.

***Loricaria* Linnaeus, 1758.** Type species: *Loricaria cataphracta* Linnaeus, 1758. Lectotype: NRM 33, In South America, also holotype of *Loricaria dura* Bleeker, 1862, Surinam. Gender: feminine. This genus is distributed east of the Andes on nearly the entire subcontinent. Species occur in a variety of habitats from the main flow of rivers on sandy and rocky bottoms to flooded areas and lakes over muddy and sandy bottoms. Sexual dimorphism includes hypertrophied development of the pectoral spines, blunt odontodes on the pelvic and anal fin spines, and tooth crowns becoming shortened and rounded in mature males (Isbrücker 1981b). Males are abdomino-lip brooders. *Loricaria* is the nominal genus of the family. Phylogenetic relationships within *Loricaria* and among other members of Loricariini remain uncertain. Its external morphology shows few shared derived characters, making comparison with other genera difficult. *Loricaria* has been hypothesized to occupy a basal position among members of the subtribe Loricariina, with the other genera possessing derived characters. Based on the characteristics of its mouth, *Loricaria* appears to maintain a close relationship with representatives of the *Pseudohemiodon* group. This hypothesis has been proposed in the studies of Rapp Py-Daniel (1997) and Montoya-Burgos *et al.* (1998), in which *Loricaria* maintained a sister relationship to the *Pseudohemiodon* group. However, these authors did not resolve relationships among species of the *Loricaria* group. Kavalco *et al.* (2005) reported karyotypic diversity ranging from 2n = 62 to 2n = 68 for the four species characterized. Under the impression that the original syntypes were lost, Isbrücker (1972) established a neotype for *L. cataphracta* (ZMA 109.616). Between 1972 and 1981, Isbrücker learned that the two syntypes did in fact exist and are currently housed at the Swedish Museum of Natural History in Stockholm. Based on a photograph of two syntypes, Isbrücker (1981b) designated one as the lectotype (NRM 33). The rediscovery of these syntypes invalidated the previous neotype according to the ICZN (1999), article 75.8. The disposition of the lectotype according to Eschmeyer (1998), which is based on Wheeler (1989), is Zoological Museum, University of Copenhagen (ZMUC 27). However, Wheeler (1989: pp. 214–215) confirmed Isbrücker's designation of the lectotype: "Two loricariid specimens from the collection of King Adolf Fredrik, one of which has been designated as the lectotype of *L. cataphracta* by Isbrücker (1981), are preserved in the Swedish Museum of Natural History, Stockholm. This specimen was certainly the specimen which Linnaeus used for the major source material of his description (Fernholm & Wheeler, 1983)". Moreover, concerning specimen ZMUC 27 deposited in Copenhagen, Wheeler (1989: p. 215) concluded: "It thus seems certain that this specimen is not one of those referred to by Linnaeus (1758) in his diagnosis of the species, or its variety

beta". *Loricaria* was revised by Isbrücker (1981b) who recognized eleven valid species. For lack of clear diagnostic features, *Proloricaria* Isbrücker, 2001 is herein considered a junior synonym of *Loricaria*. A twelfth species has been described from North-East of Argentina by Rodríguez & Miquelarena (2003).

***Loricariichthys* Bleeker, 1862.** Type species: *Loricaria maculata* Bloch, 1794. Lectotype: ZMB 3163, type locality restricted to Surinam (Isbrücker 1971). Gender: masculine. *Loricariichthys* is widely distributed in the Amazon basin, the Paraná system, and coastal rivers of the Guyana and Brazilian Shields. These species occur in a large diversity of habitat over sandy and muddy bottoms. Like other members of the *Loricariichthys* group, *Loricariichthys* species are lip brooders. Sexual dimorphism includes hypertrophied development of the lips, which are used by the male to incubate the eggs. During the spawning period, the first function of this organ (mainly adherence to the substrate) is reoriented toward a new reproductive function. Although this genus is well diagnosed, the species are very similar and difficult to identify. Moreover, missing holotypes and type localities has led to uncertain taxonomic status for several species which would benefit from a generic revision. *Loricariichthys* seems to be intermediate between *Limatulichthys* and *Pseudoloricaria* on one hand, and *Furcodontichthys* and *Hemiodontichthys* on the other. Concerning the karyotypic characteristics of *Loricariichthys*, Kavalco *et al.* (2005) reported a diploid number of 2n = 56 for the two species characterized. A ZZ/ZW sex chromosome system was also reported for *L. platymetopon* by Kavalco *et al.* (2005) and Centofante *et al.* (2006). Seventeen valid species are assigned to this genus (Ferraris 2003). A key to the southern species is available in Reis & Pereira (2000).

***Metaloricaria* Isbrücker, 1975.** Type species: *Metaloricaria paucidens* Isbrücker, 1975. Holotype: IRSNB 549, French Guiana, Maroni River drainage, Ouaqui River upstream of Bali falls. Gender: feminine. *Metaloricaria* is only known from the Guyana Shield in French Guiana and Surinam where the species occupy an ecological niche similar to that of *Harttia*. The species are rarely found in their natural environment and inhabit primarily streams over rocky and sandy substrates (Le Bail *et al.* 2000). Sexual dimorphism includes hypertrophied development of odontodes arranged in brushes along the sides of the head and on the spine and rays of the pectoral fins in mature males. Females also possess such brushes along sides of the head, but do not seem to develop pectoral-fin enlarged odontodes (pers. obs. on an adult specimen collected in Crique Voltaire, Maroni drainage, that spontaneously laid large yellowish eggs after catching). *Metaloricaria* is a curious genus because of its geographic isolation and unique combination of morphological characteristics. The length of the maxillary barbels (longer than in all other Harttiini), low number of teeth and their reduced size, reduction of the number of caudal-fin rays (i–11–i), and sexual dimorphism reminiscent of that seen in the *Rineloricaria* group, tend to support a closer relationship of *Metaloricaria* with the Loricariini. This agrees with Rapp Py-Daniel (1997), who also suggested placement in Loricariini, but outside of any clade, because of an apparent lack of affinities to other Loricariini. The phylogenetic position of *Metaloricaria* remains uncertain. It was revised by Isbrücker & Nijssen (1982), in which a key to the two species is available.

***Paraloricaria* Isbrücker, 1979.** Type species: *Loricaria vetula* Valenciennes, 1836. Holotype: MNHN A.8996, no type locality, described by Valenciennes in Cuvier & Valenciennes (1840: 466) from the surroundings of Buenos-Aires. Gender: feminine. This genus is distributed in the southern part of the continent in the Paraguay, Uruguay, and La Plata River basins. Males *Paraloricaria* are abdomino-lip brooders (Reis & Pereira 2000). It is assigned to the *Loricaria* group but it shows characteristics that possibly represent close phylogenetic relationship with representatives of the *Pseudohemiodon* group (a group established on the basis of a filamentous structure of the lips as also found in the *Loricaria* group). In particular, it shows a strongly flattened body, weak postorbital notches, long and ramified maxillary barbels, and overall, conspicuous fringed barbels. Three valid species are assigned to this genus (Ferraris 2003).

***Planiloricaria* Isbrücker, 1971.** Type species: *Pseudohemiodon (Planiloricaria) cryptodon* Isbrücker, 1971. Holotype: ZFMK 1865, Peru, Rio Ucayali drainage near Pucallpa. Gender: feminine. This monotypic genus is distributed in the upper Amazon basin, including the Ucayali, Purus, and Mamoré River drainages.

Planiloriaria inhabits sandy substrates in the main streams of large rivers (Evers & Seidel 2005). Reproductive ecology is unknown but could be reminiscent of those of other representatives of the *Pseudohemiodon* group. Evers & Seidel (2005) characterized sexual dimorphism by the shape of the genital area. The genital area in males is elongate and narrow compared with the large and roundish area of females. *Planiloricaria* shows derived features such as a reduction in size and number of teeth, premaxillary teeth absent, a circular head shape, and eyes reduced in size without iris operculum.

***Pseudohemiodon* Bleeker, 1862.** Type species: *Hemiodon platycephalus* Kner, 1853. Holotype: lost (Isbrücker 1971), Brazil, Rio Cuiaba. Gender: masculine. *Pseudohemiodon* is distributed in the Amazon and Paraná River basins. This genus is also known from the Orinoco system. Like other members of the *Pseudohemiodon* group, *Pseudohemiodon* occurs primarily over sandy substrates. This ecological specialization is reflected by the dramatic dorsoventral compression of the body and pelvic fins that are used mainly for locomotion on sand. Sexual dimorphism is unknown, but like the other genera in this group, *Pseudohemiodon* species are abdomino-lip brooders (pers. obs.). The very large eggs are incubated by the male. Considering their ecological and morphological specialization, representatives of the *Pseudohemiodon* group may represent highly derived Loricariinae. This is in agreement with Rapp Py-Daniel (1997), who found Planiloricariina nested within Loricariini, as sister group of *Loricaria*. Seven species are currently recognized (Ferraris 2003). A partial key to the species is available in Isbrücker (1975).

***Pseudoloricaria* Bleeker, 1862.** Type species: *Loricaria laeviuscula* Valenciennes, 1840. Holotype: MNHN B 365, no type locality. Gender: feminine. This monotypic genus is distributed in the middle and lower Amazon basin. *Pseudoloricaria laeviuscula* was collected over sandy bottoms, in clear waters, in the main flow of rivers, and in neighboring temporary ponds (pers. obs.). Sexual dimorphism includes hypertrophied development of the lower lip suggesting that *Pseudoloricaria* is a lip brooder. The phylogenetic position of *Pseudoloricaria* is uncertain. This genus closely resembles *Limatulichthys* in external morphology. Isbrücker & Nijssen (in Isbrücker 1979) considered *Limatulichthys* to be the most primitive representative of the *Loricariichthys* group. Consequently, *Pseudoloricaria* could occupy a basal position among its group, as the sister genus of *Limatulichthys*. Rapp Py-Daniel (1997) found *Pseudoloricaria* and *Limatulichthys* as sister groups to the Hemiodontichthyina but didn't resolve the relationships between these two genera. A key to distinguish *Pseudoloricaria* (*P. laeviscula*) from *Limatulichthys* (*Pseudoloricaria punctata* in Isbrücker & Nijssen 1976b) is available in Isbrücker & Nijssen (1976b).

***Pterosturisoma* Isbrücker & Nijssen, 1978.** Type species: *Harttia microps* Eigenmann & Allen, 1942. Lectotype: CAS 28543, Peru, Iquitos. Gender: neutral. This monotypic genus is known only from the upper Amazon River basin. *Pterosturisoma* is a rheophilic species. Evers & Seidel (2005) described this species as an open brooder. Males do not possess hypertrophied odontodes neither on the sides of head, nor on pectoral-fin spines. Nevertheless, Evers & Seidel (2005) distinguished both sexes by the width of a naked trapezoidal area framed by four bony plates in the genital region. This area appeared broader in females, and longer and narrower in males. *Pterosturisoma* appears morphologically very similar to *Lamontichthys* from which it differs primarily in the number of pectoral fin rays (i–6 in the former *versus* i–7 in the latter). These two genera share features with *Sturisoma* such as similar body depth at dorsal-fin origin, presence of filamentous extensions on caudal-fin spines, and complete abdominal plate cover extending to the lower lip margin. These features are never observed in *Harttia,* suggesting these two genera share a closer relationship with *Sturisoma* than with *Harttia* as hypothesized by Rapp Py-Daniel (1997).

***Pyxiloricaria* Isbrücker & Nijssen, 1984.** Type species: *Pyxiloricaria menezesi* Isbrücker & Nijssen, 1984. Holotype: MZUSP 26800, Brazil, Mato Grosso do Sul, Miranda. Gender: feminine. This monotypic genus is only known from the Paraguay River drainage. *Pyxiloricaria menezesi* inhabits sandy substrates and is sympatric with representatives of the genus *Pseudohemiodon*. Sexual dimorphism and reproductive ecology are unknown. In the *Pseudohemiodon* group, *Pyxiloricaria* more closely resembles *Pseudohemiodon* than other representatives of the group. Moreover, with *Loricaria*, it shares filamentous lips, inconspicuous fringed

barbels on the lower lip, and shorter maxillary barbels. The phylogenetic position of *Pyxiloricaria* remains uncertain, and its presence in the *Pseudohemiodon* group could be artificial, given the characters shared with *Loricaria*. Herein, following Isbrücker & Nijssen (1984), it is maintained in the *Pseudohemiodon* group.

***Reganella* Eigenmann, 1905.** Type species: *Hemiodon depressus* Kner, 1853. Lectotype: NMW 9438, Brazil, Rio Negro, Marabitanas. Gender: feminine. This monotypic genus is distributed in the middle Amazon River basin. Ecological and behavioral data are unavailable. The dorsoventrally flattened body suggests that *Reganella* inhabits flowing waters over sandy substrates. *Reganella depressa* is a poorly known species and uncommon in collections. Its phylogenetic position remains uncertain and relationship to other representatives of the *Pseudohemiodon* group is unclear.

***Rhadinoloricaria* Isbrücker & Nijssen, 1974.** Type species: *Loricaria macromystax* Günther, 1869. Holotype: BMNH 1869.5.21.8, Peru, Amazon River. Gender: feminine. This monotypic genus is distributed in the upper Amazon and Orinoco River basins, Essequibo, and Tocantins drainages. Although poorly known and uncommon in collections, *Rhadinoloricaria* is known to occur over sandy substrates. Ecological and behavioral data are unavailable. Most studies of *Rhadinoloricaria* are based on the holotype, which is in a poor state of preservation (particularly its mouth, which is one of the most important features for the study of this group). The holotype is an adult with complete abdominal plate cover; smaller specimens have incomplete abdominal plate cover forming a double median row of platelets. This feature, which is also observed in *Apistoloricaria*, *Crossoloricaria*, and one species of *Dasyloricaria*, could reflect a common developmental pattern of abdominal plates among these taxa. Consequently, its use as a diagnostic feature should be rejected (see *Crossoloricaria* above).

***Ricola* Isbrücker & Nijssen, 1974.** Type species: *Loricaria macrops* Regan, 1904. Lectotype: BMNH 1868.9.16.1, Argentina, Rio de la Plata. Gender: feminine. This monotypic genus is known from the lower Paraná River basin. Ecological and behavioral data are unavailable. This genus is interesting in many respects. It shares features with representatives of different groups within Loricariini. For example, it possesses conspicuous fringed barbels on the lower lip, a feature shared by the representatives of the *Pseudohemiodon* group. It also bears numerous papillae on the inner surfaces of the lips and numerous straight bicuspid teeth (approx. 15 per premaxillae) that are characteristic of the *Rineloricaria* group.

***Rineloricaria* Bleeker, 1862.** Type species: *Loricaria lima* Kner, 1853. Holotype: NMW probably lost (Isbrücker, 1979), from Brazil according to Natterer. Gender: feminine. This genus, by far the most speciose in Loricariinae, is widely distributed on nearly the entire subcontinent, from Costa Rica to Argentina, on both slopes of the Andes. The species inhabit an extremely diverse array of environments. Sexual dimorphism includes hypertrophied development of the odontodes along the sides of the head, on the pectoral spines and rays, and predorsal area of mature males. Several species also show hypertrophied development of the odontodes on the entire caudal peduncle. *Rineloricaria* are cavity brooders (pers. obs.). Numerous eggs (often more than 100) are laid attached to one another in single layer masses on the cavity floor, and are brooded by males. *Rineloricaria* exhibit high levels of karyotypic diversity with chromosome numbers ranging from 2n = 36 to 2n = 70 in the five species characterized (Kavalco *et al.* 2005). These authors also described, according to Giuliano-Cataneo (1998), a Robertsonian polymorphism between several populations of *R. latirostris*, with a variation of 2n = 36 to 2n = 48 chromosomes. If the loss of the holotype of *R. lima* is confirmed, a neotype must be designated in order to permit all the necessary clarifications for a detailed and much needed revision of this genus. The characters given by Kner (1853), although very detailed, are valid for almost all congeneric species. Without the type locality, it is presently impossible to decide which species represents *R. lima*. Isbrücker & Nijssen (1976a) and Isbrücker (1981a) proposed the revalidation of *Hemiloricaria* Bleecker, 1862 (type species: *Hemiloricaria caracasensis*), but they finally left it in the synonymy of *Rineloricaria* because of the lack of sufficient features to split these two genera. In 2001, Isbrücker *et al.* revalidated *Hemiloricaria* and created two new genera: *Fonchiiichthys* (type species: *Loricaria uracantha*) and *Leliella* (type species: *Rineloricaria heteroptera*) on the basis of subtle differences in the sexual dimorphism. These

characters are expressed only during the spawning period and are outweighed by other shared characters used to diagnose *Rineloricaria*. Moreover, as specified by Isbrücker & Nijssen (1976a: pp. 110–111) in the description of *R. heteroptera*: "As in *Spatuloricaria* Schultz, 1944 it shows strong secondary sexual dimorphism: males develop 'bristles' along sides of snout, usually also on dorsum of pectoral fin spine and rays, and often dorsum of head, on post-occipital and predorsal scutes. There are specific differences in the development of male bristles". Following this interpretation, the characters given to define *Leliella* and *Fonchiiichthys* can be regarded as species specific characters. Herein, *Hemiloricaria*, *Leliella*, and *Fonchiiichthys* are considered as synonyms of *Rineloricaria* for lack of sufficient diagnostic features. Forty nine valid species are assigned to this genus considering Ferraris (2003), Knaack (2003), and Rodríguez & Miquelarena (2005).

***Spatuloricaria* Schultz, 1944.** Type species: *Spatuloricaria phelpsi* Schultz, 1944. Holotype: USNM 121121, Venezuela, Rio Socuy. Gender: feminine. This genus is distributed in the northwestern part of the subcontinent, in drainages of the Pacific and Atlantic Slopes of the Andes. Several species occur also in the upper Amazon River basin, upper Paraguay, and São Francisco River basins. Ecological data are unavailable and reproductive biology is unknown. Sexual dimorphism includes hypertrophied development of claw-like odontodes along the sides of the head and on the pectoral spines in mature males. *Spatuloricaria* is in need of revision, as species boundaries and distributions are poorly known. The phylogenetic position of *Spatuloricaria* remains uncertain. Rapp Py-Daniel (1997) placed *Spatuloricaria* at the base of a clade including representatives of the *Loricaria* and *Pseudohemiodon* groups. Its dentition, with few teeth on the premaxillae, and its abdominal cover consisting of minute disjointed platelets resembles that of some representatives of the *Loricaria* group. Conversely, the papillose surface of the lips and sexually dimorphic features are more characteristic of the *Rineloricaria* group. Eleven valid species are currently recognized (Ferraris 2003).

***Sturisoma* Swainson, 1838.** Type species: *Loricaria rostrata* Spix & Agassiz, 1829. Brazilian rivers. Holotype: lost (Isbrücker 1979). Gender: neuter. The species of the genus *Sturisoma* are widely distributed on both slopes of the Andes, in Panama and Colombia, and in the Amazon, Orinoco, and Paraná River basins. *Sturisoma* inhabit gently to swiftly flowing white waters (Evers & Seidel 2005) where submerged wood is abundant in the main flow of rivers. Sexual dimorphism includes hypertrophied odontodes on the sides of the head of the male. As representatives of *Farlowella*, *Sturisoma* species are open brooders (pers. obs.). Kavalco *et al.* (2005) reported a diploid number of 2n = 74 chromosomes for the single species characterized: *S.* cf. *nigrirostrum*. A neotype has yet to be designated for *Sturisoma rostratum*, the type species, which was destroyed during World War II (Isbrücker 1979). Neotype designation is needed to fix the type locality, which is unspecified and may pertain to several of the currently recognized species. Ghazzi (2003) revised genus, but it remains unpublished and unavailable for the moment. *Sturisoma* has been shown to be sister to *Farlowella* according to Rapp Py-Daniel (1997) and Montoya-Burgos et al. (1998). Ghazzi (2005) confirms this relationship. Sexual dimorphism and reproductive strategy are comparable in both genera and tend to corroborate the molecular and morphological data. Fifteen valid species are currently recognized (Ferraris 2003, Ghazzi 2005). Ghazzi (2005) described a new species, *Sturisoma kneri*, replacing an unavailable name, *Loricaria kneri*, proposed by Tortonese (1940). She argued (p. 564) that "Tortonese's (1940) intention was solely to publicize the large number of species housed in Museo di Torino; it was not his intention to validate De Filippi's manuscript names." However Tortonese (1940: p. 134–135) explained in his introduction: "*Infine, ho avuto anch'io la ventura di trovare nei nuclei di materiale da me studiato diversi Pesci che ho creduto dover attribuire a specie non ancora note.*" In this citation we can see the intention of Tortonese to publish the names of new species, in part from De Filippi's manuscript. The name *Loricaria kneri* De Filippi in Tortonese, 1940 is here used as the valid name of this species, because the author refers to a holotype and because he gives a short diagnosis according to De Filippi's manuscript. Thus, Tortonese made the name *Loricaria kneri* De Filippi available. Secondly, Tortonese proposes that *L. kneri* could be a representative of the genus *Sturisoma* as he specified in his introduction: "*Ciascuna specie è elencata secondo l'ordine sistematico, col nome dell'A. che per primo la descrisse: ad esso seguono, oltre le indicacazioni bibliografiche, il nome cor-*

etto – se il primo è passato in sinonimia o vi è stato cambiamiento di genere – il numero che il materiale porta nel Catalogo della collezione e i dati relativi alla provenienza, al raccoglitore o donatore e allo stato attuale di conservatione." The statement on the validity of *Loricaria kneri* De Filippi in Tortonese, 1940 should be submitted to the International Commission on Zoological Nomenclature.

***Sturisomatichthys* Isbrücker & Nijssen, 1979.** Type species: *Oxyloricaria leightoni* Regan, 1912. Lectotype: BMNH 1909.7.23.45, Colombia, Honda. Gender: masculine. The genus *Sturisomatichthys* is distributed in the northwestern part of South America, on the Pacific and Atlantic slopes of the Andes. The species appear to occupy the same ecological niche as those in *Sturisoma*. Sexual dimorphism and reproductive biology of *Sturisomatichthys* are also similar to *Sturisoma*. *Sturisomatichthys* is distinguished from *Sturisoma* primarily by the absence of a rostrum. Only one species, *Sturisomatichthys citurensis*, from Panama, seems to be significantly different from all congeneric species in having an abdominal plate cover consisting of small platelets without any particular organization. Other species may represent a species complex with a short snout as in the genus *Farlowella* with reference to the representatives of the *F. curtirostra* group. The weakness of this diagnostic feature could lead to the synonymy of *Sturisomatichthys* with *Sturisoma*.

Conclusions

This work attempts to provide a useful tool for the identification of representatives of the subfamily Loricariinae in the laboratory as well as in the field. As laboratory apparati are rarely available in the field, an approach mainly based on the external morphology was preferred. It also provides a short summary of the present knowledge concerning this group. Major taxonomic problems are underlined and solutions are proposed. This study also tries to promote original statistic tools such as the Hill & Smith analysis (1976) and cluster analysis, still rarely used in morphology for solving complex problems of identification. Although the matrix analyzed here contains phylogenetic information, the methods used do not allow robust phylogenetic inferences. Thus, the tree proposed here must not be interpreted as a phylogeny, but as a representation of groups of individuals sharing common morphological characteristics. The results of this study reflect our present knowledge concerning the taxonomy of Loricariinae and encourage future research endeavors into the evolutionary history of this group.

