

UNIVERSIDADE ESTADUAL PAULISTA
INSTITUTO DE BIOCÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
ÁREA DE CONCENTRAÇÃO: GENÉTICA

GABRIEL DE SOUZA DA COSTA E SILVA

**ESTUDOS EVOLUTIVOS EM LORICARIIDAE
(OSTARIOPHYSI: SILURIFORMES)**



Botucatu - SP

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ORIENTADO: GABRIEL DE SOUZA DA COSTA E SILVA
COORIENTADOR: DR. FÁBIO FERNANDES ROXO
ORIENTADOR: DR. CLAUDIO DE OLIVEIRA

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“Ah, tem uma repetição, que sempre outras vezes em minha vida acontece. Eu atravesso as coias – e no meio da travessia não vejo! – só estava entretido na idéia dos lugares de saída e de chegada. Assaz o senhor sabe: a gente quer passar um rio a nado, e passa; mais vai dar na outra banda é num ponto muito mais embaixo, bem diverso do que em primeiro se pensou. Viver não é muito perigoso?” (Guimarães Rosa – Grande Sertão: Veredas)

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Introduction

Systematic history of Loricariidae with emphases in Hypostominae and Hypoptopomatinae

Loricariidae is a Neotropical freshwater fish familie of Siluriformes widespread distributed throughout most of the Neotropics, extending from Costa Rica in the north to Argentina in the south. Currently represent roughly 920 valid species (Eschmeyer & Fong, 2016) distributed in five subfamilies: Hypostominae, Loricariinae, Hypoptopomatinae, Delturinae and Rinelelinae (*sensu* Lujan et al, 2015). The Loricariidae familie have a long and complex systematic history, with morphological and molecular studies (Eigenmann and Eigenmann, 1890; Regan, 1904; Gosline, 1945, 1947; Isbrucker, 1980; Howes, 1983, Schaefer, 1987; Montoya-Burgos et al, 1998, 2002; Montoya-Burgos, 2003; Armbruster, 2004; Chiachio et al, 2008; Cramer et al, 2011; Covain and Fisch-Muller, 2012, Roxo et al, 2012, Roxo et al., 2014; and Lujan et al.,2015). However the historical evolutionary, and mechanisms that lead to diversification are poorly understood in Loricariids.

The systematics of Loricariidae started to be highlighted with works of Eigenmann and Eigenmann (1980) who divided the familiy in three subfamilies: Loricariinae, Hypoptopomatinae and Plecostominae. After Eigenmann & Eigenmann (1980), Regan (1904) divided Loricariidae in five subfamilies Argiinae, Hypoptopomatinae, Loricariinae, Neoplecostominae and Plecostominae, and Gosline (1945, 1947) recognized five subfamilies: Plecostominae, Hypoptopomatinae, Loricariinae, Astroblelinae (Argiinae) and Neoplecostominae.

Isbrucker (1980) in a revisionary study, considered six subfamilies: Loricariinae, Hypoptopomatinae, Neoplecostominae, Hypostominae, Ancistrinae and Lithogeninae. However, Howes (1983) was the first author to perform the cladistic analyses within

Loricariidae, and found six subfamilies: Loricariinae, Hypoptopomatinae, Hypostominae, Neoplecostominae, Lithogeninae and Chaetostominae. Schaefer (1987) also studies the family using cladistic methods and recognized six subfamilies in Loricariidae: Lithogeninae, Neoplecostominae, Hypoptopomatinae, Loricariinae, Ancistrinae and Hypostominae.