Acknowledgments

This work corresponds partly to the Master's work of the senior author, who would like to thank Volker Mahnert, MHNG, Geneva for his supervision. We are also grateful to Juan Montoya-Burgos, MHNG and University of Geneva, Claude Weber, Bernard Landry, Andreas Schmitz, Alain de Chambrier, Alain Merguin, and Claude Vaucher, MHNG, Stéphane Dray, UCBL, Lyon, for their helpful advices; Jan Mol, Department of Fish Ecology, CELOS, Paramaribo, Peter Rask Møller, and Tammes Menne, ZMUC, Copenhagen, Erica Pellegrini Caramaschi, UFRJ, Rio de Janeiro, Reginaldo Constantino, UNB, Brasília, Isaäc J. H. Isbrücker, Mónica S. Rodríguez, PUCRS, Porto Alegre, Gladys Monasterio de Gonzo, UNSA, Salta, and Miriam Ghazzi for providing information concerning species, specimens or publications. We would also like to thank two anonymous reviewers for their helpful comments that led to improvements in the paper, Patrick de Rham for the donation of the numerous specimens used in this study, and the G. & A. Claraz Foundation for their financial support for the mission in Surinam and French Guiana in 2001. The figures were finalized by Florence Marteau, MHNG.

Literature cited

Armbruster, J.W. (2004) Phylogenetic relationships of the suckermouth armoured catfishes (Loricariidae) with emphasis on the Hypostominae and the Ancistrinae. *Zoological Journal of the Linnean Society*, 141, 1–80.

Boeseman, M. (1971) The "comb-toothed" Loricariinae of Surinam, with reflections on the phylogenetic tendencies within the family Loricariidae (Siluriformes, Siluroidei). *Zoologische Verhandelingen (Leiden)*, 116, 1–56.

Burgess, W.E. (1989) *An Atlas of Freshwater and Marine Catfishes. A Preliminary Survey of the Siluriformes*. T.F.H. Publications, Neptune City, New Jersey, 784 pp.

Casattti, L. & Castro, R.M.C. (2006) Testing the ecomorphological hypothesis in a headwater riffles fish assemblage of the rio São Francisco, southeastern Brazil. *Neotropical Ichthyology*, 4, 203–214.

Centofante, L., Bertollo, L.A.C. & Moreira-Filho, O. (2006) Cytogenetic characterization and description of an XX/XY_1Y_2 sex chromosome system in catfish *Harttia carvalhoi* (Siluriformes, Loricariidae). *Cytogenetic and Genome Research*, 112, 320–324.

Covain, R., Le Bail, P.-Y., Sagnes, P. & Fisch-Muller, S. (2006) Les espèces du genre *Harttia* (Siluriformes : Loricariidae) en Guyane Française : morphologie, taxinomie et distribution. *Cybium*, 30, 3–18.

Cuvier, G. & Valenciennes, A. (1840) *Histoire naturelle des poissons. Tome quinzième. Suite du livre dix-septième. Siluroïdes*. Ch. Pitois & Ve Levrault, Paris & Strasbourg, xxxi + 540 pp., pls. 421–455.

Dotzer, P. & Weiner, T. (2003) *Harttia guianensis*, Die Pflege und die Zucht sind swar schwierig, aber doch möglich. *Das Aquarium*, 407, 20–26.

Eschmeyer, W.N. (1998) *Catalog of Fishes*. California Academy of Sciences, San Francisco, USA, 2905 pp.

Evers, H.-G. & Seidel, I. (2005) *Catfish Atlas Vol. 1: South American Catfishes of the Families Loricariidae, Cetopsidae, Nematogenyidae and Trichomycteridae*. Mergus Verlag Gmbh, Melle, Germany, 943 pp.

Ferraris, C.J. Jr. (2003) Subfamily Loricariinae (Armoured catfishes). In: Reis, R. E., Kullander, S. O. & Ferraris, C. J. Jr. (Eds.), *Check List of the Freshwater Fishes of South and Central America*, Edipucrs, Porto Alegre, Brazil, pp. 330–350.

Ghazzi, M.S. (2003) *Relações filogenéticas e revisão do gênero* Sturisoma *Swainson, 1838 (Siluriformes: Loricariidae, Loricariinae)*. Unpublished Ph. D. Thesis, University of São Paulo, São Paulo, Brazil, 346 pp.

Ghazzi, M.S. (2005) *Sturisoma kneri*, new species, a name for an old yet poorly-known catfish (Siluriformes: Loricariidae). *Copeia*, 2005, 559–565.

Giuliano-Caetano, L. (1998) *Polimorfismo cromossômico Robertsoniano em populações de* Rineloricaria latirostris *(Pisces, Loricariinae)*. Unpublished PhD Thesis, Federal University of São Carlos, Brazil, 78 pp.

Günther, A. (1864) *Catalogue of the Fishes in the British Museum, vol. 5. Catalogue of the Physostomi, Containing the Families Siluridae, Characinidae, Haplochitonidae, Sternoptychidae, Scopelidae, Stomiatidae in the Collection of the British Museum*. Trustees, London, xxii + 455 pp.

Hill, M.O. & Smith, A.J.E. (1976) Principal component analysis of taxonomic data with multi-state discrete characters. *Taxon*, 25, 249–255.

ICZN [International Commission on Zoological Nomenclature] (1999) *International Code of Zoological Nomenclature*. 4th Edition, International Trust for Zoological Nomenclature. London, 306 pp.

Isbrücker, I.J.H. (1971) A redescription of the South American catfish *Loricariichthys maculatus* (Bloch, 1794), with designation of the lectotype and restriction of its type locality (Pisces, Siluriformes, Loricariidae). *Bijdragen tot de Dierkunde*, 41, 10–18.

Isbrücker, I.J.H. (1972) The identity of the South American catfish *Loricaria cataphracta* Linnaeus, 1758, with descriptions of the original type specimens of four other nominal *Loricaria* species (Pisces, Siluriformes, Loricariidae). *Beaufortia*, 19, 163–191.

Isbrücker, I.J.H. (1975) *Pseudohemiodon thorectes,* a new species of mailed catfish from the Rio Mamoré system, Bolivia (Pisces, Siluriformes, Loricariidae). *Beaufortia*, 23, 85–92.

Isbrücker, I.J.H. (1979) Description préliminaire de nouveaux taxa de la famille des Loricariidae, poissons-chats cuirassés néotropicaux, avec un catalogue critique de la sous-famille nominale (Pisces, Siluriformes). *Revue Française d'Aquariologie et Herpétologie*, 5, 86–116.

Isbrücker, I.J.H. (1980) Classification and catalogue of the mailed Loricariidae (Pisces, Siluriformes). *Verslagen en Technische Gegevens*, 22, 1–181.

Isbrücker, I.J.H. (1981a) *A treatise of the Loricariidae Bonaparte, 1831, a family of South American mailed catfishes, with emphasis on the subfamily Loricariinae (Pisces, Siluriformes)*. Unpublished Ph. D. Thesis, University of Nancy, Nancy, France, 224 pp.

Isbrücker, I.J.H. (1981b) Revision of *Loricaria* Linnaeus, 1758 (Pisces, Siluriformes, Loricariidae). *Beaufortia*, 31, 51–96.

Isbrücker, I.J.H., Britski, H.A., Nijssen H. & Ortega, H. (1983) *Aposturisoma myriodon*, une espèce et un genre nouveaux de Poisson-Chat cuirassé, tribu Farlowellini Fowler, 1958 du Bassin du Rio Ucayali, Pérou (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpétologie*, 10, 33–42.

Isbrücker, I.J.H. & Nijssen, H. (1974a) *Rhadinoloricaria* gen. nov. and *Planiloricaria*, two genera of South American mailed catfishes (Pisces, Siluriformes, Loricariidae). *Beaufortia*, 22, 67–81.
Isbrücker, I.J.H. & Nijssen, H. (1974b) On *Hemiodontichthys acipenserinus* and *Reganella depressa*, two remarkable mailed catfishes from South America (Pisces, Siluriformes, Loricariidae). *Beaufortia*, 22, 193–222.
Isbrücker, I.J.H. & Nijssen, H. (1976a) *Rineloricaria heteroptera,* a new species of mailed catfish from Rio Amazonas near Manaus, Brazil (Pisces, Siluriformes, Loricariidae). *Zoologischer Anzeiger*, 196, 109–124.
Isbrücker, I.J.H. & Nijssen, H. (1976b) The South American mailed catfishes of the genus *Pseudoloricaria* Bleeker, 1862 (Pisces, Siluriformes, Loricariidae). *Beaufortia*, 25, 107–129.
Isbrücker, I.J.H. & Nijssen, H. (1978) The neotropical mailed catfishes of the genera *Lamontichthys* P. de Miranda-Ribeiro, 1939 and *Pterosturisoma* n. gen., including the description of *Lamontichthys stibaros* n. sp. from Ecuador (Pisces, Siluriformes, Loricariidae). *Bijdragen tot de Dierkunde*, 48, 57–80.
Isbrücker, I.J.H. & Nijssen, H. (1982) New data on *Metaloricaria paucidens* from French Guiana and Surinam (Pisces, Siluriformes, Loricariidae). *Bijdragen tot de Dierkunde*, 52, 155–168.
Isbrücker, I.J.H. & Nijssen, H. (1983) *Crossoloricaria rhami* n. sp., un nouveau poisson-chat cuirassé du Rio Huacamayo, Pérou (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpétologie*, 10, 9–12.
Isbrücker, I.J.H. & Nijssen, H. (1984) *Pyxiloricaria menezesi,* a new genus and species of mailed catfish from Rio Miranda and Rio Cuiabá, Brazil (Pisces, Siluriformes, Loricariidae). *Bijdragen tot de Dierkunde*, 54, 163–168.
Isbrücker, I.J.H. & Nijssen, H. (1986a) *Apistoloricaria condei,* nouveau genre et nouvelle espèce de poisson-chat cuirassé, tribu Loricariini Bonaparte, 1831, du bassin du Rio Napo, haute Amazone, Equateur (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpetologie*, 12, 103–108.
Isbrücker, I.J.H. & Nijssen, H. (1986b) New records of the mailed catfish *Planiloricaria cryptodon* from the Upper Amazon in Peru, Brazil and Bolivia, with a key to the genera of the Planiloricariina. *Bijdragen tot de Dierkunde*, 56, 39–46.
Isbrücker, I.J.H., Seidel, I., Michels, J.P., Schraml E. & Werner, A. (2001) Diagnose vierzehn neuer Gatungen der Familie Loricariidae Rafinesque, 1815 (Teleostei, Ostariophysi). In: R. Stawikowski (Ed.), *Harnischwelse 2*, Die Aquarien und Terrarien Zeitschrift, Sonderheft, Eugen Ulmer, Stuttgart, Germany, p. 17–24.
Kavalco, K.F., Pazza, R., Bertollo, L.A.C. & Moreira-Filho, O. (2005) Karyotypic diversity and evolution of Loricariidae (Pisces, Siluriformes). *Heredity*, 94, 180–186.
Knaack, J. (2003) Ein neuer Prachthexenwels aus Paraguay: *Hemiloricaria aurata* n. sp. (Pisces, Siluriformes, Loricariidae). *Aquaristik aktuell*, 11, 56–61.
Kner, R. (1853) Die Panzerwelse des K.K. Hof-naturalien-Cabinetes zu Wien. I. Abtheilung: Loricarinae. *Denkschriften der Mathematisch-Naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften in Wien*, 6, 1–34.
Kner, R. (1854) Die Hypostomiden. Zweite Hauptgruppe der Familie der Panzerfische. (Loricata vel Goniodontes). *Denkschriften der Mathematisch- Naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften in Wien*, 7, 251–286.
Langeani, F., Oyakawa O.T. & Montoya-Burgos, J.-I. (2001) New species of *Harttia* (Loricariidae, Loricariinae) from the Rio São Francisco basin. *Copeia*, 2001, 136–142.
Le Bail, P.-Y., Keith, P. & Planquette, P. (2000) *Atlas des Poissons d'eau Douce de Guyane. Tome 2, fascicule II: Siluriformes*. Patrimoines naturels (M.N.H.N./S.P.N.), 43 (II), 307 pp.
Leviton, A.E., Gibbs, R.H. Jr., Heal, E. & Dawson, C.E. (1985) Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. *Copeia*, 1985, 802–832.
Martín Salazar, F.J., Isbrücker, I.J.H. & Nijssen, H. (1982) *Dentectus barbarmatus*, a new genus and species of mailed catfish from the Orinoco Basin of Venezuela (Pisces, Siluriformes, Loricariidae). *Beaufortia*, 32, 125–137.
Mol, J.H. & Ouboter, P.E. (2004) Downstream effects of erosion from small-scale gold mining on the instream habitat and fish community of a small neotropical rainforest stream. *Conservation Biology*, 18, 201–214.
Montoya-Burgos, J.-I., Muller, S., Weber, C. & Pawlowski, J. (1998) Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences. In: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena Z.M.S. & Lucena, C.A.S. (Eds.) *Phylogeny and Classification of Neotropical Fishes*. Edipucrs Porto Alegre, Porto Alegre, Brazil, pp. 363–374.
Montoya-Burgos, J.-I., Weber, C. & Le Bail, P.-Y. (2002) Phylogenetic relationships within *Hypostomus* (Siluriformes: Loricariidae) and related genera based on mitochondrial D-loop sequences. *Revue suisse de Zoologie,* 109, 369–382.
Nijssen, H. & Isbrücker, I.J.H. (1987) *Spectracanthicus murinus*, nouveaux genre et espèce de poisson-chat cuirassé du Rio Tapajós, Est. Pará, Brésil, avec des remarques sur d'autres genres de Loricariidés (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpétologie*, 13, 93–98.
Nijssen, H. & Isbrücker, I.J.H. (1988) Trois nouvelles espèces du genre *Apistoloricaria* de Colombie et du Pérou, avec illustration du dimorphisme sexuel secondaire des lèvres de *A. condei* (Pisces, Siluriformes, Loricariidae). *Revue Française d'Aquariologie et Herpétologie*, 15, 33–38.
Oyakawa, O.T. (1993) Cinco espécies novas de *Harttia* Steindachner, 1876 da região sudeste do Brasil, de comentários sobre o gênero (Teleostei, Siluriformes, Loricariidae). *Comunicações do Museu de Ciências da PUCRS, Série Zoologia, Porto Alegre*, 6, 3–27.

Pinna, M.C.C. de (1998) Phylogenetic relationships of neotropical Siluriformes (Teleostei: Ostariophysi): historical overview and synthesis of hypotheses. In: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M. & Lucena, C.A.S. (Eds.). *Phylogeny and Classification of Neotropical Fishes*. Edipucrs Porto Alegre, Porto Alegre, Brazil, pp. 279–330.
Provenzano, R.F., Machado-Allison, A., Chernoff, B., Willink, P. & Petry, P. (2005) *Harttia merevari*, a new species of catfish (Siluriformes: Loricariidae) from Venezuela. *Neotropical Ichthyology*, 3, 519–524.
Rapp Py-Daniel, L.H. (1981) *Furcodontichthys novaesi* n. gen., n. sp. (Osteichthyes, Siluriformes; Loricariidae) na bacia Amazônia, Brasil. *Boletim do Museu Paraense Emilio Goeldi, Nova Serie, Zoologia*, 105, 1–17.
Rapp Py-Daniel, L.H. (1997) *Phylogeny of the Neotropical armored catfishes of the subfamilly Loricariinae (Siluriformes: Loricariidae)*. Unpublished Ph. D. Thesis, University of Arizona, Tucson, 280 pp.
Rapp Py-Daniel, L.H. & Oliveira, E.C. (2001) Seven new species of *Harttia* from the Amazonian-Guyana region (Siluriformes: Loricariidae). *Ichthyological Exploration of Freshwaters*, 12, 79–96.
Regan, C.T. (1904) A monograph of the fishes of the family Loricariidae. *Transactions of the Zoological Society of London*, 17, 191–350.
Reis, R.E. & Cardoso, A.R. (2001) Two new species of *Rineloricaria* from southern Santa Catarina and northeastern Rio Grande do Sul, Brazil (Teleostei: Loricariidae). *Ichthyological Exploration of Freshwaters*, 12, 319–332.
Reis, R.E., Kullander, S.O. & Ferraris, C.J. Jr. (Eds.) (2003) *Check List of the Freshwater Fishes of South and Central America*, Edipucrs Porto Alegre, Porto Alegre, Brazil, 742 pp.
Reis, R.E. & Pereira, E.H.L. (2000) Three new species of the loricariid catfish genus *Loricariichthys* (Teleostei: Siluriformes) from southern South America. *Copeia*, 2000, 1029–1047.
Reis, R.E., Pereira, E.H.L. & Armbruster, J.W. (2006) Delturinae, a new loricariid catfish subfamily (Teleostei, Siluriformes), with revisions of *Delturus* and *Hemipsilichthys*. *Zoological Journal of the Linnean Society*, 147, 277–299.
Retzer, M.E. (2006) A new species of *Farlowella* Eigenmann and Eigenmann (Siluriformes: Loricariidae), a stickcatfish from Bolivia. *Zootaxa*, 1282, 59–68.
Retzer, M.E. & Page, L.M. (1997) Systematics of the stick catfishes, *Farlowella* Eigenmann & Eigenmann (Pisces, Loricariidae). *Proceedings of the Academy of Natural Sciences, Philadelphia*, 147, 33–88.
Rodiles-Hernández, R., Hendrickson, D.A., Lundberg, J.G. & Humphries, J.M. (2005) *Lacantunia enigmatica* (Teleostei: Siluriformes) a new and phylogenetically puzzling freshwater fish from Mesoamerica. *Zootaxa*, 1000, 1–24.
Rodríguez, M.S. & Miquelarena, A.M. (2003) Una nueva especie de *Loricaria* (Siluriformes: Loricariidae) para la cuenca del río San Francisco, Jujuy, Argentina. *Anales de la Academia Nacional de Ciensas Exactas, Físicas y Naturales*, 55, 139–149.
Rodríguez, M.S. & Miquelarena, A.M. (2005) A new species of *Rineloricaria* (Siluriformes: Loricariidae) from the Paraná and Uruguay River basins, Misiones, Argentina. *Zootaxa*, 945, 1–15.
Schaefer, S.A. (1986) *Historical biology of the loricariid catfishes: Phylogenetics and functional morphology*. Unpublished Ph. D. Thesis, University of Chicago, 198 pp.
Schaefer, S.A. (1987) Osteology of *Hypostomus plecostomus* (Linnaeus) with a phylogenetic analysis of the loricariid subfamilies (Pisces: Siluroidei). *Contributions in Science, Natural History Museum of Los Angeles County*, 394, 1–31.
Schaefer, S.A. (1991) Phylogenetic analysis of the loricariid subfamily Hypoptopomatinae (Pisces: Siluroidei: Loricariidae), with comments on generic diagnoses and geographic distribution. *Zoological Journal of the Linnean Society*, 102, 1–41.
Schaefer, S.A. & Stewart, D.J. (1993) Systematics of the *Panaque dentex* species group (Siluriformes: Loricariidae), wood-eating armored catfishes from tropical South America. *Ichthyological Exploration of Freshwaters*, 4, 309–342.
Sneath, H.A. & Sokal, R.R. (1973) *Numerical Taxonomy*. Freeman Publ., San Francisco, USA, 573 pp.
Steindachner, F. (1878) Zur Fischfauna des Magdalenen-Stromes. *Anzeiger der Mathematisch- Naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften in Wien*, 15, 88–91.
Steindachner, F. (1881) Beiträge zur Kenntniss der Flussfische Südamerika's, II. *Denkschriften der Mathematisch-Naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften in Wien*, 43, 103–146.
Taphorn, D.C. & Lilyestrom, C.G. (1984) *Lamontichthys maracaibero* y *L. llanero* dos especies nuevas para Venezuela (Pisces, Loricariidae). *Revista UNELLEZ de Ciencia y Tecnologia*, 2, 93–100.
Thioulouse, J., Chessel, D., Dolédec, S., Olivier, J.M., Goreaud, F. & Pelissier, R. (2001) *ADE–4, Ecological Data Analysis : exploratory and euclidian methods in environmental sciences, Version 2001,* CNRS 1995–2000.
Tortonese, E. (1940) Elenco dei tipi esistenti nella collezione ittiologica del R. Museo di Torino. *Bollettino dei Musei di Zoologia ed Anatomia Comparata della Università di Torino (Ser. 3)*, 48, 133–144.
Weber, C. & Montoya-Burgos, J.-I. (2002) *Hypostomus fonchii* n. sp. (Siluriformes: Loricariidae) from Peru, a key species suggesting the synonymy of *Cochliodon* with *Hypostomus*. *Revue Suisse de Zoologie,* 109, 355–368.
Wheeler, A. (1989) Further notes on the fishes from the collection of Laurens Theodore Gronovius (1730–1777). *Zoological Journal of the Linnean Society*, 95. 205–218.

Annex 3

Diversity of the Ancistrini (Siluriformes: Loricariidae) from the Guianas: the *Panaque* group, a molecular appraisal with descriptions of new species.

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The variability of the first intron of the new f-rtn4 marker is here evaluated in comparison to the standard COI barcode region. RC performed the molecular analyses and wrote the related parts of the ms; SFM wrote the rest of the manuscript.

Accepted for publication in: *Cybium*. Special issue on the Guianese fish fauna [project deposited by R. Covain and S. Fisch-Muller and accepted by the Société française d'ichtyologie (RC & SFM guest editors)].

ABSTRACT. – DNA barcoding represents a reliable and powerful way to discriminate and identify species using a standardized region of the mt COI gene. However, a correct identification requires two factors: differentiation and assignment. When one component is lacking, the barcode approach usually fails. To circumvent such problem, we developed a dual approach using a nuclear marker as complementary identifier. A first step consisted in characterizing the first intron of the F-RTN4 gene. This intron was found to be the longest, the most divergent and the most variable of the different introns constituting F-RTN4, making it a candidate of choice. This dual approach was applied to a group of closely related armoured catfishes constituting the *Panaque* group within the Guianas. Three groups were found: *Pseudacanthicus*, *Hemiancistrus*, and *Peckoltia-Panaqolus*, and four new species were highlighted. Within the latter group, *Panaqolus koko* n. sp. displayed a pattern of mitochondrial introgression with *Peckoltia otali* n. sp., while *Peckoltia capitulata* n. sp. and *Peckoltia simulata* n. sp. revealed cryptic species of *Peckoltia oligospila*. *Hemiancistrus* appeared significantly distinct from *Peckoltia*. Its type species is redescribed and a neotype is designated to clarify its taxonomic status considering the loss of the holotype.

RÉSUMÉ. – Diversité des Ancistrini (Siluriformes : Loricariidae) des Guyanes : le groupe *Panaque*, une évaluation moléculaire avec descriptions de nouvelles espèces.

Le code barre ADN représente un moyen fiable et puissant de discriminer et d'identifier les espèces en utilisant une région standardisée du gène mitochondrial COI. Une identification correcte requiert toutefois deux critères : différentiation et assignation. Lorsque qu'une composante manque, l'approche code barre échoue fréquemment. Afin de circonvenir à un tel problème, nous avons développé une double approche faisant appel à un marqueur nucléaire en tant qu'identifiant complémentaire. Une première étape consista à caractériser le premier intron du gène F-RTN4. Cet intron s'est révélé le plus long, le plus divergent et le plus variable des différents introns constituant F-RTN4, en faisant un candidat de choix. Cette double approche a été appliquée à un groupe de poissons-chats cuirassés étroitement apparentés constituant le groupe *Panaque* dans les Guyanes. Trois groupes ont été trouvés : *Pseudacanthicus*, *Hemiancistrus* et *Peckoltia-Panaqolus*, et quatre espèces nouvelles ont été mises en évidence. Dans le dernier groupe, *Panaqolus koko* sp. n. montre un pattern d'introgression mitochondriale avec *Peckoltia otali* sp. n., alors que *Peckoltia capitulata* sp. n. et *Peckoltia simulata* sp. n. se révèlent espèces cryptiques de *Peckoltia oligospila. Hemiancistrus* apparaît significativement distinct de *Peckoltia*. Son espèce type est redécrite et, basé sur la perte de l'holotype, un néotype est désigné afin de clarifier son statut taxonomique.

Key words. – DNA barcode – COI gene – intron – *Hemiancistrus* – *Peckoltia* – *Panaqolus* – cryptic species

INTRODUCTION

Historical methods for identifying, naming and classifying fishes rely essentially on external morphology (Ward *et al*., 2009). Nevertheless, this approach has often proven its limitation, particularly in the detection of cryptic species (see Hillis *et al*., 1996; e. g. Emberton *et al*., 1995; Fisch-Muller *et al*., 2002). Modern techniques, including gene sequencing, appeared as complementary and relevant methods to reveal this hidden diversity (*e.g*. Hebert *et al*., 2004a; Miura *et al*., 2005; Ellis *et al*., 2006; Lara *et al*., 2010). In this context, the establishment of a standard DNA sequence devoted to the identification of species was a necessary prerequisite. This was the main goal of the Barcoding Of Life Initiative (BOLI) which established the use of a mitochondrial 648-bp 5' target region of the cytochrome *c* oxidase I (COI) gene (Hebert *et al*., 2003). The COI gene encodes part of a large enzymatic complex of the mitochondrial respiratory chain. The sequence, due to the degenerate nature of the genetic code, possesses high mutational rates in third and first positions of codons, despite relative conservation in amino acids (Ward and Holmes, 2007). These high mutational rates therefore allow the rapid accumulation of mutations between sequences that forms the conceptual basis of the barcode system. The differences accumulated are expected to be low within species due to the constant transmission of mitochondria, and high among species due to the absence of mitochondrial exchanges. The COI barcode system has already been efficiently used in quantifying and qualifying fish diversity (Ward *et al*., 2005; Hubert *et al.,* 2008; Ward *et al.,* 2009; Valdez-Moreno *et al*., 2009; Lara *et al*., 2010), and successfully highlighted cryptic species (e.g. Ward *et al*., 2008a; Ward *et al*., 2008b; Lara *et al.*, 2010). However, this method has not been without controversy, essentially because it relies on a mitochondrial gene. Particularly, doubts were voiced concerning the ability of the COI gene to discriminate recently radiated species (Moritz and Cicero, 2004; Hickerson *et al.*, 2006). Another major concern with the use of a mitochondrial marker is the lack of sensitivity to detect hybridization and mitochondrial introgression (Ward *et al.,* 2009). To circumvent this last issue, it is often recommended that comparisons be made with a nuclear marker to detect conflicting signals (Hebert *et al*., 2003; Ward and Holmes, 2007; Ward *et al.,* 2009). Different proposals have been made mostly relying on the variable regions of the nuclear ribosomal genes (e.g. Sonnenberg *et al*., 2007; Raupach *et al*., 2010). Nevertheless, no widely accepted standard nuclear marker has presently been developed as a complementary barcode in animals. A possible explanation for this gap may rely on the different natures of both genomes. Moreover, it is well accepted that the coding sequence of nuclear genes evolve

much more slowly than mitochondrial ones (Page and Holmes, 1998), what may lead to the absence of the necessary barcoding gap (Meyer and Pauley, 2005) allowing the discrimination of species. In this case, the use of non-coding regions with more relaxed evolutionary constraints such as introns may provide a solution.

The selection of appropriate introns as candidate markers for barcoding purposes can benefit from the following theoretical considerations. A recent investigation of the evolution of the exon-intron structure conducted by Zhu *et al*. (2009) revealed three main evolutionary patterns recovered in all eukaryotic genomes analysed. First, an ordinal reduction of length and divergence in both exon and intron; second, a co-variation of GC content and divergence between exons and flanking introns; and three, a decrease of average exon or intron length, GC content and divergence with the increasing number of exons in a gene. Moreover, they noted a strong complicated correlation between the GC content and the length of the introns and exons. To explain these significant trends, the authors hypothesised that these patterns were caused by factors common to either exons or introns or to both (e.g. splicing elements). They noted that the monotonic reduction of length, GC content and divergence as the ordinal variation or as a function of the total number of introns or exons, may reveal the factors that shaped this pattern, since this ordinal trend may reflect a time-orderly evolution. Zhu *et al*. (2009) thus proposed the timely-ordered model for the evolution of the intron-exon structure. This model stipulates that if the number of introns or exons follows an increasing trend, then the first exon and intron are older than the next ones. These older introns had more time to be inserted by regulatory or transposable elements and became accordingly longer. Moreover, the inserted sequences in introns have generally a lower GC content; and the later occurring introns cut the coding sequences into shorter ones except for the first and last exons which are required by splicing-related factors; the subsequent recruited exons, have a higher possibility of coming from intron sequences and therefore have a lower GC content. The first intron of eukaryotic nuclear genes therefore appeared as a possible candidate for identification purpose as that region is supposed to have accumulated enough mutations through time compared with its flanking exons, or subsequent introns.

In the present study, we used a classical barcode approach to investigate species diversity in a group of closely related catfishes belonging to the Loricariidae. The family Loricariidae is the world's largest catfish family including 716 valid species (Ferraris 2007), without considering the numerous species still awaiting for a formal description, neither the undiscovered nor cryptic ones (300 undescribed species estimated in Reis *et al.* 2003). Loricariids are mainly characterized by their body encased in rows of bony dermal plates, and

by the possession of a ventral sucker mouth. They feed by scraping the substrate to eat algae, detritus, and invertebrates. Their highly specialized morphology makes the Loricariidae one of the best characterized family among Siluriformes, recognized as a natural group since the earliest classifications for the order (de Pinna, 1998). Their exceptional diversity, usually allied to parental care and to low fecundity, are conditions that were compared to those observed for the cichlid species flocks in the East African rift lakes (Schaefer and Stewart, 1993). Genera sharing the presence of hypertrophied and movable cheek odontodes were placed in the subfamily Ancistrinae Kner, 1853 (Isbrücker 1980; Fisch-Muller 2003). Based on a phylogenetic analysis of the Loricariidae using osteological characters, Armbruster (2004) considered the Ancistrinae as one of five tribes of the Hypostominae. The Ancistrini represent the most diversified tribe including about the third of all loricariid species distributed in 26 genera (Ferraris 2007). It occurs through all main Neotropical drainages, from Panama to Chile on the Western side of the Andes, and to Argentina on the eastern side. The highest generic diversity is mainly represented by rheophilic species distributed in rivers flowing the Brazilian and Guiana Shields. The present work is restricted to a recently defined group of the Ancistrini, the *Panaque* clade (Armbruster 2008). In an updated osteological analysis Armbruster (2008) found three groups within the Ancistrini, one composed of a single undescribed taxon, the two others comprising numerous genera and named *Panaque* and *Ancistrus* clades. The *Panaque* clade included *Acanthicus*, *Baryancistrus*, *Hemiancistrus*, *Hypancistrus*, *Leporacanthicus*, *Megalancistrus*, *Panaque*, *Peckoltia*, *Pseudacanthicus*, *Spectracanthicus*, and an undescribed genus. In that analysis, which did not include the type species of *Hemiancistrus* (*H. medians*), corroborating previous studies, *Panaque* (including *Panaqolus*) was found most closely related to *Peckoltia* (Schaefer, 1986; Schaefer and Stewart, 1993; Armbruster, 2004) and to *Scobinancistrus* (Armbruster, 2004: 59). *Scobinancistrus* was also placed in synonymy of *Panaque* (Armbruster, 2004: Table I). The hypothesis of close relationship between *Pekoltia* and *Panaque* was however not supported by the analysis of 12S and 16S mitochondrial rRNA genes (Montoya-Burgos *et al.*, 1998), and several studies provided evidence that the genus Hemiancistrus forms a polyphyletic assemblage (Montoya-Burgos *et al.* 2002; Armbruster 2008) and is in need of a revision. In a recent checklist of the Siluriformes, Ferraris (2007) considered *Panaqolus* and *Scobinancistrus* as valid genera.

Within the Guianas (comprising French Guiana, Suriname and Guyana), nine species of the *Panaque* group, placed in four genera, were reported (Le Bail *et al*., 2000; Ferraris 2007; Vari *et al*., 2009): - *Hemiancistrus medians* (Kner, 1854), type species of *Hemiancistrus*,

described from a single specimen without statement of locality; - a species found in the upper Maroni River that was assigned to *Panaque* cf. *dentex* (Günther, 1868) (now *Panaqolus*); - *Peckotia braueri* (Eigenmann, 1912) known from the Amazonian Takutu and Branco River basins, and a species assigned to *Hemiancistrus* aff. *braueri* (now *Peckoltia*) found in the Maroni River basin, with a distinct form mentioned for the Oyapock River; -*Peckoltia cavatica* Armbruster & Wernecke, 2005 endemic to the Rupununi River in Guyana; and - *Peckoltia sabaji* Armbruster, 2003 from Essequibo, Branco, Negro, and Orinoco rivers drainages; - three *Pseudacanthicus* species, *P. fordii* (Günther, 1868), known from type material from Suriname, *P. serratus* (Valenciennes, 1840) from Suriname and French Guiana, and *P. leopardus* (Fowler, 1914) from the Rupununi River basin. Two additional Surinamese species that are essentially known from their respective holotypes were never, to our knowledge, collected again in Suriname. Described as *Chaetostomus megacephalus* by Günther (1868) and *C. macrops* by Lütken (1874), they were both placed in *Hemiancistrus* (Fisch-Muller, 2003; Ferraris, 2007) and in *Pseudancistrus* (Armbruster, 2004, Vari *et al*., 2009), a genus that is included in Armbruster's *Ancistrus* group. Eigenmann (1912) provided a complementary description of *H. megacephalus* from material collected in the Essequibo River basin. However, based on the examination of the holotype and one of the specimens identified by Eigenmann, *H. megacephalus sensu* Eigenmann may well prove to be distinct from the species. The assignation of species to genera such as *Hemiancistrus* and *Peckoltia* remains a problem. Both taxa are poorly defined despite a recent attempt to revise *Peckoltia* (Armbruster 2008), and their taxonomic history has for long been intimately linked (Miranda Ribeiro 1912; Isbrücker 1980; Cardoso and Lucinda 2003; Armbruster 2003, 2004, 2008), species being regularly moved from one genus to the other. In this work, we followed the taxonomy provided by Ferraris (2007), except for *H. macrops* and *H. megacephalus* that still deserve further investigations.

Recent field work in the Guianas resulted in a representative sampling of the *Panaque* group for molecular analyses, including unidentified and tentatively identified forms. Based on a dual barcode evaluation to prevent species misassignment, the systematics of the Guianese representatives of the *Panaque* group are revised here, and the new taxa highlighted are described. The methods used in the present work are primarily addressed for discriminating and identifying species, and have only limited phylogenetic resolution (Moritz and Cicero, 2004).

MATERIAL AND METHODS

DNA barcodes

For an assessment of the diversity of the Guianese Ancistrini constituting the *Panaque* group, the standard COI barcode region was amplified. A total of 15 specimens (Table I) representing all available species and populations was submitted to molecular analyses. Among the fifteen, nine represented strictly Guianese lineages and two were downloaded from GenBank to provide comparative material for a correct assignment of the taxa. In addition, because of the close resemblance of the Oyapock form of *Peckoltia* aff. *braueri* with the lower Amazonian *Peckoltia oligospila* (Günther 1864), three specimens representing two populations were added to the data set. Due to the confusing taxonomy of the group and the close relatedness of its representatives, a fragment of the Fish Reticulon-4 (F-RTN4) nuclear gene was also amplified to detect potential conflicting signals. Tissue samples were housed in MHNG and ANSP, and preserved in 80% ethanol and stored at -20°C. Total genomic DNA was extracted with the DNeasy Tissue Kit (Qiagen) following the instructions of the manufacturer. The PCR amplifications were carried out using the Taq PCR Core Kit (Qiagen), using the Fish-F1 and Fish-R1 primers (Ward *et al.*, 2005). The amplifications and sequencing processes were performed as in Covain *et al*. (in press) for the COI gene, and as in Chiachio *et al*. (2008) for the F-RTN4 gene. Sequences were deposited in GenBank, and accession numbers provided in table I.

The DNA sequences were edited and assembled using BioEdit 7.0.1 (Hall, 1999). Prior to the alignment, all sequences were confronted to GenBank database using the blastn 2.2.24 algorithm (Altschul *et al.*, 1997) to confirm the identity of the amplified genes. Additionally, F-RTN4 fragments were queried against the genome of *Danio rerio* in Ensembl database (http://www.ensembl.org/index.html) to identify the ordinal position and intervals of the amplified introns of the F-RTN4 gene. The sequences were secondarily manually aligned since the coding COI gene aligned unambiguously in a single block, and very few indels were present in the F-RTN4 introns. The GC content and base composition were computed using the seqinr 2.0-9 package (Charif and Lobry, 2007) in R 2.10.1 (R Development Core Team, 2009), and usual tests for homogeneity of nucleotide frequencies and substitution saturation (Xia *et al.*, 2003) were performed using Dambe 4.5.56 (Xia and Xie, 2001).

To evaluate the ability of the intronic regions of F-RTN4 to discriminate and assign the different species to the correct taxa, and accordingly confirm or detect conflicting signals with COI barcodes, different types of analyses were performed. These analyses were also used to

Table I. – Taxa list, specimen and sequence data for the 15 Ancistrini of the *Panaque* group analyzed for COI and F-RTN4 genes. Institutional acronyms follow Fricke and Eschmeyer (2010).

				COI					RTN4				
Species	Catalog Number	Field Number	Locality	GenBank No.	GC content	GC1	GC2	GC3	GenBank No.	GC content 1	Length 1	GC content 2	Length 2
Hemiancistrus medians [H. med.]	MHNG 2664.078	GF00-084	French Guiana, Marouini River	JF746998	0.46	0.55	0.43	0.39	JF747011	0.33	694	0.41	197
Hemiancistrus medians [H. med.]	MHNG 2717.005	SU08-173	Surinam, Tapanahony River	JF746999	0.46	0.55	0.43	0.39	JF747012	0.33	694	0.41	197
Panaqolus changae [Pn. chan.]	ANSP 181097	P6218	Peru, aquarium trade, Itaya River[1]	EU359435	0.42	0.52	0.43	0.31	JF747023	0.32	694	0.41	196
Panaqolus sp. L204 [Pn. L204]	MHNG 2710.093	PE08-900	Peru, aquarium trade, San Alexandro River[1]	EU359436[2]	0.43	0.54	0.44	0.32	JF747024	0.32	694	0.41	196
Panaqolus koko [Pn. Mar.]	MNHN 2011-0013	GF00-115	French Guiana, Marouini River	JF747003	0.42	0.53	0.43	0.30	JF747016	0.32	694	0.41	196
Peckoltia capitulata [Pc. Appr.]	MNHN 2011-0011	MUS 331	French Guiana, Approuague River	JF747000	0.41	0.52	0.43	0.28	JF747013	0.32	694	0.4	196
Peckoltia simulata [Pc. Oya.]	MHNG 2681.058	GF06-120	French Guiana, Oyapock River	JF747001	0.43	0.54	0.44	0.32	JF747014	0.32	694	0.41	196
Peckoltia simulata [Pc. Oya.]	MHNG 2681.058	GF06-119	French Guiana, Oyapock River	JF747002	0.43	0.54	0.44	0.32	JF747015	0.31	694	0.41	196
Peckoltia cavatica [Pc. cava.]	MHNG 2651.020	GY04-030	Guyana, Rupununi River	JF747004	0.42	0.52	0.43	0.30	JF747017	0.32	694	0.41	190
Peckoltia otali [Pc. Mar.]	ANSP 187118	SUR07-05	Surinam, Litani River	JF747005	0.41	0.52	0.43	0.27	JF747018	0.32	694	0.4	196
Peckoltia sabaji [Pc. saba.]	MHNG 2651.016	GY04-029	Guyana, Rupununi River	JF747006	0.42	0.53	0.43	0.29	JF747019	0.32	694	0.41	196
Peckoltia oligospila [Pc. olig.]	MHNG 2602.017	BR98-154	Brazil, Guamá River	JF747007	0.42	0.53	0.43	0.31	JF747020	0.32	694	0.41	196
Peckoltia oligospila [Pc. olig.]	MHNG 2602.017	BR98-155	Brazil, Guamá River	JF747008	0.42	0.53	0.43	0.31	JF747021	0.31	694	0.41	196
Peckoltia oligospila [Pc. olig.]	MHNG 2601.078	BR98-076	Brazil, Mãe do Rio River	JF747009	0.42	0.53	0.43	0.31	JF747022	0.31	694	0.41	196
Pseudacanthicus leopardus [Ps. leop.]	MHNG 2651.024	GY04-025	Guyana, Rupununi River	JF746997	0.42	0.55	0.43	0.28	JF747010	0.33	694	0.41	196

[1] according to the exporter
[2] specimen voucher ZSM 32728, no locality stated
(Cramer *et al.,* 2007)

verify that the selected region fitted the timely-ordered model suggesting evolutionary constraints acting on this region. The length of each intron was measured in number of bases and submitted to the upper tail Wilcoxon signed-rank test to assess significant differences in length between introns according to their ordinal position. The alignments of introns were secondarily converted into distances matrices using the Kimura 2 Parameters (K2P) metrics (Kimura, 1980) as implemented in ape 2.5 (Paradis *et al.,* 2004; Paradis 2006) in R, to evaluate sequence divergence, and submitted to the upper tail Wilcoxon signed-rank test to detect significant differences in variation between the different introns according to their ordinal position. The Spearman's rank correlation coefficient was also computed to assess the type of association recorded between GC contents and length of introns. Due to the low taxonomic level, too few or even no variation was observed in our data to compute this last statistic. To enlarge the range of variation of introns and allow the computation of the coefficient of correlation, all F-RTN4 sequences deposited in GenBank from previous studies were downloaded (Chiachio *et al*., 2008; Cardoso and Montoya-Burgos, 2009).

Subsequently, Shannon's information theoretic entropy (Shannon, 1948) was computed for both markers, COI and selected intron of F-RTN4, to measure the diversity of bases and hence bases' conservation in the alignments using the bio3d 1.0-6 package (Grant *et al*., 2006) in R. To detect potential conflicting phylogenetic signals, both alignments were submitted to the Incongruence Length Difference (ILD) test (Farris *et al*., 1994) as implemented in PAUP* 4.0b10 (Swofford, 1998), and after conversion of both alignments into distances matrices using the K2P metrics to the Mantel test (Mantel, 1967) using the ade4 1.4-14 package (Dray and Dufour, 2007) in R. The ILD test was conducted using a heuristic search with 100 replicates, TBR branch swapping, and random addition of taxa, and the Mantel test was performed using 9,999 random permutations of both matrices. The pattern of selection pressure acting on mt COI gene and the selected intron of F-RTN4 was assessed using a global estimation of $\omega = d_N/d_S$ for coding regions and ζ for non coding regions to detect differences in the selective forces acting on silent versus replacement changes (Pybus and Shapiro, 2010). The parameter ζ (Wong and Nielsen, 2004) assuming that neutral (i.e. synonymous) nucleotide substitution rate is constant in both the coding and non-coding regions of the same gene, represents the nucleotide substitution rate in the non-coding region, normalized by the synonymous nucleotide substitution rate in the coding region. Therefore, the interpretation of ζ becomes identical to that of ω. The computation of ω was performed with HyPhy 2.0 (Kosakovsky Pond *et al*., 2005) following the methodology proposed by Kosakovsky Pond *et al*. (2010). The parameter ζ was estimated using a batch file developed

for HyPhy 2.0 by O. Fedrigo (http://www.duke.edu/~ofedrigo/Olivier_Fedrigo/HyPhyScripts.html). Assuming the timely-ordered model, the computation of ζ for the selected intron of F-RTN4 was performed using synonymous changes of a flanking exon as neutral proxy. Both estimates require a topology, which was obtained from a different study currently in progress and is not presented here.

Finally Neighbour Joining (NJ) trees (Saitou and Nei, 1987) were reconstructed based on the K2P distances matrices to provide a cluster ordination of the species. The NJ algorithm has the advantage over other agglomerative partitioning methods to translate distances into branch lengths. To estimate robustness of the groupings, a nonparametric bootstrap analysis (Efron 1979) was performed following Felsenstein's (1985) methodology using 9,999 pseudoreplicates. In addition, levelplot graphs allowing a graphical representation of both distance matrices were computed using the lattice 0.18-3 and colorRamps 2.3 packages (Sarkar, 2010; Keitt, 2009) in R.

Taxonomy

Material belonging to the new species described here is deposited in the Muséum National d'Histoire Naturelle, Paris (MNHN), the Muséum d'histoire naturelle, Geneva (MHNG), and the Academy of Natural Sciences, Philadephia (ANSP). Comparative material includes primary type-specimens of *Hemiancistrus macrops* (Lütken, 1874), *Hemiancistrus megacephalus* (Günther, 1868), *Peckoltia braueri* (Eigenmann, 19129, *Peckoltia oligospila* (Günther, 1864), *Panaqolus dentex* (Günther, 1868), *Pseudacanthicus leopardus* (Fowler, 1914) *Pseudacanthicus serratus* (Valenciennes, 1840) and *Pseudacanthicus spinosus* (Castelnau, 1855), and twelve specimens collected in the Maroni/Marowijne River basin that we assigned to *Hemiancistrus medians* (Kner, 1854) (see COMPARATIVE MATERIAL). Institutional acronyms follow Fricke and Eschmeyer (2010).

Measurements and counts indicated in descriptions are based on all specimens listed, except those less than 30 mm SL and one individual with end of caudal peduncle and fin missing (ANSP187118, estimated size 64.5 mm SL). Specimens were measured with a digital calliper to the nearest 0.1 mm. Measurements follow Fisch-Muller *et al.* (2001), and are expressed as percents of standard length (SL) except for subunits of the head, which are expressed as percents of head length (HL). Counts follow Schaefer and Stewart (1993), excluding the marginal caudal plates, and with the addition of the counts of plates along the dorsal-fin base, plates between the anal and the caudal fins, and hypertrophied cheek odontodes.

RESULTS

DNA barcodes analysis of Guianese Ancistrini, *Panaque* group.

The obtained sequences reached a total length of 652 bp for the COI gene and 1,797 to 1,813 bp for the F-RTN4. Comparisons made against the GenBank database using blastn 2.2.24 produced high similarity scores ranging between 1,169 and 767 for the 100 first Blast Hits indicating homology between our sequences and the COI sequences deposited in the database. The E-value was null for all comparisons which indicated that the obtained scores were not due to chance. For F-RTN4 sequences, similarity scores ranged between 2,892 and 255 with E-values ranging between 0 and $7x10^{-64}$. These results also indicated that the amplified segments were homologs of the F-RTN4 gene with high probability. Comparison made between our F-RTN4 fragments and the *Danio rerio* genome in Ensembl located the *D. rerio* homolog gene rtn4rl2a-001 on chromosome 1, region 37,951,786-37,976,785. The amplified fragments comprised partial exons 1 (positions 1 to 5) and 3 (pos. 1,173: 1,823), and complete introns 1 (pos. 6: 746) and 2 (pos. 974: 1,172), and exon 2 (pos. 747: 973) (Fig. 1).

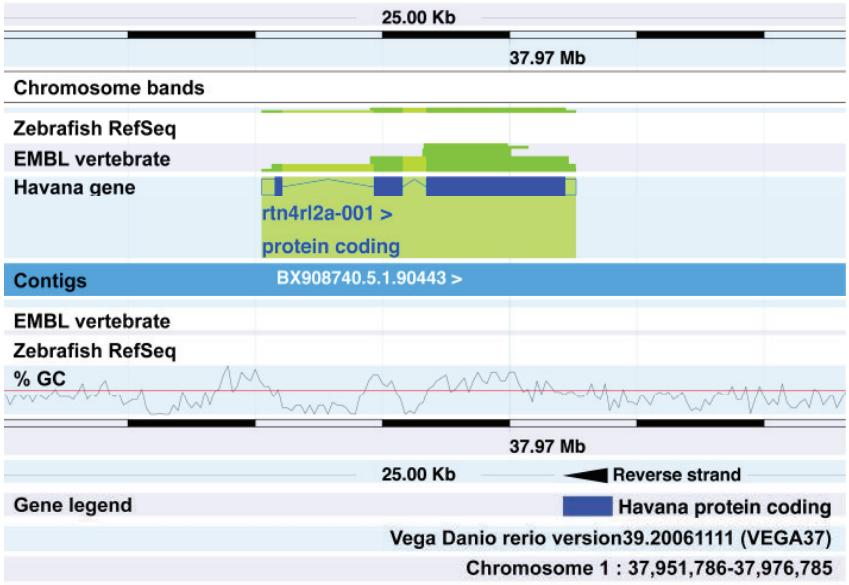

Figure 1. – Localization and main characteristics of the F-RTN4 gene homolog in *Danio rerio*.