Schaefer (1991, 1998) based in morphological characters, presented a phylogenetic analysis in subfamily Hypoptopomatinae, provided phylogenetic diagnosis for included genera plus an undescribed genus from Venezuela, described as *Niobichthys* (Schaefer and Provenzano, 1998). The study of Schaefer (1991) was based in 55 osteological and myological features used to construct a phylogenetic analysis of subfamily Hypoptopomatinae (genera *Acestridium*, *Hypoptopoma*, *Microlepdogaster*, *Otocinclus*, *Otothyris*, *Oxyropsis*, *Parotocinclus*, *Pseudotothyris*, *Schizolecis*, and a new genus from Venezuela). This study found the genus *Otocinclus* paraphyletic since the type species *Otocinclus vestitus* are more closely related to others Hypoptopomatinae than to other *Otocinclus* species. Additionally, Schaefer (1991), created the tribe Otothyrinae to include the genera *Otothyris*, *Pseudotothyris*, *Parotocinclus*, *Schizolecis* and *Pseudotocinclus*. Schaefer (1998) prompted a re-evaluation of the phylogeny and classification of schaefer (1991) with the inclusion of six new genera. The phylogeny is based in 46 morphological characters and species 17 genera of Hypoptopomatinae. The classification remain the same of Schaefer (1991), except for the inclusion of *Microlepdogaster* in the ter group Otothyrinae. *Nannoptoma* is the sister group of *Hypoptopoma*, and *Nioblichthys* is the sister group of the all Hypoptopomatini, except *Otocinclus*. Whithin Otothyriini the new taxon 3 from Tocantins and Xingu basin are sister-group of all others members of the tribe. *Microlepdogaster* was sister group of the clade composed of the genera *Schizolecis*,

Pseudotothyris and *Otothyris*, and *Eurychelichthys* was sister group of new taxon 2 from Coastal rivers of Sergipe state.

Montoya-Burgos et al. (1998) performed the first molecular phylogenetic study of the family Loricariidae. This study was based in partial sequences of 12S and 16S rRNA genes. The results suggested that the subfamilies recognized previously by Isbrücker (1980) and Schaefer (1987) did not form monophyletic groups, the species *Pareiorhina* sp., *Kronichthys* sp., Hypostominae unidentified *Hemipsilichthys splendens*, *Hemipsilichthys* sp., and *Isbrueckerichthys duseni*, before placed in Hypostominae were close related with Hypoptopomatinae species and *Neoplecostomus* sp.

Armbruster (2004) presented a hypothesis of loricariids interrelationship, emphasizing the subfamilies Hypostominae and Ancistrinae, based on external morphology, osteology and digestive tract anatomy. The old Subfamily Ancistrinae was recognized as the tribe Ancistrini, Hypostomini was revalidated, and three new tribes were created (Corimbophanini, Rhinelepini, and Pterigoplichthini). Armbruster (2004) also recognized the monophyly of the subfamily Loricariinae, sister group of Hypostominae, the genus *Isbrueckerichthys*, *Kronichthys* and *Pareiorhina* appeared more related to Neoplecostominae than Hypostominae.

Chiachio et al. (2008), in a molecular study of Hypoptopomatinae found that the Hypoptopomatinae (*sensu* Schaefer et al. 1998) was not monophyletic and proposed the recognition of three monophyletic lineages named Hypoptopomatinae (former tribe Hypoptomatini), Otothyrinae (former tribe Otothyrinae) and Neoplecostominae, including the genus *Pseudotocinclus*. The close relationship between *Pseudotocinclus* and *Pareiorhina* was previously recognized by Montoya-Burgos (1998) and posterior works of Cramer et al. (2008, 2011) and Roxo et al. (2012). In the results of Chiachio et al. (2008) the new subfamily Otothyrinae forms is sister group with Neoplecostominae

and both subfamilies sister group of Hypoptopomatinae. Ootothyrinae forms is sister group with Neoplecostominae and both subfamilies sister group of Hypoptopomatinae. Ootothyrinae is composed for the genera *Hisonotus*, *Corumbataia*, *Schizolecis*, *Pseudotothyris*, *Microlepdogaster*, *Ootothyropsis*, *Ootothyris*, *Parotocinclus*, *Eurychelichthys* and *Epactionotus*, and Hypoptopomatinae for the *Otocinclus*, *Lampiella*, *Macrotocinclus*, *Hypoptopoma*, *Nannoptyopoma*, *Oxyropsis* and *Acestridium*. Within Ootothyrinae the genus *Hisonotus* was not found as monophyletic, since there was species related with *Corumbataia*, others with *Epactionotus* and others with *Eurycheilichthys*.