The sequence alignment of the 15 COI barcodes reached a total length of 652 positions. No insertions, deletions, or stop codons were observed in any sequence. The base composition was: A = 0.251, T = 0.322, G = 0.172, and C = 0.255. The χ^2 test of heterogeneity of nucleotide frequencies among OTUs failed to reject the null hypothesis ($\chi^2 = 5.3$, p-value = 1) implying that the data set is not at base-composition equilibrium. A slight tendency toward

AT enrichment was present in the data since the GC content per sequence (Table I) was always below 0.5 (mean = 0.43±0.014). In first codon position (GC1) the GC content reached a mean value of 0.53±0.01, versus 0.43±0.004 in second position (GC2), and 0.31±0.035 in third position (GC3). The maximum in GC content was thus observed in first position, with a mean value above 0.5, whereas a minimum was reached in third position with a significant enrichment in AT bases (0.69). The test on the Index of substitution saturation (Iss) resulted in Iss = 0.0869 significantly smaller than Iss.c assuming both a symmetrical ($Iss.c_{sym}$ = 0.73) and an asymmetrical ($Iss.c_{asym}$ = 0.5368) topology (p-value < 0.0001), implying little substitution saturation in the data.

The alignment of the first intron of F-RTN4 reached a total length of 694 bases. Insertions and deletions consisted in two deletions of one base, and one insertion of two bases in the sequence of *Pseudacanthicus leopardus*. The base composition was: A = 0.309, T = 0.371, G = 0.154, and C = 0.166. The χ^2 test of heterogeneity of nucleotide frequencies among OTUs failed to reject the null hypothesis (χ^2 = 2.38, p-value = 1) implying that the first intron of F-RTN4 is not at base composition equilibrium. A significant trend toward AT enrichment was present in the data since the GC content per sequence (Tab. I) was always below 0.5 (mean = 0.32±0.007). The test on the Index of substitution saturation (Iss) resulted in Iss = 0.0482 significantly smaller than Iss.c assuming both a symmetrical ($Iss.c_{sym}$ = 0.734) and an asymmetrical ($Iss.c_{asym}$ = 0.5419) topology (p-value < 0.0001), implying little saturation in the data.

Comparisons between intron 1 and intron 2 of F-RTN4 (Tab. I) revealed significant difference in length between both introns, intron 1 being the longest (Wilcoxon test: V = 120, p-value = 0.0002), as well as significant differences in K2P divergences (V = 4216, p-value < 0.0001), intron 1 being the most divergent. A significant negative correlation between length and GC content was also recorded for both introns (ρ_1 = -0.65, p-value < 0.0001 for intron 1 and ρ_2 = -0.44, p-value < 0.0001 for intron 2). In addition, comparisons between intron 1 and the 3' flanking exon 2 revealed a significant positive correlation between their respective K2P divergences (ρ = 0.75, p-value < 0.0001) but no significant correlation between their respective GC content (ρ = 0.20, p-value = 0.4849). Since intron 1 showed patterns meeting the general patterns observed in the evolution of intronic regions, subsequent analyses were performed with this marker.

The pattern of base diversity provided by the Entropy plots (Fig. 2) of the COI gene and F-RTN4 intron 1 showed a regular pattern of substitutions all along both sequence alignments even though the intronic region displayed less variation. This pattern implies that the

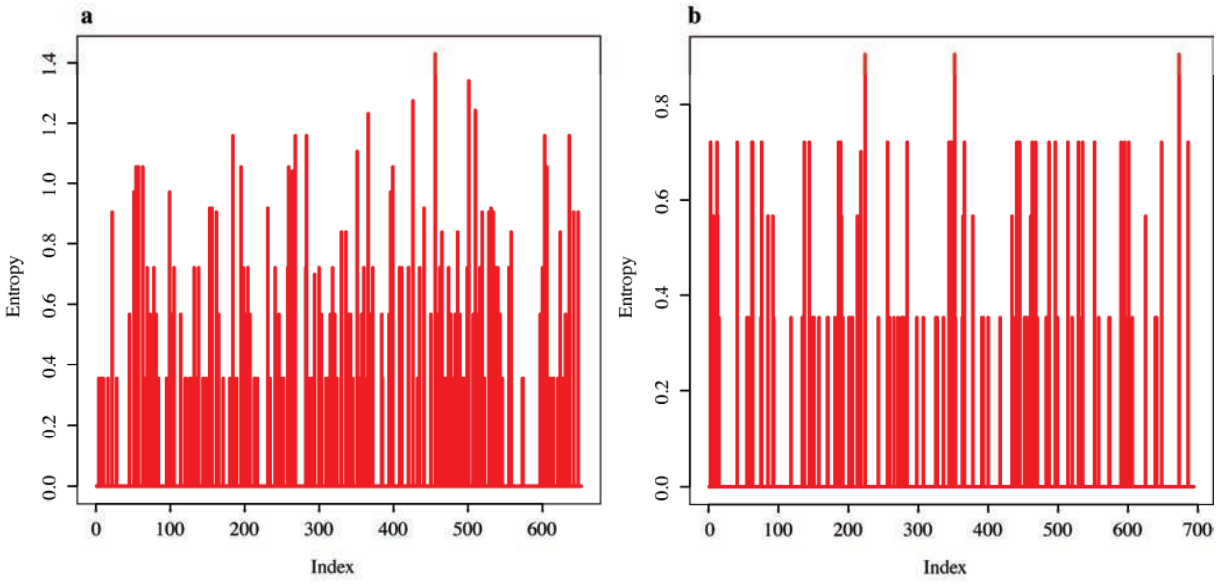

Figure 2. – Entropy plots of each position in alignments for 15 Ancistrini of the *Panaque* group. **A**: COI gene (652 bp). **B**: F-RTN4 intron 1 gene (694 bp).

information was regularly distributed along sequences and is not restricted to a particular region of the alignment.

No conflicting phylogenetic signal was detected between COI and F-RTN4 intron 1 as the ILD test failed to reject the null hypothesis of congruence between data partitions (ILD: $p(X>X_{obs}) = 1$) and that K2P distances matrices were highly correlated ($r = 0.97$, p-value = 0.0001). In the COI alignment, the rate of synonymous substitution d_S was much higher than the rate of non-synonymous substitutions d_N leading to a very small value of $\omega = 0.0459$ implying strong negative (= purifying) selection acting on this marker. For the first intron of F-RTN4, the parameter ζ computed using the 3' flanking exon 2 (length = 225 bp; $\omega = 0.388$) as neutral proxy was very high ($\zeta = 4.79$) implying positive selection acting on this marker. The likelihood ratio tests used in the Wong-Nielsen test confirmed this hypothesis in significantly rejecting the null hypothesis of neutral or negative selection (p-value = 0.0039).

The NJ tree reconstruction computed with the K2P distance matrix of COI sequences grouped the different species within three strongly supported clusters (100% bootstrap) corresponding to *Pseudacanthicus leopardus*, *Hemiancistrus medians*, and a mix of *Peckoltia-Panaqolus* representatives (Fig. 3a). The first diverging species corresponded to *P. leopardus* which possessed the deepest genetic divergences to other representatives of the *Panaque* group with K2P corrected distances ranging between 0.122 and 0.146. The second diverging group gathered the two barcoded populations of *H. medians*. The within species

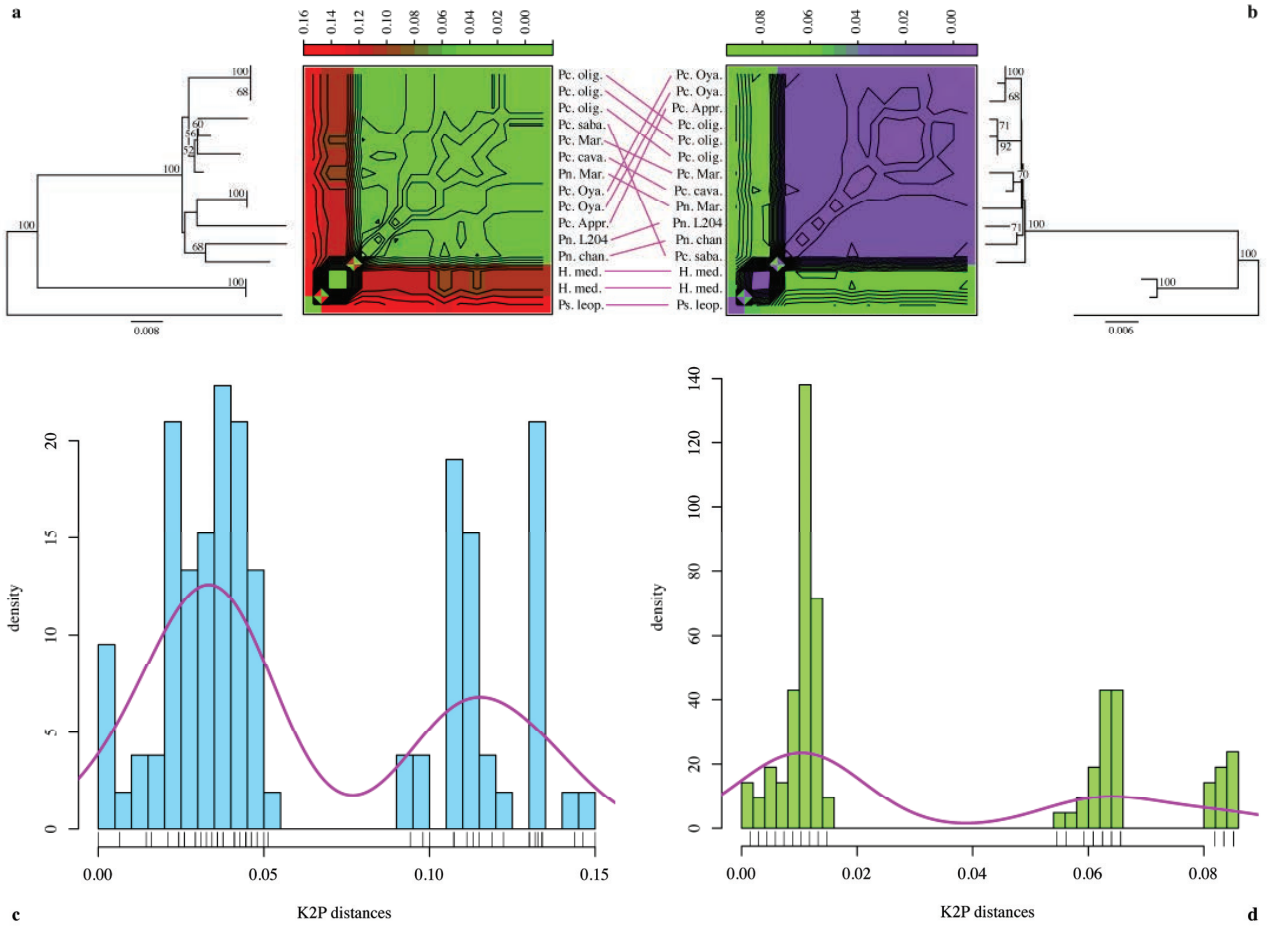

Figure 3. – Analysis of COI and F-RTN4 intron 1 genes of 15 Ancistrini of the *Panaque* group. **A:** NJ tree reconstructed from the K2P distances matrix computed on 652 bases of the mitochondrial COI gene facing the levelplot representation of the ordinated K2P matrix with scale indicating the levels of variation in K2P distances; numbers above branches indicate bootstrap support using 9,999 pseudoreplicates; scale indicates K2P distances; tips labelled as in Table 1. **B**: NJ tree reconstructed from the K2P distances matrix computed on 694 bases of the F-RTN4 gene intron 1 facing the levelplot representation of the ordinated K2P matrix with scale indicating the levels of variation in K2P distances; numbers above branches indicate bootstrap support using 9,999 pseudoreplicates; scale indicates K2P distances; tips labelled as in Table 1. **C**: Histogram of variation of the K2P distances matrix of COI gene using 105 paiwise comparisons; scale indicates the frequencies of pairwise comparisons in a definite range. **D**: Histogram of variation of the K2P distances matrix of F-RTN4 intron 1 gene using 105 paiwise comparisons; scale indicates the frequencies of pairwise comparisons in a definite range.

variation recorded was null between the specimen from Marouini River in French Guiana and the specimen from Tapanahony River in Suriname, whereas between species variation ranged from 0.094 to 0.119. The *Peckoltia-Panaqolus* group was split into two poorly supported groups (< 50% bootstrap), one comprising *Pn. changae* and *Pn.* sp. L204 in a sister position to *Pc.* sp. Approuague and the two specimens of *Pc.* aff. *braueri* Oyapock, and the second comprising the three specimens of *Pc. oligospila* in a sister position to *Pn.* cf. *dentex* plus *Pc. cavatica*, *Pc.* aff. *braueri* Maroni, and *Pc. sabaji*. Within the first group, *Pn. changae* formed the sister species of *Pn.* sp. L204 with low statistical support (64% bootstrap), the two species diverging by 0.038 K2P distances. The sister group of *Panaqolus* grouped the three species from Eastern French Guiana with only the two specimens of *Pc.* aff. *braueri* Oyapock displaying significant support (100% bootstrap). The K2P divergence between the two specimens from Oyapock was null whereas a divergence of 0.036 was recorded between *Pc.* aff. *braueri* Oyapock and *Pc.* sp. Approuague implying divergence of between species level. The divergence between *Panaqolus* representatives and their sister *Peckoltia* species ranged between 0.044 and 0.051. Within the second group, the only strongly supported grouping comprised the different populations of *Pc. oligospila* from the Capim River drainage (100% bootstrap). The within species variation recorded was null whereas divergence to other sister species ranged between 0.029 and 0.031. The first diverging species in the sister group of *Pc. oligospila* was *Pn.* cf. *dentex* in a position weakly supported (52% bootstrap). *Panaqolus* cf. *dentex* displayed small divergence with its sister species with K2P distances ranging between 0.006 (*Pc.* aff. *braueri*) and 0.016 (*Pc. sabaji*). The sister group of *Pn.* cf. *dentex* was also poorly supported (56% bootstrap) and recovered *Pc. cavatica* in a sister position to *Pc.* aff. *braueri* and *Pc. sabaji*, this last grouping being also weakly supported (60% bootstrap). *Peckoltia cavatica* diverged from *Pc.* aff. *braueri* by a K2P distance of 0.014 and from *Pc. sabaji* by a distance of 0.024. These latter diverged by K2P distances of 0.016.

Due to the lack of resolution and the poor generic assignment obtained within the *Peckoltia-Panaqolus* group using the COI K2P matrix (i. e. mixing of species belonging to different genera), a new NJ tree ordination was computed using the K2P matrix of the F-RTN4 gene intron 1 (Fig. 3b). The topological results were highly congruent with the previous analysis and the three highly supported main clusters (100% bootstrap) corresponding to *P. leopardus*, *H. medians* and *Peckoltia-Panaqolus* were recovered. Within the *Peckoltia-Panaqolus* group, deeper relationships were not supported (50% < bootstrap). *Peckoltia sabaji* was the first diverging species and connected at base of the group. The second diverging group comprised *Pn. changae* and *Pn.* sp. L204. The sister relationship

between these two species was moderately supported (71% bootstrap). The third diverging species was *Pn*. cf. *dentex* in a sister position to all remaining *Peckoltia*. The last group of *Peckoltia* was split into two groups, one strongly supported (100% bootstrap) comprising *Pc*. aff. *braueri* Oyapock and *Pc.* sp. Approuague, and a second comprising the remaining species. Within this last group, the three specimens of *Pc. oligospila* were highly supported (92% bootstrap) and formed the sister group of *Pc.* aff. *braueri* Maroni and *Pc. cavatica*. The sister relationship between the two latter species was well supported (70% bootstrap).

Using these two species ordinations, both matrices were reordinated and levelplots reconstructed (Fig. 3a). Even though three levels of variation were present in the COI matrix corresponding to within species (between populations), between species, and between genera levels, the pairwise distances followed a bimodal distribution (Fig. 3c). The within-species level (light green) displayed indeed no variation (K2P = 0). Following the distribution of pairwise distances, the between species level (green to khaki) ranged from 0.006 to 0.051 (mean = 0.033±0.01), and the between genera level (red) from 0.094 to 0.146 (mean = 0.117±0.013). Assuming the current taxonomy, the between species range of variation became 0.014 to 0.04 (mean = 0.031±0.008), and the between genera 0.006 to 0.146 (mean = 0.083±0.043). The smallest between genera K2P distance was recorded between *Pn.* cf. *dentex* and *Pc.* aff. *braueri* (d_{K2P} = 0.006). The mitochondrial signature of *Pn.* cf. *dentex* was thus very similar to that of *Pc.* aff. *braueri*, and smaller divergences between *Pn.* cf. *dentex* and other *Peckoltia* representatives were indeed observed (0.014 to 0.032) compared to divergences observed between *Panaqolus* and *Peckoltia* (0.029 to 0.051). Comparison made to the levelplot representing the F-RTN4 intron 1 K2P matrix (Fig. 3b), revealed three levels of variation corresponding to within species, between species, and between genera levels. The global rate of variation of F-RTN4 intron 1 was half of that of COI. The within species level (pink) ranged from 0.0014 to 0.004 (mean = 0.0024±0.0011), the between species level (purple) from 0.0043 to 0.014 (mean = 0.014±0.0022), and the between genera level (green) from 0.0546 to 0.0851 (mean = 0.069±0.01). Two maxima were observed within the between genera level (Fig. 3d), one located at 0.062±0.003, and a second at 0.084±0.001. Using F-RTN4 intron 1, *Pn.* cf. *dentex* displayed variations to *Peckoltia* representatives (range between 0.0103 and 0.0132) comparable to that observed with the two other species of *Panaqolus* (d_{K2P} = 0.0147) whereas within *Peckoltia* variations ranged between 0.003 and 0.0118.

Taxonomic implications

Based on these results, *Hemiancistrus* is valid, but only represented by the type species *H. medians* within the Guianas. Species here placed in *Peckoltia* but considered in *Hemiancistrus* either previously (*P. braueri*) or presently by some authors (*P. sabaji*) do not cluster with *H. medians* but belong to the *Peckoltia-Panaqolus* group. With a COI K2P distance of 11 % between *Hemiancistrus* and the *Peckoltia-Panaqolus* group (*versus* an intra-group K2P distance ranging from 0 to 3.8 %), *Hemiancistrus* clearly appears very divergent from both *Peckoltia* and *Panaqolus*. It has a similarly high degree of divergence with *Pseudacanthicus* (d_{K2P} = 0.13). The identity of *H. medians* is clarified below accordingly, and the species is redescribed. The twelve populations included in the *Peckoltia-Panaqolus* group represent nine distinct species according to the genetic and morphological divergences. Four new species are recognized for the Guianas, three *Peckoltia* and one species that we assigned to *Panaqolus* for the time being, and described here.

Identity of *Hemiancistrus medians*

Hemiancistrus medians is the type species of *Hemiancistrus* as originally designated by Bleeker (1862:2). The name of *Ancistrus medians* was made available by Kner (1854: 256; 6 of separate) with an unusual diagnosis placed in the general introduction of his main group named “Loricaten” or “Goniodonten”. Kner mentioned that the royal Museum from Stuttgart possessed a wrongly named *barbatus* hypostomid, that was absent from “Hof-Naturalien-Cabinet” (Vienna) before proceeding with the description of this specimen (holotype). Although the description by Kner did not mention its origin, the historical catalog of the Museum of Stuttgart’s collection shows that the only material available to Kner was a specimen registered under the number SMNS 186. Confirming its typical status, it was first registered as *Hypostoma barbatum* Cuv., as mentioned by Kner. This identification was then changed to *Chaetostomus medians* Kner, thus probably only later than the complementary description of the species by Günther (1864: 242) who placed it in the genus *Chaetostomus* [=*Chaetostoma*]. The catalog indicates that it is one dried specimen, locality “Surinam”, collected by “Kappler”, and received Feb. 1849. More ancistrine specimens were obtained later from the same collector, including: two alcohol specimens registered as *Chaetostoma medians* Kner (SMNS 791; received 1860), and one dried specimen originally identified as *Chaetostomus serratus* (SMNS 1729; received 1870). August Kappler was a German researcher and entrepreneur in Suriname. He founded the settlement of Albina on the Marowijne (Surinamese) or Maroni (French) River, where he lived for several years, and

according to our knowledge he collected his materials in the vicinity of Albina (R. Fricke, pers. comm.).

The holotype SMNS 186 was searched for, without success, in 1991 by Ronald Fricke, Curator of Fishes in the Staatliches Museum für Naturkunde, who concluded that it had to be considered lost (see Isbrücker, 1992). In the same publication, Isbrücker invalidly restricted the type-locality on the base of the specimens SMNS 791 ("Rivière Marouini", Maroni system, French Guiana, mentioning that the area was Surinamese during Kappler's time and not French). He provided illustrations based on more recently collected specimens. His view of *H. medians* is the same as that of previous authors, in particular Günther (1864) and Regan (1904) who provided complementary descriptions of the species based on two specimens also collected in Suriname by Kappler, but sent to the British Museum.

Recently, Ronald Fricke found a dried specimen with label indicating SMNS 186, *Pseudacanthicus serratus*, 1 ex, Surinam, Kappler (type-written) and also "Holotype of *Chaetostomus medians* Kner, 1854" (hand-written). It has the inventory number 186 written in ink on its lower side. The specimen, considered as putative holotype, was photographied by N. Khardina and is illustrated on the All Catfish Species Inventory Image Base (Morris, Jager & Sabaj Pérez, 2010; images available at http://acsi.acnatsci.org/base/image_show_wrapper.html?target=589063, accessed on the 2nd Feb. 2011).

Because the typical status of the specimen labelled SMNS 186 has only recently been claimed and, if confirmed, renders the identity of the species different from current usage, and because the original description is crucial but in German language from the mid-nineteenth century, we repeat it here followed by an English translation. It is described in these terms: "Er ist ein *Ancistrus* von gedrungener Gestalt mit wenig strahliger Rückenflosse, gekielten und grobzähnigen Rumpfschildern, einem Bündel sehr langer Haken von Form wie bei *Anc. mystacinus m.* und den folgenden Arten, mit kurzem Kopfe, breiter Schnauze, grossen Augen, sehr langen, bis hinter die Anale reichenden Bauchflossen und sehr stachliger Pectorale; Rumpf und Flossen sind mit grossen, dunklen Flecken besetzt, die Bauchseite ist dicht und klein beschildert. Schon das letzte Merkmal allein unterscheidet ihn als eine von allen mir bekannten verschiedene Art, indem ich keinen *brachypteren Ancistrus* mit beschildertem Bauche kenne, welcher dagegen allen *macropteren Lictoren* eigen ist. Da somit diese Art das vermittelnde Glied zwischen beiden Gruppen darstellt, so dürfte die Benennung *Anc. medians* vielleicht nicht unpassend erscheinen." A literal translation of this description is: "It is an *Ancistrus* of stocky stature with dorsal fin having few rays [Kner's *Brachypteri* subgroup],

keeled and rough-toothed trunk plates, a tuft of very long hooks whose form is like in *Anc. mystacinus m.* and the following species [*A. pictus*, *A. brachyurus*, and *A. scaphirhynchus*, species at present ranged in *Lasiancistrus* and in *Dekeyseria*], with a short head, broad snout, large eyes, very long pelvic fins, which reach behind the anal, and a very pointed pectoral; trunk and fins covered by large dark spots, the ventral side is densely and finely plated. The last character alone already distinguishes it as a different species from all the ones I know, because I do not know any *brachypteren Ancistrus* [defined by Kner as having dorsal fin with few rays, meaning 7 to 9 considering the species included in this sub-group, and belly usually naked] with a plated belly, which on the other hand is particular for all *macropteren Lictoren* [defined by Kner as having dorsal fin with more rays, meaning 11 to 13 considering the species included in this sub-group, and belly constantly plated]. As this species therefore represents an intermediate link between the two groups, perhaps the name *Anc. medians* doesn't seem inappropriate". On page 281, the author briefly placed *Ancistrus medians* according to his systematic position, between *Brachypteri* and *Macropteri Ancistrus*, together with another species that he listed as *Hyp*. (*Anc*.) *itacua*, based on ZMB specimens that would later become the type material of *Hemiancistrus braueri* Eigenmann, 1912, now included in *Peckoltia*.

The specimen SMNS 186 indicated as putative holotype of *H. medians* is a representative of a species of *Pseudacanthicus*. Based on Kner's original description of species, there are several reasons to reject it as the holotype of *H. medians*. It does not agree to the description in the following characters: - stocky structure, or stout body (SMNS 186 not much elevated, and elongated head and body); - rough-toothed trunk plates (plates with particularly long and strong spines); - a tuft of very long hooks (jugal hooks not very long); - broad snout (elongated and more or less pointed, as in all *Pseudacanthicus*); - trunk and fins covered by large dark spots (specimen at present uniformly coloured; one cannot exclude that it was spotted at time of description, however both Surinamese species *Pseudacanthicus serratus* (Valenciennes, 1840) and *P. fordii* (Günther, 1868) are white spotted, and no other known species of *Pseudacanthicus* has large dark spots on the trunk and fins; small dark spots are present in the Amazonian *P. spinosus* (Castelnau, 1855), and irregular dark spots and vermiculations in *P. leopardus* (Fowler, 1914) from the Rupununi River in Guyana); - ventral side densely and finely plated (specimen has no more abdominal skin at all, showing skeleton; *Pseudacanthicus* species generally have no plates on the abdomen; very small plates with odontodes are sometimes present, but restricted to some areas, and generally widely separated from one another; often only odontodes are visible). In addition, as just mentioned, the

specimen has no skin on the abdomen. It appears very unlikely that skin was removed from a dried specimen subsequent to Kner's description, especially for a holotype. We conclude that the specimen is not the holotype, and that it is not SMNS 186. It was very likely labelled as such subsequently, having been confused with SMNS 1729: one dried specimen received from Kappler in 1870 and originally registered as *Chaetostomus serratus*, now *Pseudacanthicus*. The SMNS does not claim to have another specimen listed as number 1729 in their collection.

Considering the previous efforts by Isbrücker and Fricke to find the type specimen, combined with the observation that the recently discovered putative type was incorrect, we believe that the holotype of *Hemiancistrus medians* is really missing in SMNS collection.

Hemiancistrus medians as recognized until recently, and redescribed and illustrated by previous authors (Günther 1864, Regan 1904, Isbrücker 1992) agrees with Kner's original description of the species. In order to clarify the taxonomic status and fix the type locality of *Hemiancistrus medians*, and in order to preserve the stability of nomenclature, the designation of a neotype is needed. We thus designate here the following specimen as the neotype of *Ancistrus medians* Kner, 1854: SMNS 26503 (ex MHNG 2675.094), 164.1 mm SL mm SL, French Guyana, Maroni River basin, Grand Inini River, Saut "S", 3°36'19''N 53°48'25''W, P.-Y. Le Bail *et al.,* 1 Oct. 1997. The specimen is illustrated in Figure 4.

As described by Kner, *H. medians* has a stocky structure, body being stout, deep and wide. Trunk plates are keeled and rough-toothed, with odontodes horizontally aligned on lateral plate series, odontodes of the central line on each plate longer than others. The snout is broad and rounded. The eye is large (23.3% HL for neotype; 18.6-26.9, mean 23.9±2.4 for 12 specimens of 61.9- 196.5 mm SL), dorsal margin of the orbit forming a crest. Jugal odontodes are strong and hooked, longest largely behind posterior margin of orbit in large specimens. Their numbers vary from 20 in a small specimen (61.9 mm SL) up to 60 in a large one (196.5 mm SL) (neotype: 49/53). The mouth is broad. The tooth row cup is medium sized (dentary: 16.9% HL, 15.1-19.8, mean 17.1±1.3; premaxillary 16.9% HL, 15.5-19.6, mean 16.9±1.1), bearing strong teeth with two elongated cusps very similar in size and shape. The number of teeth is variable, slightly higher on dentary (32/25; 14-41, mean 24.1± 6.6) than on premaxillary (21/24; 12-35, mean 20.6 ± 6.2). Dorsal fin is high, with a long spine (30.6% SL; 28.3-37.2, mean 32.7±2.7) and seven branched rays. Pectoral-fin spine is long (35.6% SL; 35.0-37.5, mean 36.1±0.7) and strong, its distal part bearing dorsally elongated odontodes. Pelvic fin is very long (29.0% SL; 27.2-31.0, mean 29.4±1.2), but in examined specimens reaching only to behind middle of anal fin, not passing anal fin as described for the holotype.

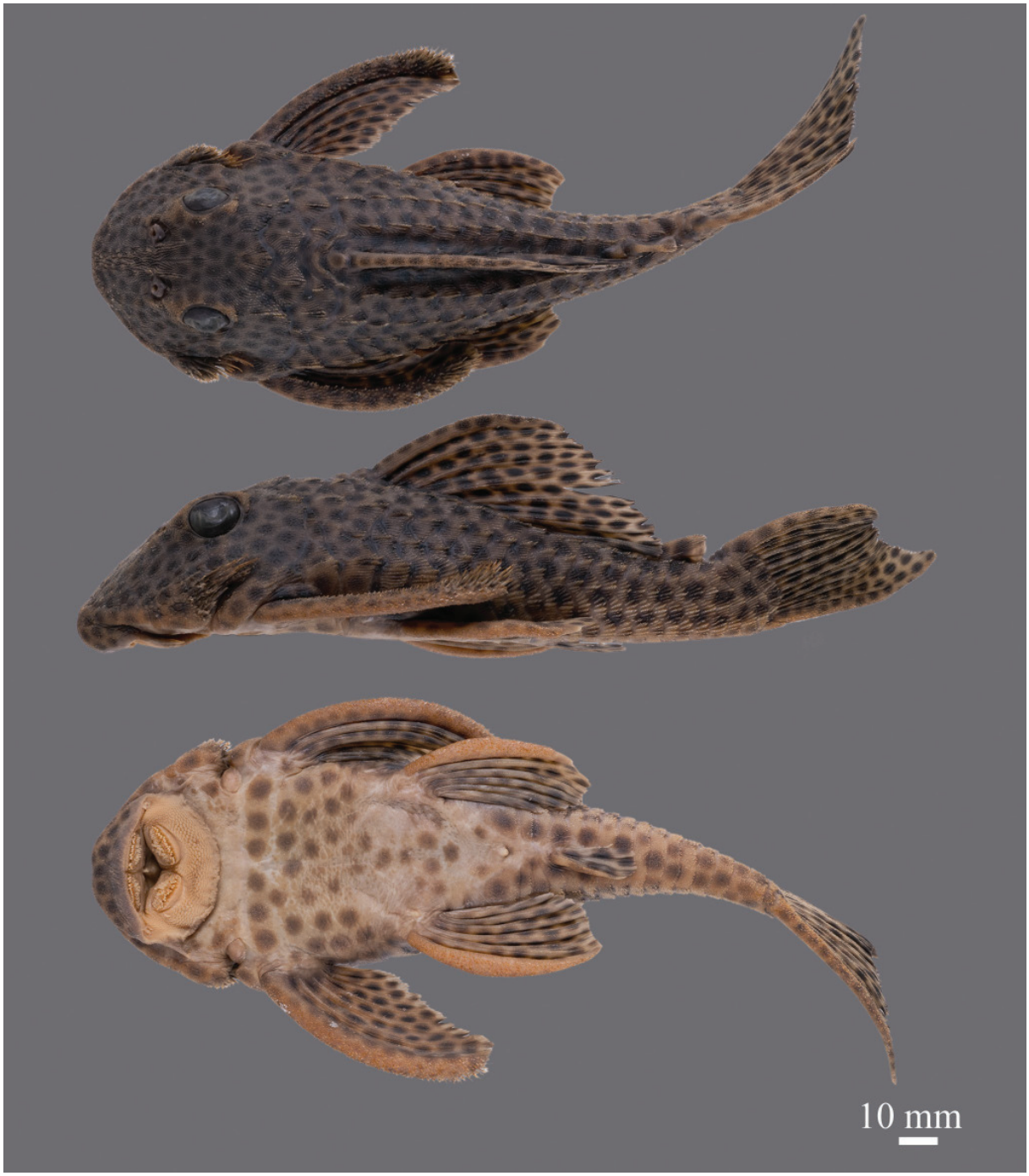

Figure 4. – Neotype of *Ancistrus medians* Kner, 1854, SMNS 26503 (ex MHNG 2675.094), 164.1 mm SL.

Body and fins are covered by numerous large, dark brown, roundish spots. Spots are less numerous and comparatively larger in juveniles (Fig. 5A). They appear black on a yellowish background in living specimens (Fig. 6A). Ventrally, spots are generally missing behind the lip and in the area surrounding the pelvic-fin origin (incl. neotype), and often more broadly. The ventral covering of the species is extremely variable. It may be densely and finely plated

Figure 5. – Juvenile specimens of **A:** *Hemiancistrus medians*, MNHN 2002.0854, 28.8 mm SL; and **B:** *Peckotia otali* n. sp., MNHN 1988.1851, paratype, 26.5 mm SL.

as described by Kner, although not covered completely in any of the examined specimens. The neotype has the abdomen largely covered with very small plates that are mostly not contiguous. Platelets are contiguous and form a dense granular cover in restricted areas under the cleithrum and on sides of the abdomen. Most other specimens, even of large size, show a less densely covered abdomen. Small individuals and some large ones have even the abdomen almost plateless: few platelets, sometimes small granular areas, are present close to the pectoral-fin origin, under the cleithrum, and/or on side of the abdomen, none in central part of abdomen. The high variability of this character may explain the difference shown by several

Figure 6. – Colouration in life. **A**: *Hemiancistrus medians*, MHNG 2717.005, Suriname, Tapanahony River, Kumaru Konde Sula (R. Covain).**B**: *Peckoltia otali* n. sp., French Guiana, Tampoc River (P.-Y. Le Bail). **C**: Holotype of *Peckotia capitulata* n. sp., MHNG 2723.086 (F. Naneix). **D**: Holotype of *Peckoltia simulata* n. sp., MNHN 2011-0012 (R. Covain).

conspecific specimens with Kner's description of the holotype, as was already highlighted by Günther (1864: 242) who nevertheless "had no doubt that our specimens are identical with *Ancistrus medians* of Kner".

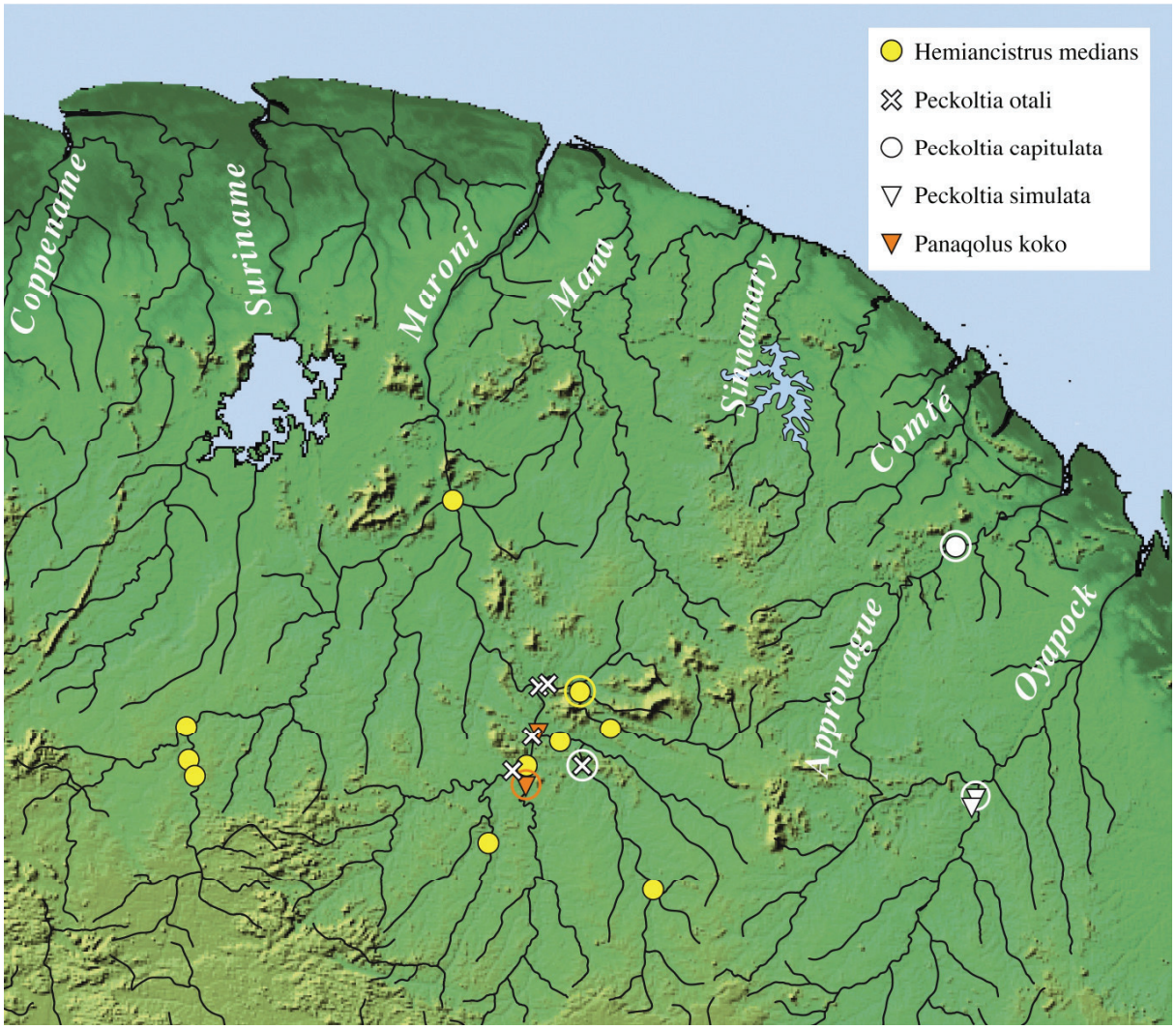

Figure 7. – Geographic distribution of *Hemiancistrus medians*, *Peckotia otali*, *Peckotia capitulata*, *Peckotia simulata*, and *Panaqolus koko*. Circled symbols refer to type localities. One symbol may overlap distinct localities.

Hemiancistrus medians was mostly found in the main channel of rivers within the upper Maroni/Marowijne basin in French Guiana and Suriname (Fig. 7). The species was collected in fast flowing waters in the main channel of the river in the immediate vicinity of waterfalls or rapids. In all places, the substrate was mainly boulders and stones, with gravels in the shallows, sand in the deeper, still water areas, and mud and decayed organic litter in the deepest holes. Exposed wet rocks were covered by the Podostemaceae *Mourera fluviatilis* (Fig. 8). *Hemiancistrus medians* was collected sympatrically with the Hypostominae

Hypostomus gymnorhynchus, *Ancistrus* cf. *leucostictus*, *Ancistrus temminckii*, *Guyanancistrus brevispinis*, *Lithoxus planquettei*, *Panaqolus koko* n. sp., *Peckoltia otali* n. sp., *Pseudancistrus barbatus*, and the Loricariinae *Cteniloricaria platystoma, Harttia guianensis, Metaloricaria paucidens*, and an unidentified Hypoptopomatinae (n. gen. aff. *Parotocinclus*).

Figure 8. – Saut Pierkuru, Tampoc River, Maroni basin.

DESCRIPTIONS OF NEW SPECIES

***Peckoltia otali* Fisch-Muller and Covain, new species**

(Fig. 5B, 7, 9)

Hemiancistrus aff. *braueri* Eigenmann, 1912: Le Bail *et al*., 2000: 232 (description), figs p. 233 (living specimen; map of distribution).

Holotype. - MNHN 2011-0005 (ex MHNG 2723.082, 76.5 mm SL), French Guiana: Tampoc River in Saut Tampoc, tributary of Lawa River, Maroni basin, 3°19’27’’N 53°50’12’’W, P.-Y. Le Bail, P. Keith, P. Gaucher and C. Richard-Hansen, 17 Nov. 1998.

Paratypes. – All from Maroni River basin. French Guiana: - MHNG 2723.082 (6, incl. 1 cleared & stained, 50.6-68.8 mm SL), MNHN 2011-0006 (5, ex MHNG 2723.082, 57.8-66.4 mm SL), MNHN 2011-0007 (1, ex MHNG 2723.082, 68.3 mm SL), same data as holotype. – MHNG 2723.083 (1, 52.9 mm SL), MNHN 2011-0008 (1, ex MHNG 2723.083, 59.8 mm SL), Tampoc River, Elahé, same collectors, 21 Nov. 1998. – MNHN 1988.1851 (3, 26.5-49.3 mm SL), Litani River, Saut Tetombé, upstram of Pilima, N3°14'54'', W54°09'28'', M. Jégu, 4 Oct. 1998. – MNHN 2002.0848 (1, 74.4 mm SL), Marouini River, 2 hours of boat from Antecume Pata, Wayana Amerindian ichthyocide fishing, Y. Fermon, R. Commergnat and R. Ksas, 18 Dec. 2001. - MHNG 2723.084 (1, 20.4 mm SL), MNHN 2011-0009 (2, ex MHNG 2723.084, 19.2 - 63.7 mm SL), Grand Inini River, downstream Saut Batardeau, 3°29'23''N, 53°42'50''W, P.-Y. Le Bail, P. Keith & M. Jégu**,** 28 Sept. 1997. - MHNG 2723.085 (1, 69.0 mm SL), MNHN 2011-0010 (1, ex MHNG 2723.085, 65.0 mm SL), ZMA 119.859 (2, 60.3-63.9 mm SL), Maroni River, Saut Singatetei, 4°23'N, 54°26'W, P.-Y. Le Bail, 9 July 1983. Suriname: Sipaliwini: ANSP 187118 (1, SUR07-05, tag 7023, estimated 64.5 mm SL, end of caudal peduncle and fin missing), Litani River at mouth and confluence with Marowijne/Maroni River, just upstream from settlement of Konya Kondre, 03°17'24''N, 54°04'38''W, J. Lundberg, M. Sabaj, P. Willink, J. Mol *et al.*, 21 April 2007.

Diagnosis

Peckoltia otali is distinguished from all congeneric species by a unique colour pattern of adults, and from Guianese species by its specific barcode sequence (JF747005). It shows numerous blackish-brown spots of irregular size and shape, distributed on head and on entire body except naked ventral areas, resulting in a mottled aspect of dorsum, while spots are aligned to form transverse bands on fins, at least on caudal fin. Juvenile specimens present large transversal blackish bands, or dorsal saddles, on the body that are similar to those characteristic of several *Peckoltia* including the type species *P. vittata*. Brown spots on posterior part of the body are also observed in *Peckoltia oligospila*, *P. sabaji*, *P. capitulata* and *P. simulata*, but in these species spots are rounded, comparatively larger and regularly spaced, and they usually do not form bands on fins. *Peckoltia otali* is further distinguished from these species by a deeper body (22.5-25.7 % SL, mean 23.8, versus less than 23.4 at occiput; 12.4-13.8 % SL, mean 13.1, versus less than 11.7 at caudal peduncle) and a wider body (33.8-37.1 % SL, mean 35.2, versus less than 32.7 at cleithrum). It is distinguished from *P. bachi* that is also mottled, by having the eye high on the head (versus low) and a much narrower interorbital (29.8-34.4 % HL, mean 32.6, versus 57.9-59.9, mean 58.8).

Figure 9. – Holotype of *Peckotia otali* n. sp., MNHN 2011-0005, 76.5 mm SL.

Description

Morphometric and meristic data given in Table II. Small-sized species (largest specimen observed 76.5 mm SL, holotype, breeding male). Body stout, deep and wide. Dorsal profile

gently convex from snout tip to dorsal-fin origin, then sloped ventrally to procurrent caudal-fin rays, and rising straight to caudal fin. Ventral profile flat to caudal fin.

Snout rounded anteriorly, slight rounded ridge from antero-lateral corner of nostril to end of dorsal margin of orbit, supraoccipital with very slight rounded crest. Eye moderately large. Dorsal margin straight flattened from base of first branched dorsal-fin ray to base of adipose fin between light ridges formed with lateral plates of dorsal series. First lateral plates of mid-ventral series forming slight lateral ridge. Caudal peduncle roughly ovoid in cross section, slightly flattened ventrally, and more compressed posteriorly.

Lips covered with short, wide papillae. Buccal papilla generally small, sometimes absent. Lower lip wide, not reaching pectoral girdle, upper lip much narrower. Maxillary barbel reaching posteriorly one-third to two-third of distance to gill opening, sometimes bifurcated. Teeth bicuspid, lateral lobe up to one third smaller than medial lobe.

Head and body plated. Tip of snout naked. Two rows of plates and curved nuchal plate between triangular supraoccipital process and dorsal fin. Five series of lateral plates extending to caudal fin. Abdominal region naked in juveniles, and largely naked in adults. Patches of platelets usually restricted to regions close to pectoral-fin base, pectoral girdle, and, by largest specimens, also anteriorly to anal pore. First anal-fin pterygiophore exposed to form a small platelike structure.

Head and body plates covered by odontodes of relatively uniform size and distribution. Odontodes on lateral series of plates not arranged in distinct longitudinal rows and not forming keels on sides. In breeding males (including holotype), odontodes on plates of postero-dorsal part of body and on adipose fin enlarged to confer hirsute appearance. Odontodes on posterior third of pectoral-fin spine generally enlarged but more in mature males. Opercle supporting odontodes in juveniles but not in most of large specimens (more than 60 mm SL). Posterodorsal margin of opercle covered by one or two plates. Hypertrophied cheek odontodes straight with tips curved, the longest reaching posterior margin of cleithrum in large specimens. Cheek plates evertible to approximately 90^{o} from head.

Dorsal-fin origin slightly anterior to pelvic-fin origin; when adpressed, dorsal-fin tip reaching adipose fin or the plate before. Dorsal-fin spinelet V-shaped, dorsal-fin spine locking mechanism functional. Adipose fin roughly triangular, preceded by single median unpaired plate, short and raised. Adipose spine straight or slightly curved. Pectoral-spine tip reaching one-fourth to one-third of pelvic spine, somewhat longer and more robust in large males. Anal fin with weak spine of approximately same length of first branched ray. Caudal fin slightly

concave, ventral lobe longer than dorsal lobe. Fin-ray formulae: dorsal II,7; pectoral I,6; pelvic i,5; anal i,4; caudal i,14, i.