Roxo et al. (2012) performed a molecular phylogeny of the subfamily Neoplecostominae using a multiple approach a maximum parsimony, maximum likelihood and a bayesian methods. Their results showed that the subfamily Neoplecostominae is monophyletic including *Pseudotocinclus*, and three clades were recognized. The first one is composed of *Pareiorhina rudolphi* the type species of the genus, *P. cf. rudolphi* and *Pseudotocinclus*. The second is composed of *Isbrueckerichthys*, *Pareiorhaphis*, *kronichthys* and *Neoplecostomus ribeirensis*. The third is composed of the species of the genera *Neoplecostomus*, except *N. ribeirensis*, *Pareiorhina carrancas*, *P. cf. carrancas*, *Pareiorhina* sp.1 and an undescribed taxon referred as New genus 2.

Covain and Fisch-muller (2012) in a molecular phylogenetic study suggested the paraphyly of *Pseudancistrus*, presenting five independent lineages within the *Pseudancistrus*. Close related with the *Pseudancistrus barbatus* (type species of *Pseudancistrus*) were the true *Pseudancistrus*. Other two lineages comprise *Guyanancistrus* and *Lithoxancistrus* that were resurrected. The clades formed by *Pseudancistrus pectegenitor* plus *P. sidereus* and *P. orinoco* represent genera not

described yet. Silva et al (2013) with a description of a new *Pseudancistrus* (*P. zawadzki*) and its phylogenetic position, using molecular data confirmed the results found by Covain and Fisch-Muller (2012) about the paraphyly of *Pseudancistrus*.

Roxo et al. (2014) in a molecular phylogenetic study recognized the same three subfamily-level found by Chiachio et al. (2008) (Hypoptopomatinae, Otothyriinae and Neoplecostominae). Moreover, several genera were not monophyletic groups (*Hisonotus*, *Parotocinclus*, *Neoplecostomus*, *Pareiorhina*). The genera *Eurychelichthys*, *Epactionotus* and *Otothyropsis* were closely related with *Hisonotus notatus* (type species of *Hisonotus*) and in future these genera should be synonymized in *Hisonotus*. *Hisonotus insperatus*, *H. oliveirai*, *H. piracanjuba*, *Hisonotus* sp.4, *Hisonotus parsi* and *Hisonotus* sp.5, formed a monophyletic group not related with *Hisonotus notatus*. In Roxo et al (2015) the species of these group was relocated and described in a new genus, named *Curculionichthys*.

Lujan et al (2015) in the most extensive work with a molecular phylogeny of the Loricariidae with emphases in Hypostominae treated the subfamilies Hypoptopomatinae, Neoplecostominae and Otothyriinae as tribes Hypoptopomatini, Neoplecostomini and Otorhyrini, within of the subfamilie Hypoptopomatinae. Moreover, proposed that the tribe Hypostomini, which has traditionnally been recognized as sister to tribe Ancistrini based on morphological data, was nested within Ancistrini. Additionaly they recognized seven additional tribe-levels clades: The *Chaetostoma* Clade, the *Pseudancistrus* Clade, the *Lithoxus* Clade, the '*Pseudancistrus*' Clade, the *Acanthicus* Clade, the *Hemiancistrus* Clade, and *Peckoltia* Clade. Moreover Rinelepini previously recognized as tribe of Hypostominae was suggested as a new subfamily within Loricariidae, the Rhinelepinae.

Objectives

The main objective are: (1) Test the hypotheses of occurrence of several historical river-capture events in South America and investigate the role in the formation of the modern species richness and geographical distribution of the Hypostominae; (2) Test the hypothesis of correlation between headwater capture and adaptive radiation in a *Hypostomus* genus (3); Test the hypothesis of correlation between a lineage's occupation of fast-vs. slow-flowing habitats and rate of diversification in Hypoptopomatinae (4); Described new genera and species of Loricariidae, as a result of collecting expeditions in different South America rivers.

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Capítulo 1

Systematic and historical biogeography of Hypostominae: a highly diverse clade of Neotropical armored catfishes (Siluriformes: Loricariidae)

Target jornal: BMC Evolutionary Biology

Systematic and historical biogeography of Hypostominae: a highly diverse clade of Neotropical armored catfishes (Siluriformes: Loricariidae)

Gabriel S. C. Silva*, Fábio F. Roxo, Luz E. Ochoa, and Claudio Oliveira

Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, IB-UNESP, Campus de Botucatu, 18618-970, Botucatu, São Paulo State, Brazil.