Colouration

In life (Fig. 6B), base colour yellow-orange, except whitish abdominal region without plates. Base colour tan in alcohol, head darker tan. Sometimes a hardly distinct lighter band between both eyes, and faint dorsal saddles on body. In adults, numerous small dark blackish-brown spots of irregular size and shape distributed on head and entire body except naked abdominal regions; spotting pattern resulting in an irregularly mottled aspect (Fig. 9). Spots may form few irregular transverse bands on posterior part of body of medium-sized specimens. Juveniles show contrasted pattern of colouration with five transversal blackish bands, or dorsal saddles, along body (Fig. 5B). Dark spots present on all fins, centred on fin rays and often combined to form transverse bands, more generally on caudal fin, and especially in smaller specimens.

Distribution and habitat

Peckoltia otali was collected from several localities in the upper Maroni River basin (Fig. 7). It lives in same biotopes as the loricariids *Guyanancistrus brevispinis*, *Hemiancistrus medians*, *Lithoxus planquettei*, *Panaqolus koko* n.sp., *Pseudancistrus barbatus*, *Hypostomus gymnorhynchus*, and *Cteniloricaria platystoma*. In rapids, it is mostly found in sunny and shallow clear water, swiftly flowing currents, with medium-sized rocks substrate. It is a discreet species due to its colouration that resembles its natural environment.

Etymology

Named *otali*, a Wayana Amerindian name meaning secret, in reference to the colouration of the species, similar to its biotope, making it difficult to observe. Wayana Amerindians live on the sides of the Upper Maroni River basin where the new species was found. A noun in apposition.

***Peckoltia capitulata* Fisch-Muller and Covain, new species**

(Fig. 7, 10)

*Holotype***. -** MHNG 2723.086 (75.9 mm SL), French Guiana: Approuague River, rapids of Saut Athanase, 4°11'12''N 52°20'03''W, F. Naneix, 24-27 Feb. 2004.

*Paratype***.** - MNHN 2011-0011 (ex MHNG 2723.086, MUS 331, 1, 59.5 mm SL), same origin as holotype.

Diagnosis

Peckoltia capitulata is characterized by its specific barcode sequence (JF747000), distinguishing it from Guianese species, and by a spotted pattern of colouration of posterior part of body, distinguishing it from all congeners except *Peckoltia oligospila*, *P. bachi*, *P. sabaji*, *P. simulata*, and *P. otali*. In contrast to these five species, no spot is present on the head of *Peckoltia capitulata*. It is additionally distinguished from the spotted species as well as from most other *Peckoltia* species by a shorter head (length 33.4-33.6 % SL versus more than 33.7).

Peckoltia capitulata is also easily separated from both *P. bachi* and *P. otali* by rounded spotting (versus mottling); from *P. bachi* by a much narrower interorbital (34.4-34.5 % HL, mean 34.5, versus 57.9-59.9, mean 58.8); from *P. otali* by several measurements including those listed in diagnosis of the latter; from *P. sabaji* by smaller spots on caudal peduncle and less slender body. It is further distinguished from *P. oligospila* by lower occipital depth (18.4-20.4% SL, mean 19.4, versus 21.1-23.4, mean 21.9), smaller cleithral width (30.3-30.5% SL, mean 30.4, versus 30.9-32.8, mean 32.1) and shorter orbital diameter (6.5-7.0% SL, mean 6.8, versus 6.9-8.1, mean 7.4); from *P. oligospila* and *P. simulata* by a shorter dorsal-fin spine (27.7-27.9 % SL, mean 27.8, versus more than 28.4) and higher caudal peduncle (11.3-11.7% SL, mean 11.5, versus less than 10.6); and from *P. simulata* by tooth shape and length of hypertrophied cheek odontodes (detailed in diagnosis of the latter species).

Description

Morphometric and meristic data given in Table II. Small to medium-sized species (largest specimen examined 75.9 mm SL, no breeding male). Body moderately stout. Dorsal profile gently convex from snout tip to supraoccipital process, then straight to dorsal-fin origin, sloped ventrally to procurrent caudal-fin rays, and rising straight to caudal fin. Ventral profile flat to caudal fin.

Figure 10. – Holotype of *Peckotia capitulata* n. sp., MHNG 2723.086, 75.9 mm SL.

Snout slightly pointed (holotype) to rounded (paratype) anteriorly, slight rounded ridge from antero-lateral corner of nostril to end of dorsal margin of orbit, supraoccipital with very slight rounded crest. Eye moderately large. Dorsal margin straight flattened from base of first branched dorsal-fin ray to base of adipose fin between light ridges formed with lateral plates

of dorsal series. First lateral plates of mid-ventral series forming slight lateral ridge. Caudal peduncle roughly ovoid in cross section, slightly flattened ventrally, and more compressed posteriorly.

Lips covered with short, wide papillae. Buccal papilla small. Lower lip wide, far from reaching pectoral girdle, upper lip much narrower. Maxillary barbel reaching posteriorly halfway the distance to gill opening. Teeth bicuspid, lateral lobe up to one-half smaller than medial lobe.

Head and body plated. Tip of snout naked. Two rows of plates and curved nuchal plate between triangular supraoccipital process and dorsal fin. Five series of lateral plates extending to caudal fin. Abdomen naked. Few patches of platelets below pectoral girdle. First anal-fin pterygiophore exposed to form a small platelike structure.

Head and body plates covered by odontodes of relatively uniform size and distribution. Odontodes on lateral series of plates not arranged in distinct longitudinal rows and not forming keels on sides. Odontodes on plates of postero-dorsal part of body and on adipose fin slightly enlarged. Odontodes on posterior third of pectoral-fin spine enlarged in holotype. Opercle supporting few odontodes. Posterodorsal margin of opercle covered with one or two plates. Hypertrophied cheek odontodes straight with tips curved, not reaching posterior margin of cleithrum. Cheek plates evertible to approximately 90^{o} from head.

Dorsal-fin origin slightly anterior to pelvic-fin origin; when adpressed, dorsal-fin tip not reaching preadipose plate. Dorsal-fin spine locking mechanism fonctional. Adipose fin preceded by single median unpaired plate, short and raised. Adipose spine thin and very slightly curved. Pectoral-spine tip reaching about one-fourth (paratype, left spine, right fin cut close to origin) to quite half (holotype) of pelvic spine, somewhat longer and more robust in large males. Anal fin with weak spine slightly shorter than first branched ray. Caudal fin apparently concave, damaged in both specimens. Fin-ray formulae: dorsal II,7; pectoral I,6; pelvic i,5; anal i,4; caudal i,14, i.

Colouration

Base colour light tan in life (Fig. 6C), somewhat darker in alcohol (Fig. 10). Head with darker areas, and without spot. Three or four faint dorsal saddles on body. Body and fins brown spotted. Spots very small and numerous at dorsal-fin origin level, but becoming rapidly larger posteriorly, about the size of the pupil before end of dorsal-fin base, and less numerous on caudal peduncle. Spots few in number, darker, larger and more rounded on dorsal and caudal fins. No spot on ventral face, abdomen with diffuse pigmentation.

Table II. – Morphometric and meristic data for the type series of *Peckoltia otali*, *Peckoltia capitulata*, *Peckoltia simulata* and *Panaqolus koko*. H: holotype. N: number of specimens measured. P: paratype. SD: standard deviation. Computed statistics include holotype.

	Peckoltia otali					***Peckoltia capitulata***				***Peckoltia simulata***					***Panaqolus koko***				
	H	N	Range	Mean	SD	H	P	Mean	SD	H	N	Range	Mean	SD	H	N	Range	Mean	SD
Standard length (mm)	76.5	23	39.7 – 76.5	62.1	8.4	75.9	59.5	68.4		83.4	3	80.4 – 83.4	82.3	1.6	90.1	6	62.7 – 90.1	77.6	11.1
Percents of standard length																			
Total length	133.1	20	130.2 – 140.4	133.9	3.0	–	–	–	–	136.8	2	132.4 – 136.8	134.6	3.1	132.1	6	126.2 – 132.4	130.9	2.4
Predorsal length	44.3	23	42.7 – 47.7	45.0	1.1	40.6	40.5	40.6	0.1	42.8	3	40.5 – 42.8	41.8	1.2	40.2	6	38.9 – 42.2	40.4	1.4
Head depth at supraoccipital	24.4	23	22.5 – 25.7	23.8	1.0	20.4	18.4	19.4	1.4	20.4	3	19.8 – 20.4	20.0	0.3	19.5	6	18.7 – 21.5	19.9	1.0
Cleithral width	35.2	23	33.8 – 37.1	35.2	0.9	30.5	30.2	30.4	0.2	30.7	3	29.5 – 30.7	30.2	0.6	31.5	6	30.6 – 32.1	31.4	0.5
Head length	36.9	23	35.4 – 40.1	37.2	1.2	33.6	33.4	33.5	0.1	34.4	3	33.7 – 34.4	34.0	0.4	33.8	6	32.9 – 35.1	33.9	0.7
Dorsal spine length	31.2	21	27.5 – 34.3	30.0	1.5	27.9	27.7	27.8	0.1	28.8	3	28.4 – 31.1	29.4	1.4	28.9	6	28.6 – 30.7	29.5	0.8
Dorsal–fin base length	26.8	23	25.1 – 28.2	26.6	0.9	25.8	26.0	25.9	0.1	26.4	3	26.1 – 26.4	26.3	0.1	28.7	6	27.6 – 29.0	28.4	0.6
Interdorsal distance	16.3	23	13.0 – 17.4	15.2	1.0	17.6	18.1	17.8	0.3	16.3	3	16.0 – 16.5	16.3	0.2	16.7	6	16.4 – 18.4	17.1	0.7
Pectoral spine length	33.3	23	30.0 – 34.3	32.2	1.2	30.7	30.6	30.6	0.1	31.2	3	29.4 – 32.2	30.9	1.4	29.8	6	29.8 – 31.3	30.3	0.5
Pelvic spine length	29.0	23	24.8 – 29.6	27.3	1.1	25.9	25.0	25.5	0.6	27.2	3	24.5 – 27.2	26.2	1.5	26.4	6	25.9 – 27.1	26.6	0.5
Thoracic length	22.4	23	22.1 – 26.2	23.9	1.0	21.5	24.3	22.9	2.0	22.8	3	20.4 – 22.8	21.4	1.3	23.7	6	21.6 – 26.7	23.7	1.6
Abdominal length	24.1	23	20.9 – 24.6	23.3	0.9	22.8	22.2	22.5	0.5	24.1	3	23.5 – 24.3	23.9	0.4	24.4	6	22.2 – 24.4	23.7	0.8
Caudal peduncle length	29.4	23	26.5 – 29.8	28.2	1.0	32.2	30.9	31.1	1.5	29.6	3	29.6 – 30.5	30.2	0.5	29.0	6	27.2 – 29.1	28.1	0.9
Caudal peduncle depth	12.9	23	12.4 – 13.8	13.1	0.4	11.3	11.7	11.5	0.3	10.2	3	10.2 – 10.5	10.3	0.1	13.1	6	12.0 – 13.4	12.9	0.5
Adipose spine length	11.1	23	8.4 – 12.1	9.8	0.8	10.5	10.6	10.5	0.9	10.4	3	9.0 – 11.8	10.4	1.4	7.3	6	5.9 – 7.5	6.9	0.6
Anal fin length	19.2	22	14.4 – 19.2	17.0	1.3	14.1	13.9	14.0	0.1	15.9	3	15.3 – 15.9	15.7	0.3	15.9	6	14.4 – 16.3	15.3	0.7
Upper caudal spine length	27.1	18	25.7 – 31.6	28.5	1.6	–	–	–	–	33.6	3	31.5 – 33.6	32.4	1.1	19.5	6	19.5 – 26.3	24.2	2.4
Lower caudal spine length	32.2	20	28.2 – 37.5	32.4	2.3	–	–	–	–	23.3	2	23.3 – 25.8	24.5	1.8	31.0	6	27.1 – 33.3	30.3	2.1
Body width at dorsal–fin origin	29.5	23	28.2 – 32.4	30.5	1.1	26.5	26.0	26.3	0.4	27.0	3	25.6 – 27.0	26.4	0.7	28.5	6	26.3 – 29.5	27.9	1.1
Percents of head length																			
Supracleithral width	79.8	23	76.1 –84.4	80.0	2.4	77.5	80.4	78.9	2.1	74.2	3	71.1 – 74.3	73.2	1.8	77.2	6	75.5 – 80.7	77.6	1.8
Snout length	58.2	23	52.2 – 59.3	56.7	1.9	52.4	59.1	55.7	4.7	61.3	3	57.9 – 61.3	59.5	1.7	60.8	6	56.1 – 60.8	58.1	1.9

Interorbital width	32.3	23	29.8 – 34.4	32.6	1.2	34.5	34.4	34.5	0.1	33.8	3	33.8 – 37.1	35.5	1.7	33.2	6	29.4 – 33.2	31.6	1.5
Plated internostril distance	11.7	23	10.6 – 14.8	12.3	1.1	12.9	13.8	13.4	0.6	13.2	3	11.8 – 13.2	12.3	0.8	10.8	6	8.3 – 10.9	9.9	1.0
Orbital diameter	19.9	23	19.9 – 23.6	21.6	1.0	19.4	20.9	20.1	1.0	18.8	3	17.5 – 20.6	19.0	1.5	18.9	6	18.9 – 20.8	19.8	0.8
Dentary tooth cup length	13.8	23	11.0 – 14.6	13.3	0.8	11.4	12.1	11.7	0.5	10.1	3	10.1 – 11.0	10.6	0.5	10.3	6	9.5 – 11.2	10.3	0.6
Premaxillary tooth cup length	13.8	23	11.0 – 15.0	13.4	0.9	12.2	12.1	12.1	0.1	10.1	3	10.1 – 11.1	10.6	0.5	8.1	6	6.4 – 8.4	7.7	0.7
Interbranchial distance	53.6	23	48.2 – 56.4	53.1	2.2	51.0	51.8	51.4	0.6	51.2	3	49.6 – 52.2	51.1	1.3	51.6	6	51.2 - 54.6	52.9	1.4
Counts																			
Lateral plates	26	23	24 –26	25	1	25	25	25	0	25	3	25 – 26	26	1	25	6	25 – 26	25	1
Plates along dorsal–fin base	8	23	7 – 8	8	1	8	8	8	0	8	3	8	8	0	8	6	8 – 9	8	1
Dorsal to adipose plates	6	23	5–7	6	1	6	6	6	0	5	3	5 – 6	6	1	7	6	6 – 7	7	1
Anal to caudal plates	12	23	11 – 12	12	1	11	12	12	1	9	3	8 – 9	8	1	8	6	7 – 8	7	1
Dentary teeth	–/23	20	17 – 28	25	3	14/14	–	–	–	19/20	3	19 – 29	25	6	4/5	7	4 – 6	5	1
Premaxillary teeth	24/24	22	17 –33	25	4	15/17	–	–	–	17/16	3	17 – 22	20	3	4/4	8	4 – 6	5	1
Hypertrophied cheek odontodes	25/22	23	10–25	19	4	26/29	19/17	23	5	24/25	3	24 – 25	25	1	29/30	9	10 – 29	22	7

Distribution and habitat

Peckoltia capitulata was collected with a cast net at a single place of the Approuague River in swift current of Saut Athanase (Fig. 7). Numerous specimens of *Guyanancistrus brevispinis*, *Hypostomus gymnorhynchus*, and the Loricariinae *Harttia guianensis*, *Rineloricaria platyura*, and *Loricaria* sp., were also found. Water at Saut Athanase is slightly acidic (pH 5-6.4), soft (20-22 $\mu s.cm^{-1}$), and relatively warm (27-30°C). At time of collection, the river was highly turbid as a result of illegal gold mining activities.

Etymology

The specific epithet *capitulata* is Latin and means having a small head.

***Peckoltia simulata* Fisch-Muller and Covain, new species**

(Fig. 7, 11)

Hemiancistrus aff. *braueri* forme Oyapock: Le Bail *et al*., 2000: 232.

*Holotype***.** – MNHN 2011-0012 (ex MHNG 2681.032, GF06-062, 83.4 mm SL), French Guiana, Crique Moulou Koulou, small tributary of the Oyapock River, 3°06'05"N 52°20'34''W, R. Covain, S. Fisch-Muller, P.-Y. Le Bail & J.I. Montoya-Burgos, 4 Nov. 2006.

*Paratypes***.** - MHNG 2681.058 (GF06-119 & 120, 2, 80.4 - 83.0 mm SL), French Guiana, Crique Fifine, left side tributary of the Oyapock River, 3°04'44''N, 52°20'34''W, R. Covain, S. Fisch-Muller, P.-Y. Le Bail & J.I. Montoya-Burgos, 5 Nov. 2006.

Diagnosis

Peckoltia simulata is characterized by its specific barcode sequences (JF747001-JF747002), distinguishing it from Guianese species, and by a spotted pattern of colouration of body including posterior part, distinguishing it from all congeners except *Peckoltia oligospila*, *P. bachi*, *P. sabaji*, *P. otali* and *P. capitulata*. It is distinguished from the latter by teeth shape, with both lobes similar, long (unless if worn), lateral lobe being only very slightly smaller than medial lobe (versus distinctly smaller). Longer hypertrophied cheek odontodes, longest one passing posterior end of cleithrum (versus not reaching) additionally separate *P. simulata* from spotted species. In addition to several measurements, it is further separated from *P.*

bachi and *P. otali* by rounded spotting (versus mottling), from *P. sabaji* by smaller spots on caudal peduncle, and from *P. capitulata* by presence of spots on head (versus absence). It can be further distinguished from *P. oligospila* by having a smaller body depth (19.8-20.4% SL versus 21.1-23.4), narrower body (29.5-30.7 % SL versus 30.9-32.8) and shorter orbital diameter (5.9-7.0 % SL versus 6.9-8.1).

Description

Morphometric and meristic data given in Table II. Fairly medium sized species (largest specimen examined 83.4 mm SL, no breeding male). Body moderately stout. Dorsal profile gently convex from snout tip to supraoccipital process, then straight to dorsal-fin origin, sloped ventrally to procurrent caudal-fin rays, and rising straight to caudal fin. Ventral profile flat to caudal fin.

Snout slightly pointed, low median ridge in front of nostrils, slight rounded ridge from antero-lateral corner of nostril to end of dorsal margin of orbit supraoccipital with distinctly elevated crest. Eye moderately large. Dorsal margin straight flattened from base of first branched dorsal-fin ray to base of adipose fin between light ridges formed with lateral plates of dorsal series. First lateral plates of mid-ventral series forming slight lateral ridge. Caudal peduncle roughly ovoid in cross section, slightly flattened ventrally, and more compressed posteriorly.

Lips covered with short, wide papillae. Buccal papilla small. Lower lip wide, far from reaching pectoral girdle, upper lip much narrower. Maxillary barbel reaching posteriorly half the distance to gill opening or slightly more. Teeth bicuspid, both lobes very similar, lateral lobe only slightly smaller than medial lobe.

Head and body plated. Tip of snout naked. Two rows of plates and curved nuchal plate between pointed tip of supraoccipital process and dorsal fin. Five series of lateral plates extending to caudal fin. Abdominal region largely naked. Patches of platelets restricted to regions close to pectoral girdle, pectoral-fin base, and between pelvic fins posteriorly to anal pore. Some large specimens more largely plated. First anal-fin pterygiophore exposed to form a small platelike structure.

Head and body plates covered by odontodes of relatively uniform size and distribution. Odontodes on lateral series of plates not arranged in distinct longitudinal rows and not forming keels on sides. Odontodes on plates of postero-dorsal part of body and on adipose fin slightly enlarged. Odontodes on tip of pectoral-fin spine generally enlarged, longest in males. Opercle supporting few odontodes. Posterodorsal margin of opercle covered with one or two

Figure 11. – Holotype of *Peckotia simulata* n. sp., MNHN 2011-0012, 83.4 mm SL.

plates. Hypertrophied cheek odontodes straight with tips curved, reaching first plate of mid-ventral lateral series. Cheek plates evertible to approximately 90° from head.

Dorsal-fin origin slightly anterior to pelvic-fin origin; when adpressed, dorsal-fin tip reaching preadipose plate. Dorsal-fin spine locking mechanism functional. Adipose fin preceded by single median unpaired plate, short and raised. Adipose spine straight or slightly curved. Pectoral-spine tip reaching past middle of pelvic spine. Anal fin with weak spine slightly shorter than first branched ray. Caudal fin forked, ventral lobe longer than dorsal lobe. Fin-ray formulae: dorsal II,7; pectoral I,6; pelvic i,5; anal i,4; caudal i,14, i.

Colouration

Base colour brownish orange-coloured in life (Fig. 6D), tan in alcohol, lighter on lower part of caudal peduncle and ventrally, abdomen whitish (Fig. 11). Faint dorsal saddles. Dark rounded spots on head, body and fins. Spots small to medium –sized (smaller or equal to pupil) on head, larger (less than eye) posteriorly. Spots rather irregularly distributed on head as well as on body, where they often superimpose. Similar spots on ventral surface, rarer on naked areas. Spots more contrasted, rounded and spaced on fins.

Figure 12. – Crique Moulou Koulou, Oyapock River tributary.

Distribution and habitat

Peckoltia simulata was collected in two small forest creek tributaries of the Oyapock River in the vicinity of Camopi (Fig. 7), with cast net and dip net on sandy and gravelled bottom with rocks, woods and leaves (Fig. 12). One specimen was hidden in a hollow piece of wood oriented against the current. The new species was collected with representatives of *Ancistrus* cf. *leucostictus*, *A.* aff. *temminckii*, *Guyanancistrus longispinis*, *Farlowella reticulata*, *Rineloricaria stewarti*, and *Otocinclus mariae*. Water parameters were: temperature 25.0-25.7°C, pH 6.1-6.2, and conductivity 13-14 $\mu S.cm^{-1}$.

Etymology

Named *simulata*, a Latin adjective meaning counterfeit, in reference to its similarity with *Peckoltia oligospila*.

***Panaqolus koko* Fisch-Muller and Covain, new species**

(Figs. 7, 13, 14)

Panaque cf. *dentex* (Günther, 1868): Le Bail *et al.*, 2000: 248 (description), figs p. 249 (living specimen).

Holotype. - MNHN 2011-0013 (ex MHNG 2675.096, GF00-115, 90.1 mm SL, male), French Guiana, Marouini River, vicinity of Antecume Pata, Wayana Amerindian fisherman ("Nivrée 2000" mission),19 Oct. 2000.

Paratypes. - All from Maroni River basin. French Guiana: MHNG 2723.088 (1, 73.5 mm SL), MNHN 2011-0014 (1, ex MHNG 2723.088, 79.9 mm SL), French Guiana, Lawa River, Elahé, Le Bail, P. Keith, P. Gaucher and C. Richard-Hansen, 21 Nov. 1998. - MHNG 2723.089 (1, ex MNHN 2002-0851, 89.8 mm SL), MNHN 2002-0851 (2, 61.2-69.4 mm SL), Marouini River, 2 hours of boat from Antecume Pata, Wayana Amerindian ichthyocide fishing, Y. Fermon, R. Commergnat and R. Ksas, 18 Dec. 2001.

Diagnosis

Panaqolus koko is diagnosed by its large and almost spoon-shaped teeth characteristic of *Panaqolus* but bifid instead of most generally unicuspid in congeneric species, and is characterized by its specific barcode sequence (JF747003). It is additionally distinguished

from all *Panaqolus* except *P. dentex* and *P. nocturnus* by a uniformly blackish-brown colouration, versus banded pattern of colouration (*P. purusiensis*, *P. gnomus*, *P. maccus*, and *P. changae*) or spotted pattern of colouration (*P. albomaculatus*). The dark pigment on membrane and branched rays of all fins distinguishes *P. koko* from *P. dentex*, as well as a smaller interorbital width (29.4-33.2 % HL, mean 31.6, versus 38.7), a shorter pectoral spine (29.8-31.3 % HL, mean 30.3, versus 34.8) and a greater caudal peduncle depth (12.0-13.4 % SL, mean 12.9, versus 10.8). The large eye distinguishes it easily from *P. nocturnus* (orbit length 18.8-20.8 % HL versus 13.7-15.9).

Description

Morphometric and meristic data given in Table II. Body moderately deep, head and body depressed. Dorsal profile gently convex from snout tip to dorsal-fin origin, straight and posteroventrally slanted to adipose-fin origin, slightly concave up to first procurrent caudal-fin rays, then rising straight to upper caudal-fin ray. Ventral profile flat to caudal fin. Ventral margin of caudal peduncle rounded.

Snout tapering anteriorly to a largely blunted point, slight rounded ridge from antero-lateral corner of nostril to end of dorsal margin of orbit, tip of supraoccipital pointed and slightly elevated. Eye large. Dorsal margin straight from base of first branched dorsal-fin ray to base of adipose fin between light ridges formed with lateral plates of dorsal series. First lateral plates of mid-ventral series forming slight lateral ridge. Caudal peduncle ovoid in cross section, slightly flattened ventrally.

Oral disk circular to diamond shape, lips covered with short and wide papillae. No buccal papilla. Maxillary barbel larger than one-half orbital diameter. Premaxillary and dentary teeth few (4-6), strong and thick, close to spoon-shape but bicuspid, major cusp large, moderately elongated, lateral cusp short, triangular to rounded (Fig. 14). Teeth slightly larger on dentary, those in middle of tooth row slightly larger than those on either end.

Head and body plated. Snout with a very small naked area near tip. Snout covered by numerous small platelets, with discreet naked interspaces. Posterodorsal margin of opercle covered by two or three plates. A single plate on midline between supraoccipital process and curved nuchal plate preceding dorsal fin. Two plates between ventral suppraoccipital and dorsal peterotic-supracleithrum margins. Five series of lateral plates extending to caudal fin. Branchial and abdominal region generally naked, without plates except for area adjacent to branchial opening, rarely on larger area below pectoral girdle. Area dorsal to pelvic-fin base below ventral margin of lateral plates with 0-4 small plates (1 and 2 on each side in holotype),

Figure 13. - Holotype of *Panaqolus koko* n. sp., MNHN 2011-0013, 90.1 mm SL.

area otherwise naked. First anal-fin pterygiophore covered by skin, except for one specimen (73.5 mm SL) that exhibits small plate-like structure.

Head and body plates covered by odontodes of relatively uniform size and distribution. Odontodes on lateral series of plates not arranged in distinct longitudinal rows and not forming keels on sides. Odontodes on plates of postero-dorsal part of body and on adipose fin slightly enlarged. Odontodes on posterior third of pectoral-fin spine generally enlarged, longest in males. Hypertrophied cheek odontodes straight with tips curved, the longest

Figure 14. - Dentition of *Panaqolus koko* n. sp., MHNG 2723.089, paratype, 69.4 mm SL.

reaching posterior margin of cleithrum in large specimens. Cheek plates evertible to approximately 90^{o} from head.

Dorsal-fin origin slightly anterior to pelvic-fin origin; when adpressed, dorsal-fin tip reaching one or two plates before adipose fin. Dorsal-fin spinelet V-shaped, dorsal-fin spine locking mechanism functional. Adipose fin roughly triangular, preceded by single median unpaired plate, short and raised. Adipose spine slightly curved. Pectoral-spine tip reaching one fourth to one-third of pelvic spine, somewhat longer and more robust in large males. Anal fin with weak spine, approximately same length of first branched ray or shorter. Caudal fin slightly concave, ventral lobe longer than dorsal lobe. Fin-ray formulae: dorsal II,7; pectoral I,6; pelvic i,5; anal i,4; caudal i,14,i. Dorsal procurrent caudal rays: 4-5 (mean 4; holotype 4). Ventral procurrent caudal rays: 3-5 (mean 4; holotype 5).

Colouration

Body and fins uniformly blackish-brown, dark brown ventrally (Fig. 13). In life (see fig. in Le Bail *et al*., 2000: p. 233), dorsum nearly black with some diffuse lighter areas, that probably correspond to the three light brown saddles or blotches described by Schaefer and Stewart (1993) for *P. dentex* and *P. nocturnus*. These light areas probably reflect a stress pattern, as commonly observed in the Loricariidae.

Distribution and habitat

Despite several fish collections, *Panaqolus koko* is only known by a few specimens collected in three stations in the surroundings of Antecume Pata in the upper Maroni River basin (Fig. 7). It was collected in main river channel on a stony substrate at two meters depth. It was further caught with ichthyocide by Wayana Amerindians together with the hypostomins *Hemiancistrus medians*, *Peckoltia otali* and *Pseudancistrus barbatus*, and with the loricariins *Harttia guianensis, Loricaria cataphracta* and *Rineloricaria stewarti*.

Etymology

Named *koko*, a Wayana Amerindian name meaning night, in reference to the dark colouration of the species, and in allusion to the similarly coloured and named *Panaqolus nocturnus*. A noun in apposition.

DISCUSSION

This assessment of the diversity of the Ancistrini constituting the *Panaque* group within the three countries of the Guianas unambiguously reveals the presence of four new species in French Guiana and Suriname. The barcode approach appears as a relevant tool to characterize such diversity, and in revealing hidden diversity, or complex evolutionary patterns. However, despite an effective differentiation between sequences, and accordingly between species, a correct assignment to congeneric species is missing in the present case.Ward (2009) indeed demonstrated that the “10-fold” rule (Hebert *et al*., 2004b) classically used as threshold to distinguish different species (e.i. ten times the mean intraspecific variation for the group under study) was correct, but rather conservative especially considering cryptic speciation. Ward (2009) refined the “10-fold” rule and proposed that specimens with divergences greater than 2% were likely to be different species with a probability greater than 0.95. This threshold

applies to most of our results since interspecific variations were generally greater than 2.1%. Nevertheless between species divergences of 1.4% and 1.6% were observed between *Pc. otali* and *Pc. cavatica* on one hand, and between *Pc. otali* and *Pc. sabaji* on the other hand. Moreover, at the between genera level, *Pk. koko* displayed a divergence of 0.6% with *Pc. otali* (and accordingly divergences of 1.4% and 1.6% with *Pc. cavatica* and *Pc. sabaji*, respectively). Considering Ward's criterion, this very small divergence recorded between *Pk. koko* and *Pc. otali* would lead to consider the former as a distinct population of the latter, whereas both belong to distinct genera. Thus, if one considers that a correct identification necessitates first differentiation and second, a correct assignment (e.g. to congenerics), then the COI marker was not sufficient in the present case to distinguish *Panaqolus koko* from *Peckoltia otali*. To circumvent this issue, we used a nuclear marker to detect potential conflicting signals.

The first step consisted thus in identifying, selecting and characterizing the first intron of the F-RTN4 gene to verify that it formed a candidate of choice for a correct assignment of the species. A preliminary assessment consisted thereby in evaluating that its pattern of evolution fitted the timely-ordered model proposed by Zhu *et al*. (2009). Since this intron is larger, presumably because it is older, it accumulates more variations due to the more numerous indels and mutations, the quantity of information accumulated along the sequences is expected to be more important than in other regions of the gene. Our results corroborate in great part the pattern of intronic evolution observed by Zhu *et al*. (2009), and particularly the first point (ordinal reduction of length and divergence in both exon and intron). Comparisons made between introns 1 and 2 confirmed indeed that intron 1 is significantly longer (in mean 3.5 times longer) and more variable (about two times) than intron 2. Concerning the second point (co-variation of GC content and divergence between exons and flanking introns), a strong and significant correlation is also observed between divergence of intron 1 and its 3' flanking exon. However the co-variation in GC content between these two entities appeared independent and contrasts with Zhu *et al*. (2009) study. This absence of co-variation is very probably due to the lack of variation in GC content in both intron 1 and exon 2, and to the small size of our data set and close relatedness of the species that constitutes it. Larger sampling and higher taxonomic levels would probably correct for this potential artefact. A strong negative correlation between both introns of F-RTN4 and their respective GC content was also observed in our data and those obtained from GenBank. The pattern of co-variation between GC content and intron length appears complex, and if no universal pattern of co-variation between intron length and GC content is observable across taxa, a significant

correlation of both parameters seems always present. Significant negative correlation was indeed observed in primate genomes (Gazave *et al*., 2007) whereas a significant positive correlation was observed in fruit flies (Haddril *et al.*, 2005). The third point was not estimated since it implies comparisons to multiple genes. The within gene characteristics of the selected intron meet generally the global pattern of evolution of intronic regions and can fit accordingly the timely-ordered model.

A second step consisted in evaluating the quality of the signal of the first intron of the F-RTN4 by making between-genes comparisons to the mt COI gene, since a good candidate for barcoding process is expected to contain a significant amount of variation for discrimination and identification purposes as well as a significant amount of phylogenetic signal for a correct assignment of the species (e.g. to the correct genus). The maximum parsimony and distances based tests of congruence of both molecular markers did not detect any conflicting phylogenetic signal between COI and the first intron of the F-RTN4 genes, revealing that a significant common signal was present in both data sets. This result is reinforced by the significance of the tests of substitution saturation that highlighted little saturation in both markers, making each of them good candidates for phylogenetic reconstructions.

Our F-RTN4 fragment was also expected to share the main qualities of the COI gene as characterized in Ward and Holmes (2007). The Shannon's information theoretic entropy plots computed for both markers revealed that each of them was highly variable, especially the COI gene. The information was distributed all along sequences providing enough variation for identification and discrimination purposes. This pattern of variation is essentially due to the degenerate nature of the genetic code that allows numerous substitutions in positions 1 and above all 3 of codons for COI. In F-RTN4, the three maxima observed corresponded to the three insertion-deletions of the *Pseudacanthicus leopardus* sequence. Moreover, important variations were also regularly distributed along sequences. A close examination of the alignment reveals that the substitutions occurring in those positions were not obtained at random but display variations that were lineage dependent. The observed mutations are indeed preserved through lineages implying inheritance from common ancestors. The lack of variation of the first intronic region of F-RTN4 is intriguing compared to that of the COI gene. Non coding regions are indeed expected to be highly variable, or at least more variable than coding regions due to presumably less evolutionary constraints acting on them. Different studies demonstrated that the first intron of genes tends to be the most conserved of all introns (e.g. Keightley and Gaffney, 2003; Chamary and Hurst, 2004; Gaffney and Keightley, 2006; Vinogradov, 2006), implying that they are more selectively constrained. Bradnam and Korf

(2008) demonstrated that early introns (e.g. the first intron) were in average significantly longer than subsequent ones, and hypothesized that this increase in length was probably due to an increase in the presence of functional elements that may be involved in controlling gene expression. This hypothesis is not necessarily in contradiction with the timely-ordered model that stipulates that first introns had more time to be inserted. These inserted elements could effectively be new regulatory elements responsible for new gene functions (e.g. due to alternative splicing). The Wong and Nielsen test (2004) conducted on the first intron of F-RTN4 using its 3' flanking exon as neutral proxy confirmed that this intron was under strong positive selection. However, if the 3' flanking exon appears as the best choice as neutral proxy, assuming the observed co-variation in divergence between intron and flanking exons as stipulated by Zhu *et al*. (2009), its short length could have biased the test. Indeed, a second test using exon 3 of F-RTN4 as neutral proxy (length = 651 bp, $\omega = 0.034499$) resulted in a neutral evolution of the first intronic sequences ($\zeta = 1$; p-value = 0.9999).

Several attempts have been made to develop alternative nuclear markers, and the use of the variable regions of the nuclear ribosomal genes has been proposed (Sonnenberg *et al*., 2007; Raupach *et al*., 2010). Nuclear ribosomal genes are usually considered highly conserved and are classically used to resolve deep phylogenetic relationships (e.g. Le *et al*., 1993; Zardoya and Meyer, 1996). The nuclear ribosomal genes consist of a succession of conserved and variable regions. Among the latter, the D1-D2 LSU (28S) region was proposed by Sonnenberg *et al*. (2007), and the D3 (28S) and V4-V7 (18S) by Raupach *et al*. (2010) as supplement for barcoding purpose. However, these regions remain highly conserved (at least in vertebrates), and the observed mutations remain scarce. For example, using accession numbers provided by Sonnenberg *et al*. (2007) for four European cyprinids belonging to four distinct genera (EF417161: *Alburnoides bipunctatus*; EF417162: *Alburnus alburnus*; EF417165: *Leuciscus cephalus*; EF417167: *Rutilus rutilus*), we obtained a mean K2P divergence of 0.003±0.001 between these four genera for an alignment of 1,052bp of the D1-D2 LSU. By comparison, using four closely related species of the same genus, *Pc. oligospila*, *Pc. otali*, *Pc. simulata*, and *Pc. capitulata*, we obtained a mean divergence of 0.009±0.003 for an alignment of 692 bp of the first intron of F-RTN4, i.e. three times more variable for a fragment one third shorter. These data are not directly comparable since they imply different taxa in the comparison of both makers. However, the fact of obtaining a between genera divergence three times smaller than a between species divergences sustains the hypothesis of the intronic regions to be more variable, and consequently reinforces the relevance of such markers in barcoding comparisons. The first intron of genes thus appears as good candidate

for barcoding purposes. Despite complex evolutionary mechanisms (for a review see Roy and Gilbert, 2006), it possesses sufficient variability for a correct identification and discrimination of the different species; it contains a significant amount of phylogenetic signal for a correct assignment of species to their respective taxa (genus, family, order…); it is functional and evolutionary constrained so that mutational pattern is not obtained at random, but rather preserved through lineages preserving thus the quality of the phylogenetic signal (e.g. limitation of multiple substitutions saturation). One of the main concern using intronic regions, especially early introns, relies however on the occurrence, origin and size of inserted elements responsible for size polymorphism. At higher taxonomic level (e.g. familial rank) these multiple insertions can reach several hundred bases making detection of homology difficult, if not impossible when inserted elements are not homolog. A possible solution to overcome this problem may consist in the selection of coding regions. In this frame, Montoya-Burgos *et al*. (2010) developed recently the Inter-Specific Selective Hybridization (ISSH) method to enrich cDNA libraries in fast evolving genes in non model organisms. This method could therefore allow the detection of exonic regions with high mutational rates providing good nuclear markers for species identification.

The dual approach used in the present study was particularly useful in the resolution of species level taxonomy of genera such as *Peckoltia*. *Peckoltia* species were indeed said to show no morphometric or meristic differences and no obvious difference in morphology, the only difference between species being the colour pattern (Armbruster, 2008: 51). Adults of all three new Guianese species of *Peckoltia* show the presence of dark spots on posterior part of body instead of dark saddles that are present in most of congeneric species. *Peckoltia otali* is clearly distinguished from the five known dorsally dark-spotted species (*Pc. oligospila, Pc. bachi*, *Pc. sabaji*, *Pc. simulata,* and *Pc. capitulata*) by additional colouration characteristics, and by morphometry. On the contrary *Pc. simulata* and *Pc. capitulata* represent cryptic species, both being very similar in colour pattern and morphologically close to *Pc. oligospila*. Nevertheless the divergence recorded between these two sister species (d_{K2P} = 0.036) and between each of them and *Pc. oligospila* (d_{K2P} = 0.048 for *Pc. capitulata*, and d_{K2P} = 0.036 for *Pc. simulata*) coupled with the topological results that never connected them within or in sister position to *Pc. oligospila* clearly demonstrate that *Pc. simulata* and *Pc. capitulata* represent distinct taxa. At the generic level, the barcode approach also unambiguously shows that none of those *Peckoltia* species, like those previously included in *Hemiancistrus* and in *Peckoltia* (e. g. *Pc. braueri*, *Pc. sabaji*), can be assigned to *Hemiancistrus* due to the very high genetic divergence recorded between representatives of both genera.

Conversely, an unexpected result of the DNA barcode analysis was obtained considering *Panaqolus koko*. The COI sequence of this species was indeed highly similar to that of *Pc. otali* whereas these two taxa represent undoubtedly distinct species, and even distinct genera. Only five silent transitions where recorded between sequences (positions 372, 385, 396, 426, and 624) leading to a between genera K2P distance of 0.6%. *Panaqolus koko* also showed smaller divergence with representatives of *Peckoltia* based on the COI gene (K2P distance ranging between 0.6% and 3.3%) whereas to other *Panaqolus* it displayed variations ranging between 2.6% and 3.1%. These intriguing results coupled to the fact that *Pn. koko* and *Pc. otali* are sympatric and endemic to the Maroni/Marowijne basin, and that both frequent the same biotopes, suggest that *Pn. koko* and *Pc. otali* may hybridise. *Panaqolus koko* shares indeed the mitochondrial signature of *Pc. otali* implying mitochondrial introgressive hybridization. This hypothesis was reinforced by the dual approach used herein. The F-RTN4 NJ tree placed indeed *Pn. koko* in a sister position to all *Peckoltia* representatives, except *Pc. sabaji*, and *Pc. otali* in a sister position of *Pc. cavatica*. *Panaqolus koko* does not group with other *Panaqolus* species, even though this topological result is not supported by bootstrap value.

Panaqolus was described by Isbrücker and Schraml (in Isbrücker *et al.*, 2001) based on a group of small species previously included in *Panaque* but defined as the *Panaque dentex* group by Schaefer and Stewart (1993). *Panaque* and *Panaqolus* are Ancistrini diagnosed by acutely angled rows of robust spoon-shaped teeth. *Panaqolus* is notably distinguished from *Panaque* by the absence of a posterior orbital notch and of a ventrolateral keel on caudal peduncle (Schaefer and Stewart 1993). These characters are shared by the new species *Pn. koko*, but the latter has morphological differences with congeneric species that have to be underlined. Teeth in *Pn. koko* approach spoon shape, but some may be more elongated, approaching the condition observed in *Scobinancistrus*. In addition they are always bicuspid, with a lateral cusp smaller but not absent or minute. Schaefer and Stewart (1993) highlighted a large polymorphism in shape and number of teeth for the Venezuelan *Pn. maccus*, and tentatively included in that species two specimens from the Guiana shield drainage having bicuspid teeth similar to those of the new species. In addition to dentition, head and body shape, *Pn. koko* appears quite different from other *Panaqolus* species. It is notably more elongated and narrower, with a smaller interorbital distance and a larger eye. However direct comparison of morphometric data with some previously described species is made difficult when not impossible because the data provided in recent literature (Schaefer and Stewart, 1993; Chockley and Armbruster, 2002) do not correspond to standard measurements. Waiting

for further evidence for its placement into a distinct genus if needed, we prefer to take a conservative position in placing the new species within *Panaqolus*.

The diversity of the *Panaque* group revealed in this study exceeds what was previously recorded for the Guianas. Here, four new species are added to the seven valid taxa. Among the latter, two were described very recently (*Pc. cavatica* and *Pc. sabaji*), one seems to have never been collected again (*Ps. fordii*), and one is confirmed from the Maroni drainage by only few specimens (*Ps. serratus*). Despite several decades of sampling throughout the Guianas countries, species constituting the *Panaque* group remain scarce and poorly known, as attested by literature and poor representation in collections (MNHN, RMNH and ZMA collections examined by SFM and RC). Apart from *Hemiancistrus medians*, that was collected in several places in the Maroni river basin, other members were sporadically collected. It appears that no specimen of the *Panaque* group was collected in rivers from Central Suriname (see this volume). Within the Essequibo drainage, the area of distribution of *Peckoltia cavatica*, described from the Upper Rupununi River close to Massara in Guyana, is here extended to Siparuni River, a left tributary of the Rupununi. *Peckoltia cavatica* was also found again close to its type locality in sympatry of *Pc. sabaji*. Excluding the representatives of *Peckoltia* within the Essequibo drainage, each species of each genus is allopatric. The Essequibo region is indeed still under the strong influence of the Amazon drainage (see de Souza *et al*., in press) and consequently exhibits the highest diversity within *Peckoltia* representatives (*Pc. braueri*, *Pc. cavatica*, and *Pc. sabaji*). From East to West, *Peckoltia simulata* is found in the Oyapock River, *Pc. capitulata* in the Approuague River, and *Pc. otali* in the Maroni/Marowijne River drainages. This latter basin exhibits also the highest diversity of genera of the *Panaque* group, including *Hemiancistrus medians*, *Pseudacanthicus serratus*, *Panaqolus koko* and *Peckoltia otali*. It makes the Maroni River the richest strictly coastal drainage of the Guianas for this group of species. Even if it corroborates other studies (e.g. Covain *et al.*, in press), additional comparisons to other groups have to be conducted to confirm this observation.

COMPARATIVE MATERIAL

Hemiancistrus medians: all from Maroni River basin: Neotype, SMNS 26503 (ex MHNG 2675.094), Grand Inini River, Saut «S». MNHN 2011-0015 (ex MHNG 2675.094) (1) same locality. MHNG 2593.085 (1)(GenBank number AJ318368), Grand Inini River, creek upstream Saut S. MHNG 2593.86 (1), Grand Inini River, dead end branch of Saut S. MHNG 2717.005 (1), Suriname, Tapanahony River, Kumaru Konde Sula. MNHN 1998.1905 (2), Grand Inini River. MNHN 1998.16 (1), Litani River, Saut Tetombé. MNHN 1998.1616 (1), Marouini River, vicinity of Antecume Pata. MHNG 2675.095 (1), MHNG 2675.096 (2, inc.1 c&s), MNHN 2000.5740 (1), MNHN 2000.5752 (3), Litani River upstream of Antecume pata. MCP 38715 (1, ex MHNG 2664.078), MHNG 2664.078 (4), MNHN 2011-0016 (3, ex MHNG 2664.078), Marouini River, vicinity of Antecume Pata. MNHN 2002.0854 (3), Marouini River, 2 hours of boat from Antecume Pata. ZMA 119.868 (6), French Guiana, Maroni River, Saut Singatetei just N of confluence with Tapanahony River. IRD Cayenne (1), Tampoc River, Kayodé. ZMA 115.301 (1), French Guiana, Maroni basin, Marouini River downstream of Epoia.

Panaqolus changae: ANSP 181097 (1, P6218), Peru, vicinity of Iquitos, Itaya River, Amazonas basin. ***Panaqolus dentex***: Holotype, BMNH 1867.6.13.37, Peru, Xeberos, upper Aipena River system, Huallaga basin. ***Panaqolus* sp. L204**: MHNG 2710.093 (1, PE08-900), Peru, aquarium trade, San Alexandro River, tributary of Aguaytia, Ucayali basin.

Peckoltia bachi: Holotype, BMNH 1897.12.1.61, Brazil, Jurua River. Paratypes of *P. ucayalensis*, ANSP 68652-68653 (2), Peru, Ucayali River, Contamana. MEPN unnum. (1), Ecuador, Condor Yacu. MHNG 2358.059 (1), Peru, Ucayali River, Pucallpa. MHNG 2721.054 (5), Peru, aquarium trade. ***Peckoltia braueri***: Holotype, ZMB 3174, paratype, ZMB 3174, Guyana [? Takutu River, Negro River basin]. MHNG 2624.091 (2) aquarium trade, export Boa Vista. ***Peckoltia cavatica***: CSBD xxx (3, ex MHNG 2651.020), MHNG 2651.020 (2, GY04-030), Guyana, Rupununi River, Pregogo. MHNG 2651.044 (1), Guyana, Siparuni River, tributary of middle Essequibo, Iwokrama Forest. ***Peckoltia oligospila***: all from Brazil: Holotype, BMNH 1849.11.8, Brazil, Capin (=Capim) River, tributary of Guamá River, lower Amazon basin. MHNG 2546.097 (8 inc. 1 c&s), MHNG 2552.007 (4), Guamá River at Ourem. MHNG 2550.027 (1), Guamá River 20 km downstream of Ourem. MHNG 2601.078 (1, BR98 076), Mãe do Rio River, tributary of Guamá River. MHNG 2602.006 (1), Guamá River, MHNG 2602.017 (6, BR 98 154-155), Guamá River near Ourem. ***Peckoltia sabaji***: CSBD xxx (1, ex MHNG 2651.016), MHNG 2651.016 (1, GY04-029), Guyana, Rupununi River, Pregogo.