*gabriel_biota@hotmail.com

Abstract

Background: The main objectives of this study are estimate a species-dense, time-calibrated molecular phylogeny of Hypostominae, a large group of armored catfishes that is widely distributed across South America, to place the origin of major clades in time and space, and to demonstrate the role of river capture on patterns of diversification of these taxa.

Results: We used Bayesian methods to estimate a time-calibrated phylogeny with 220 Hypostominae species, using two mitochondrial and four nuclear genes, and used parametric biogeographic analyses (DEC and DECj models) to estimate ancestral geographic ranges, to infer the colonization routes and distribution of these taxa. Hypostominae is composed by ten tribe-level clades, and our time-calibrated phylogeny and ancestral-area estimations indicate an origin of Hypostominae during the Middle Eocene in the ancestral area corresponding to actual Pacific Coastal Drainages and Magdalena Basin, Amazon Basin, Orinoco Basin and Atlantic Guyana drainages.

Conclusion: The speciation in Hypostominae coincides with important geological events in South America.

Keywords: Diversification, Molecular Phylogeny, DEC model, Headwater capture

Background

The Neotropical region comprises one of the highest ichthyological diversity on the globe [1] with more than 5,600 species, representing about 10% of all vertebrate biodiversity according to Vari & Malabarba [2], Lundberg [3] and Reis [4]. This huge

species richness has been the focus of many scientific studies [5,6] regarding to all areas of science. However, until the past years, the taxonomy, systematics and historical geographic distributions of fishes, mainly the Amazonian species, remains unknown [7]. As well as, the main mechanisms responsible to generate this huge diversity and influence the distribution of species on earth still remains very poorly known [1].

In this context, one of the principal process that affect local fish fauna is river capture events (also known as stream capture or headwater capture), an important landscape-level mechanism that can isolate lineages and promote diversification [8,9,10] by changing the connectivity of adjacent river basin [11,12,13,14]. Ribeiro [15] described river capture as a complex process associated with the ancient faults systems, which are more susceptible to erosion process. In this case, small or sometimes large portions of a river are *captured* (i.e. changes its course direction) to an adjacent drainage or river basin. This process was very common during formation of the South America hydrographic systems over the last 100 My (million of years) [15, 1].

The consequences of river capture events for the local fauna can be profound, changing watershed boundaries and allowing previously isolated species to disperse and colonize new environments [16,17,18]. Furthermore, with the pools of aquatic species in adjacent basins becoming mixed, it can elevate regional levels of species richness [1]. However, the repeated separation and merging of stream basins through watershed boundaries serves to both *split* (i.e., vicariance) and *join* (i.e., geodispersal) species populations. Headwater capture enriches faunas at local allowing the mixing of previously isolated faunas on either side of a watershed divide, but also isolate populations across the new divide basins [19,1]. However, the different ways in which head water capture events could affect the evolutionary history in aquatic biota remains an open area of research [20,21].

The origin of the modern Neotropical freshwater fish fauna was influenced by tectonic and orogenic events such as the fragmentation of Gondwana and the rise on the Andes for a period that comprises part of Mesozoic and the entirely of the Cenozoic [22]. In the last 15 to 10 My the western was covered by freshwater Lake Pebas that connected to the Paraná basin to the Proto-Amazon basin. The western Amazon occupying the lake Pebas was separated from the central and eastern Amazon by the Purus arch. The uplift of the Venezuelan Andes and the Vuapes arch between 8 and 5 Mya (million of years ago) [23,24] resulted in the breach of the Purus Arch and the connection of the western Amazon with the central and eastern amazon, a process that

can promote allopatric speciation. At the same time, the reconnection of adjacent basins would allow the endemic species to disperse and colonize new habitats.