Pseudacanthicus fordii: Syntype, BMNH 1866.8.14.150, Suriname. ***Pseudacanthicus leopardus***: Holotype, ANSP 39345, Guyana, Rupununi River. CSBD xxx (3, ex MHNG 2651.024), MHNG 2651.024 (3), Guyana, Rupununi River, Pregogo. MHNG 2588.050 (2), MHNG 2624.096 (3), MHNG 2677.047 (3), aquarium trade (Negro River basin, probably Demini River). ***Pseudacanthicus serratus***: Holotype, RMNH 3125, Suriname. MHNG 1223.014 (1, dried), French Guiana, Mana River, Saut Sabbat. RMNH 6915 (1), Suriname. RMNH uncat. (1), French Guiana, Maroni River basin, Litani River. ZMA 106.331 (2), Suriname, Suriname River, rapid 1 km. South of Botopasi village. ZMA 106.523 (2), Suriname, Marowijne River, ca. 3 km. N of Albina. ***Pseudacanthicus spinosus***: Holotype, MNHN A.9577, Amazon River.

Acknowledgments. – We are grateful to Michel Jégu (IRD) and Hélène Pagesy (CNRS) for the "Nivrée 2000" campaign; Wayana Amerindians of Antecume Pata, Yves Fermon, Philippe Keith, and François Meunier (MNHN), Jan Mol and Kenneth Wan Tong You (CELOS), Pedro Hollanda Carvalho, Alexandre Fort and Claude Weber (MHNG) for help in different field collections, and Fabien Naneix for the collect and gift of *P. capitulata*.
We gratefully acknowledge for their welcome in collections François Meunier and Philippe Keith (MNHN), Jan Mol and Martien Van Oijen (RMNH), Hielke Praagman and Ronald Vonk (ZMA), and for loan of specimens and tissue samples Mark H. Sabaj (ANSP), James Maclaine (BMNH), Ramiro Barriga (MEPN), Romain Causse and Patrice Pruvost (MNHN), Peter Bartsch (ZMB).

We are indebted to Ronald Fricke for information on SMNS collection, to Jan Pawlowski and José Fahrni (UNIGE) for laboratory facilities and sequencing process, to Stéphane Dray (CNRS, Université de Lyon), Olivier Fedrigo (Duke University), Sergei Kosakovsky Pond (University of California), and Wendy Wong (Cornell University) for statistical assistance, and to Carl Ferraris (Portland) and Dirk Steinke (University of Guelph) for helpful comments on the manuscript and for improvement of English language. In MHNG, Andreas Schmitz helped in German to English translations, Claude Weber provided data on the syntype of *Pseudacanthicus fordii*, Florence Marteau realized the illustrations and Philippe Wagneur the photographs of preserved specimens, and Alain Merguin provided significant logistical help.

We thank Calvin Bernard (CSBD), the North Rupununi District Development Board (NRDDB), and the Iwokrama organization (Guyana); Jan Mol (CELOS) (Suriname); Philippe Gaucher (CNRS; station des Nouragues), Colin Niel and Vincent Piron (Parc de Guyane), Régis Vigouroux and Philippe Cerdan (Hydreco) (French Guiana) for logistic assistance.

For their financial support, we acknowledge the G. and A. Claraz Foundation for the missions in French Guiana in 2006 and in Suriname in 2008; the Académie Suisse des Sciences Naturelles (ScNat) for the missions in Guyana 2004 and French Guiana 2006; and the Constantin Topali Found for the acquisition of lab material in 2009. We also thank the Swiss National Science Foundation, the Département de génétique et évolution of the University of Geneva and the State of Geneva.

The Guyana Environmental Protection Agency, and Ministry of Amerindian affairs; the French Guiana Diren, and Préfecture; and the Surinamese Ministry of Agriculture, Animal Husbandry and Fisheries provided the necessary authorizations and collecting permits.

REFERENCES

ALTSCHUL S.F., MADDEN T.L., SCHÄFFER A.A., ZHANG J., ZHANG Z., MILLER W. & LIPMAN D.J., 1997. - Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389-3402.

ARMBRUSTER J.W., 2003. - *Peckoltia sabaji*, a new species from the Guyana Shield (Siluriformes: Loricariidae). *Zootaxa*, 344: 1-12.

ARMBRUSTER J.W., 2004. - Phylogenetic relationships of the suckermouth armoured catishes (Loricariidae) with emphasis on the Hypostominae and Ancistrinae. *Zool. J. Linn. Soc.*, 141: 1-80.

ARMBRUSTER J.W., 2008. - The genus *Peckoltia* with the description of two new species and a reanalysis of the phylogeny of the genera of the Hypostominae (Siluriformes: Loricariidae). *Zootaxa*, 1822: 1-76.

BLEEKER P., 1862. - Atlas ichtyologique des Indes orientales néêrlandaises, publié sous les auspices du gouvernement colonial néêrlandais: Siluroîdes, Characoîdes et Hétérobranchoides. 112 p., 52 pls. Amsterdam: Fr. Müller.

BLEEKER P., 1864. - Description des espèces de Silures du Suriname conservées aux Musées de Leide et Amsterdam. Mémoire publié par la Société Hollandaise des Sciences à Harlem. 104 p., 16 pls. Harlem: Les Héritiers Loosjes.

BRADNAM K.R. & KORF I., 2008. - Longer first introns are a general property of eukaryotic gene structure. *PLoS One*, 3(8): e3093.

CARDOSO A.R. & LUCINDA P.H.F., 2003. - Three new species of *Hemiancistrus* (Teleostei: Siluriformes: Loricariidae) from the rio Tocantins basin with comments on the genus. *Ichthyol. Explor. Freshwaters*, 14: 73-84.

CARDOSO, Y. P. & MONTOYA-BURGOS J. I., 2009. - Unexpected diversity in the catfish *Pseudancistrus brevispinis* reveals dispersal routes in a Neotropical center of endemism: the Guyanas Region. *Mol. Ecol.* 18: 947–964.

CHAMARY J.V. & HURST L.D., 2004. - Similar rates but different modes of sequence evolution in introns and at exonic silent sites in rodents: evidence for selectively driven codon usage. *Mol. Biol. Evol*., 21*:* 1014–1023.

CHARIF D. & LOBRY J.R., 2007. - Seqin R 1.0-2: a contributed package to the R project for statistical computing devoted to biological sequences retrieval and analysis. *In*: Structural approaches to sequence evolution: Molecules, networks, populations (Bastolla U., Porto M., Roman H.E. & Vendruscolo M., eds.), pp 207-232. New York: Springer Verlag.

CHIACHIO, M. C., OLIVEIRA C. & MONTOYA-BURGOS J. I., 2008. - Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). *Mol. Phyl. Evol.,* 49: 606-617.

CHOCKLEY B.R. & ARMBRUSTER J.W., 2002. - *Panaque changae*, a new species of catfish (Siluriformes: Loricariidae) from eastern Peru. *Ichthyol. Explor. Freshwaters*, 13: 81-90.

COVAIN R., FISCH-MULLER S., MONTOYA-BURGOS J.I., MOL J. LE BAIL P-Y. & DRAY S., in press. - The Harttiini (Siluriformes, Loricariidae) from the Guianas: a multi-table approach to assess their diversity, evolution, and distribution. *Cybium*

DRAY S. & DUFOUR A-B., 2007. - The ade4 package: implementing the duality diagram for ecologists. *J. Stat. Soft*., 22(4): 1-20.

EFRON B., 1979. - Bootstrap methods: Another look at the jackknife. *Ann. Statist*., 7: 1–26.

EIGENMANN C.H., 1912. - The freshwater fishes of British Guiana, including a study of the ecological grouping of species and the relation of the fauna of the plateau to that of the lowland. *Mem. Carneg. Mus.*, 5: 1-578, 103 pls.

ELLIS J.S.**,** KNIGHT M.E., CARVELL C. & GOULSON D., 2006. - Cryptic species identification: a simple diagnostic tool for discriminating between two problematic bumblebee species. *Mol. Ecol. Notes,* 6: 540-542.

FARRIS J. S., KÄLLERSJÖ M., KLUGE A. G. & BULT C., 1994. - Testing significance of congruence. *Cladistics*, 10: 315-319.

FELSENSTEIN J., 1985. - Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39: 783–791.

FERRARIS JR. C.J., 2007. - Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. *Zootaxa*, 1418: 1-628.

FISCH-MULLER S., 2003. - Subfamily Ancistrinae (Armored catfishes). *In*: Check List of the Freshwater Fishes of South and Central America. (by O., Reis R.E., Kullander S.O.& Carl J. Ferraris J., eds.), pp. 373-400. Porto Alegre: EDIPUCRS.

EMBERTON K.C., 1995. - Cryptic, genetically extremely divergent, polytypic, convergent, and polymorphic taxa in Madagascan *Tropidophora* (Gastropoda: Pomatiasidae). *Biol. J. Linn. Soc.*, 55: 183-208.

FISCH-MULLER S., MAZZONI R. & WEBER C., 2001. - Genetic and morphological evidences for two new sibling species of *Ancistrus* (Siluriformes: Loricariidae) in upper rio Tocantins drainage, Brazil. *Ichthyol. Explor. Freshwaters*, 12: 289-304.

FRICKE, R. & ESCHMEYER W. N., 2010. - A guide to Fish Collections in the Catalog of Fishes database. On-line version of 15 January 2010.

GAFFNEY D.J. & KEIGHTLEY P.D., 2006. - Genomic selective constraints in murid noncoding DNA. *PLoS Genet*, 2(11): e204.

GAZAVE E., MARQUES-BONET T., FERNANDO O., CHARLESWORTH B. & NAVARRO A. 2007. - Patterns and rates of intron divergence between humans and chimpanzees. *Genome Biol*., 8(2): R21.

GRANT B.J., RODRIGUES A.P.C., ELSAWY K.M., MC CAMMON A. & CAVES L.S.D., 2006. - Bio3D: An R package for the comparative analysis of protein structures. *Bioinformatics*, 22: 2695-2696.

GÜNTHER A., 1864. Catalogue of the Physostomi, containing the families Siluridae, Characinidae, Haplochitonidae, Sternoptychidae, Scopelidae, Stomiatidae, in the collection of the British Museum. xxii, 455p. London: Trustees.

GÜNTHER A., 1868. Description of fresh-water fishes from Surinam and Brazil. *Proc. Zool. Soc. London*, 1868: 229-247, pls. 20-22.

HADDRILL P.R., CHARLESWORTH B., HALLIGAN D.L. & ANDOLFATTO P. 2005. - Patterns of intron sequence evolution in *Drosophila* are dependent upon length and GC content. *Genome Biol.*, 6: R67.

HALL T.A., 1999. - BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.*, 41: 95–98.

HEBERT P.D.N., CYWINSKA A., BALL S.L. &. DE WAARD J.R., 2003. - Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B*., 270: 313–322.

HEBERT P.D.N., PENTON E.H., BURNS J.M., JANZEN D.H. & HALLWACHS W., 2004a. - Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proc. Natl. Acad. Sci. USA*, 101: 14812-14817.

HEBERT P.D.N., STOECKLE M.Y., ZEMLAK T.S. & FRANCIS C.M., 2004b. - Identification of birds through DNA barcodes. *PLoS Biol.,* 2:1657–1663.

HICKERSON M. J., MEYER C. P. & MORITZ C., 2006. - DNA barcoding will often fail to discover new animal species over broad parameter space. *Syst. Biol*., 55 (5): 729-739.

HILLIS D.M., MORITZ C. & MABLE B.K. (eds.), 1996. - Molecular systematics. Second edition. 655 p. Sunderland, Mass., U. S. A.: Sinauer Associates, Inc.

HUBERT N., HANNER R., HOLM E., MANDRAK N.E., TAYLOR E., BURRIDGE M., WATKINSON D., DUMONT P., CURRY A., BENTZEN P., ZHANG J., APRIL J. &

BERNATCHEZ L., 2008. - Identifying Canadian freshwater fishes through DNA barcodes. *Plos One*, 3: e2490.

ISBRÜCKER I.J.H., 1980. - Classification and catalogue of the mailed Loricariidae (Pisces, Siluriformes). *Versl. tec. Geg., Inst. taxon. Zoöl. (zoöl. Mus.)*, 22: 1-181.

ISBRÜCKER I.J.H., 1992. - Ein fish findet nach 138 Jahren sein Zuhause. Der verborgene Fundort von *Hemiancistrus medians* (Kner, 1854). *In*: Harnischwelse. Aquarien- und Terrarien-Zeitschrift, Sonderheft (Stawikowski R., ed., pp. 56-57. Stuttgart: Eugen Ulmer.

ISBRÜCKER I.J.H., SEIDEL I., MICHELS J.P., SCHRAML E. & WERNER A., 2001. - Diagnose vierzehn neuer Gatungen der Familie Loricariidae Rafinesque, 1815 (Teleostei, Ostariophysi). *In*: Harnischwelse 2 (Stawikowski R., ed.), pp. 17-24. Die Aquarien- und Terrarienzeitschrift, Sonderheft, Eugen Ulmer, Stuttgart.

KEIGHTLEY P.D. & GAFFNEY D.J., 2003. - Functional constraints and frequency of deleterious mutations in noncoding DNA of rodents. *Proc. Natl. Acad. Sci*., 100 (23): 13402-13406.

KEITT T., 2009. - colorRamps: Builds color tables. R package version 2.3. http://CRAN.R-project.org/package=colorRamps.

KIMURA M., 1980. - A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*., 15: 111–120.

KNER R., 1854. - Die Hypostomiden. Zweite Hauptgruppe der Familie der Panzerfische (Loricata vel Goniodontes). *Denkschriften der Akademie der Wissenschaften, Wien, mathemathische-naturwissenschaftlische Klasse*, 7: 251-286, 255 pls.

KOSAKOVSKY POND S.L., FROST S.D.W & MUSE S.V., 2005. - HyPhy: hypothesis testing using phylogenies. *Bioinformatics*, 21: 676–679.

KOSAKOVSKY POND S.L., POON A.F.Y. & FROST S.D.W. - Estimating selection pressures on alignments of coding sequences. *In: Phylogenetic Handbook. A Practical Approach to Phylogenetic Analysis and Hypothesis Testing*, 2nd Ed. (Lemey P., Salemi M. & Vandamme A.-M., eds), pp. 407-418. Cambridge: University Press.

LARA A., PONCE DE LEÓN J.L., RODRÍGUEZ R.L., CASANE D.L., CÔTÉ G., BERNATCHEZ L. & GARCIA-MACHADO E., 2010. - DNA barcoding of Cuban freshwater fishes: evidence for cryptic species and taxonomic conflicts. *Mol. Ecol. Res.*, 10 : 421-430.

LE H.L.V., LECOINTRE G. & PERASSO R., 1993. - A 28S rRNA-Based Phylogeny of the Gnathostomes: First Steps in the Analysis of Conflict and Congruence with Morphologically Based Cladograms. *Mol. Phyl. Evol.*, 2: 31-51.

LE BAIL P.-Y., KEITH P. & PLANQUETTE P., 2000. - Atlas des poissons d'eau douce de Guyane. Tome 2, fascicule II: Siluriformes. 307 p. Paris: M.N.H.N./S.P.N.

LÜTKEN C., 1874. - Ichthyographiske Bidrag. I. Nogle nye eller mindre fuldstaendigt kjendte Pandsermaller, isaer fra det nordlige Sydamerika. *Vidensk. Meddr. dansk naturh. Foren.*, 1873: 202-220, pl. 204.

MANTEL N., 1967. - The detection of disease clustering and a generalized regression approach. *Cancer Res.*, 27: 209-220.

MEYER C.P. & PAULAY G., 2005. - DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol.,* 3: 2229–2238.

MIURA O., KURIS A.M., TORCHIN M.E., HECHINGER R.F., DUNHAM E.J. & CHIBA S., 2005. - Molecular-genetic analyses reveal cryptic species of trematodes in the intertidal gastropod, *Batillaria cumingi* (Crosse). *Int. J. Parasit.*, 35:793-801.

MIRANDA RIBEIRO A., 1912. - Loricariidae, Callichthyidae, Doradidae e Trichomycteridae. *In*: Commissao de Linhas Telegraphicas Estrategicas de Matto-Grosso ao Amazonas, Annexo 5, Historia Natural, Zoologia, Rio de Janeiro: 5-31, 31 pls.

MONTOYA-BURGOS J.-I., MULLER S., WEBER C. & PAWLOWSKI J., 1998. - Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences. *In*: Phylogeny and Classification of Neotropical Fishes. Part 3 - Siluriformes. (Malabarba L.R., Reis R.E., Vari R.P., Lucena Z.M.S.& Lucena C.A.S., eds.), pp. 363-374. Porto Alegre, Brasil: Edipucrs.

MONTOYA-BURGOS J.I., WEBER C. & LE BAIL P.-Y., 2002. - Phylogenetic relationships within *Hypostomus* (Siluriformes: Loricariidae) and related genera based on mitochondrial D-loop sequences. *Revue suisse Zool.*, 109: 369-382.

MONTOYA-BURGOS, J. I., FOULON A. & BAHECHAR I., 2010. - Transcriptome screen for fast evolving genes by Inter-Specific Selective Hybridization (ISSH). *BMC Genomics*, 11: 126.

MORITZ C. & CICERO C., 2004. - DNA barcoding: promise and pitfalls. PLoS Biol., 2(10): e354.

MORRIS, P.J., YAGER, H.M. (progs) & SABAJ PÉREZ, M.H. (ed.) 2006. - ACSImagebase: A digital archive of catfish images compiled by participants in the All Catfish Species Inventory. [WWW image Database] URL http://acsi.acnatsci.org/base .

PAGE R.D.M. & HOLMES E.C., 1998. - Molecular Evolution: A phylogenetic approach. 346 p. Oxford : Blackwell Scientific.

PARADIS E., 2006. - Analysis of Phylogenetics and Evolution with R. 211 p. New York: Springer.

PARADIS E., CLAUDE J. & STRIMMER K., 2004. - APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20: 289-290.

PINNA M.C.C. de, 1998. - Phylogenetic relationships of Neotropical Siluriformes (Teleostei: Ostariophysi): Historical overview and synthesis of hypothesis. *In*: Phylogeny and Classification of Neotropical Fishes. Part 3 - Siluriformes. (Malabarba L.R., Reis R.E., Vari R.P., Lucena Z.M.S.& C.A.S. Lucena, eds.), pp. 279-330. Porto Alegre, Brasil: Edipucrs.

PYBUS O.G. & SHAPIRO, B. - The Natural selection and adaptation of molecular sequences. In: Phylogenetic Handbook. A Practical Approach to Phylogenetic Analysis and Hypothesis Testing, 2nd ed. (Lemey P., Salemi M. & Vandamme A.-M., eds), pp. 407-418. Cambridge: University Press.

R DEVELOPMENT CORE TEAM, 2009. - R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

RAUPACH M.J., ASTRIN J.J., HANNIG K., PETERS M.K., STOECKLE M.Y. & WÄGELE J.-W., 2010. - Molecular species identification of Central European ground beetles (Coleoptera: Carabidae) using nuclear rDNA expansion segments and DNA barcodes. *Front. Zool.*, 7 : 26.

REGAN C.T., 1904. - A monograph of the fishes of the family Loricariidae. *Trans. zool. Soc. Lond.*, 17: 191-350, pls 199-121.

REIS R.E., KULLANDER S.O. & FERRARIS C.J. (eds.), 2003. - Check List of the Freshwater Fishes of South and Central America (CLOFFSCA). 728 p. Porto Alegre: EDIPUCRS.

ROY S.W. & GILBERT W., 2006. - The evolution of spliceosomal introns: patterns, puzzles and progress. *Nat. Rev. Genet.*, **7** (3): 211–21.

SAITOU N. & NEI M., 1987. - The neighbor-joining method; a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406-425.

SARKAR D., 2010. - lattice: Lattice Graphics. R package version 0.18-3. http://CRAN.R-project.org/package=lattice.

SCHAEFER S.A., 1986. - Historical biology of the loricariid catfishes: phylogenetics and functional morphology. 198 p., 46 figs. Unpubl. Ph. D. thesis, University of Chicago.

SCHAEFER S.A. & S STEWART D.J., 1993. - Systematics of the *Panaque dentex* species group (Siluriformes: Loricariidae), wood-eating armored catfishes from tropical South America. *Ichthyol. Explor. Freshwaters*, 4: 309-342.

SHANNON C. E., 1948. - A mathematical theory of communication. *Bell System Tech. J.,* 27: 379-423, 623-656.

SONNENBERG R, NOLTE A.W., TAUTZ D., 2007. An evaluation of LSU rDNA D1-D2 sequences for their use in species identification. *Front. Zool.,* 4: 6.

SOUZA DE L.S., ARMBRUSTER J.W. & WERNEKE D.C., in press. - The influence of the Rupununi portal on distribution of freshwater fish in the Guiana Shield. *Cybium*

VALDEZ-MORENO M., IVANOVA N.V., ELÍAS-GUTIÉRREZ M., CONTRERAS-BALDERAS S. & HEBERT P.D.N., 2009. - Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. *J. Fish Biol*., 74: 377–402.

SWOFFORD D. L., 1998. - PAUP*. Phylogenetic Analysis Using Parcimony (*and Other Methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

VARI R.P., FERRARIS JR C.J., RADOSAVLJEVIC A. & FUNK V.A., 2009. - Checklist of the Freshwater Fishes of the Guiana Shield. *Bull. Biol. Soc. Wash.*, 17: i-vii, 1-95.

VINOGRADOV A.E., 2006. - Genome design' model and multicellular complexity: golden middle. *Nucleic Acids Res.*, 34(20): 5906-14.

WARD R.D., 2009.- DNA barcode divergence among species and genera of birds and fishes. *Mol. Ecol. Res*., 9: 1077-1085.

WARD R.D. & HOLMES B.H., 2007. - An analysis of nucleotide and amino acid variability in the barcode region of cytochrome c oxidase I (cox1) in fishes. *Mol. Ecol. Notes*, 7: 899–907.

WARD R.D., ZEMLAK T.S., INNES B.H., LAST P.R. & HEBERT P.D.N., 2005. - Barcoding Australia's fish species. *Phil. Trans. R. Soc. B*, 360: 1847–1857.

WARD R. D., COSTA F. O., HOLMES B. H. & STEINKE, D. 2008a. - DNA barcoding of shared fish species from the North Atlantic and Australasia: minimal divergence for most taxa, but *Zeus faber* and *Lepidopus caudatus* each probably constitute two species. *Aquat. Biol.*, 3: 71-78.

WARD R. D., HOLMES B. H. & YEARSLEY G. K., 2008b. - DNA barcoding reveals a likely second species of Asian seabass (barramundi) (*Lates calcarifer*). *J. Fish Biol*., 72: 458–463.

WARD R.D., HANNER R. & HEBERT P.D.N., 2009. - REVIEW PAPER: The campaign to DNA barcode all fishes, FISH-BOL. *J. Fish Biol*., 74: 329-356.

WONG W.S.W. & NIELSEN R., 2004. - Detecting Selection in noncoding regions of Nucleotide Sequences. Genetics, 167: 949-958.

XIA X. & XIE Z., 2001. - DAMBE: Data analysis in molecular biology and evolution. *J. Hered*., 92: 371-373.

XIA X., XIE Z., SALEMI M., CHEN L. & WANG Y., 2003. - An index of substitution saturation and its application. *Mol. Phylogenet. Evol.*, 26: 1-7.

ZARDOYA R. & MEYER A., 1996. - Evolutionary relationships of the coelacanth, lungfishes, and tetrapods based on the 28S ribosomal RNA gene. *Proc. Natl. Acad. Sci. USA*, 93: 5449–5454.

ZHU L., ZHANG Y., ZHANG W., YANG S., CHEN J.-Q. & TIAN D., 2009. - Patterns of exon-intron architecture variation of genes in eukaryotic genomes. *BMC Genomics*, 10: 47.