Furthermore, fish species diversity in South America hydrographic systems may also be affected by sea level oscillations that have permitted the dispersal among adjacent rivers via delta connections in coastal drainages or lead species to local extinctions [25,26,27,28,29]. Bloom and Lovejoy [30] predicted that broad areas of South America, mainly the Amazon basin, have low elevations (<100 m) and are thus likely to be impacted by sea level fluctuations. Montoya-Burgos [31] hypothesized that this last process is as a result of climate changes that affected lineages of *Hypostomus* inhabiting Amazon drainages about 5-6 Mya. Moreover, from the Miocene to the present, the climate has become frosty and has undergone periodic climatic oscillations that were responsible for several marine transgression and regression over South America continent and that may have affected local freshwater fish fauna leading them to extinction or migration to other areas. Albert and Reis [1] documented several marine regressions over the last 100 My (e.g. 76–80, 66–71, 45–50, 35–42, 23–34, 9–12, and from 3.5 Mya to the present) that according to Budyko [32], Petit [33], and Rothman [34] may be the result of the tilt of the planet's axis, thermohaline circulation, atmospheric CO₂ concentration, and the relative position of the continents.

Hypostominae has been recognized as natural group using morphological [35] and molecular data [36,37] (in the last two studies Rineleporini tribe, sensu Armbruster [35], shared a new subfamily within Loricariidae). Currently the Hypostominae is composed by about 466 species divided in 43 genera [38], widely distributed across the Neotropical freshwater rivers. Rheophilic fishes, such as Hypostominae, are specially good candidates to biogeography studies associated with stream captures, because species movements among drainages and habitats would be expected to be hindered by deep, main-channel habitats, and facilitated by river captures or reductions in river base level during oceanic low stands [39].

In the present study, we present a time-calibrated phylogenetic analysis of the Hypostominae subfamily with the most species-dense taxon sampling for this group using a multilocus approach. We use parametric biogeographic methods to estimate ancestral geographic ranges to document historical river-capture events in Hypostominae, and understand how this process and climate oscillation over the last 100 My affected the distribution of species of this subfamily across the main hydrographic systems of the South America.

Methods

Taxon sampling

Our study includes the most comprehensive sampling of Hypostominae to date with 238 terminal taxa spanning 220 species in 43 genera (Table 1). To test the monophyly of Hypostominae we compiled a broad range of outgroups representing all other Loricariidae subfamilies, as well as samples of the families Astroblepidae (*Astroblepus* sp.) and Callichthyidae (*Hoplosternum littorale*, *Corydoras imitator* and *C. oiapoquensis*) based on relationships established in previous larger scale phylogenetic analyses of both morphological [40,41] and molecular [42] data. Sequence data for members of the subfamilies Loricariinae, Hypoptopomatinae and Rhinelepininae were obtained from previously published studies [43,44,45,46,47,36].

Vouchers of all sequenced samples were deposited in the Auburn University Natural History Museum, Auburn, Alabama, U.S.A. (AUM), Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brasil (LBP), Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Paraná, Brasil (NUP), Museum d'Histoire Naturelle, Département d'Herpétologie et Ichthyologie, Ville de Genève, Genève, Switzerland (MHNG), or at the Smithsonian Tropical Research Institute, Balboa, Ancón, Panama (STRI). Specimens were identified directly by the author GSCS and Dr. Claudio Henrique Zawadzki, except in the case of GenBank identifications, which were not changed.

DNA extraction and sequencing

Whole genomic DNA was extracted from muscle, fin or liver samples preserved in 95% ethanol using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI). Partial sequences of the genes were amplified by polymerase chain reaction (primers listed in Table 2). Amplifications were performed in a total volume of 12.5 μ L with 1.25 μ L of 10X buffer (10 mM Tris-HCl+15 mM MgCl₂), 0.5 μ L dNTPs (200 nM of each), 0.5 μ L of each 5 mM primer, 0.05 μ L Platinum® *Taq* Polymerase (LTI: Life Technologies Inc., Carlsbad, CA), 1 μ L template DNA (approximately 12 ng), and 8.7 μ L ddH₂O. PCR reactions consisted of an initial step of 30 s at 95°C, 35-45 cycles of 15-30 s at 48-58°C and a final step of 90 s at 72°C. Nested PCRs were used to amplify the

nuclear marker RTN4; the first amplification was performed using the primers Freticul4-D and Freticul4-R with a total volume of 12.5 μ L for 30–40 cycles (30 s at 95°C, 30 s at 48°C and 135 s at 72°C); the second amplification was performed using the primers Freticul4 D2 and Freticul4 R2 with a total volume of 12.5 μ L for 30–40 cycles (30 s at 95°C, 30 s at 53–54°C and 135 s at 72°C). All PCR products were first visually identified on a 1% agarose gel and then purified using ExoSap-IT® (USB Corporation, Cleveland, OH) following manufacturer instructions. Purified PCR products were sequenced using the Big Dye® Terminator v3.1 Cycle Sequencing Ready Reaction Kit (ABI: Applied Biosystems Inc., Foster, CA), purified again by ethanol precipitation and sequenced on a 3130 Genetic Analyser (ABI) in the Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo State.

Sequence alignment and phylogenetic inference

Two intronic regions of the RTN4 gene, totalling 63% of total locus length, were amplified and sequenced by our primers. All loci, including the entire RTN4 sequence, were first aligned using the MUSCLE algorithm (default parameters; [47]) followed by visual inspection and correction of the alignment requiring almost no changes in base position. The total matrix consisted of 295 individuals with 5,816 base pairs, of which 53.9% were missing data. To detect potential sequencing errors due to contamination or paralogy, alignments for each gene were separately analysed by maximum likelihood [48] using the web server RAxML BlackBox [49]. Sequences that resulted in obviously misplaced taxa in resulting gene trees were re-sequenced or eliminated from subsequent analyses.

We estimated the index of substitution saturation (Iss) and rates of transitions/transversions for each gene separately in DAMBE 5.2.31 [50], as described by Xia [51] and Xia and Lemey [52]. Iss was estimated without considering gaps because unresolved sites reduce the ability of the method to test for phylogenetic signal.

Maximum likelihood (ML) analyses [48] were performed using RAxML on the CIPRES Science Gateway computing cluster [53]. RAxML implements a fast algorithm of heuristic searches using bootstrap (BS) resampling [54]. Support for individual nodes were assessed using random start trees, the GTR+G model for all partitions of the matrix as recommended by the software PartitionFinder [55], and several independent ML tree searches consisting of 1,000 pseudoreplicates each with all other parameters set to default values.

Time calibration tree

The uncorrelated relaxed molecular clock (log-normal) was estimated using BEAST 1.7.5. [56]. We used a clade-based approach that included each Hypostominae lineage in our analysis. The GTR+G model was used for all partitions. The calibration prior was implemented using a log-normal distribution offset to 55 My with a mean and standard deviation of 1 for the origin of the genus *Corydoras* lineage (node including all Callichthyidae: *Corydoras imitator*, *C. oiapoquensis* and *Hoplosternum littorale*). The oldest known loricarioid fossil, *Corydoras revelatus* Cockerell [57] was dated by Marshall [58] as Paleocene, which we used to assign a 55 My minimum age for the family Callichthyidae. We used a birth–death model for speciation likelihood and a starting tree obtained from ML. The analysis was run for 10 million generations and sampled every 1,000th generation. Stationary and sufficient mixing of parameters (ESS > 200) was checked using Tracer v1.5 [59]. A consensus tree was built using TreeAnnotator v1.7.5 [60].

Ancestral area estimation

Data on the geographic distributions of species in the Hypostominae were taken from the original species descriptions and information available from the online Catalog of Fishes [38]. To maintain consistency with previous biogeographic analyses of the subfamily Hypoptopomatinae (*sensu* Lujan [36]) by Chiachio [43] and Roxo [45], we assigned taxa to geographic areas using the ecoregion classification of Vari [61], which was derived from patterns of endemism in the Neotropical endemic characiform family Curimatidae. Our samples were drawn from the following seven ecoregions: (A) Pacific Coastal Drainages and Magdalena Basin, (B) Amazon Basin, (C) Orinoco Basin, (D) Atlantic Coastal Drainages, (E) Atlantic Guyana Drainages, (F) Paraguay, Lower Paraná and Uruguay Basin, and (G) Upper Paraná Basin.

We estimated the likelihood of ancestral range evolution using the Dispersal-Extinction-Cladogenesis (DEC: Ree and Smith [62]) and “jumping” (DECj: Matzke [63]) models of species range evolution. These models are composed of two (DEC) or three (DECj) parameters including: 1) dispersal (D), where ancestral ranges expand by adding new geographic units, 2) extinction (E), where ancestral ranges are reduced by extirpating geographic units, and 3) “jumping” events (j), where j specifies the weight of the “jumping” events beyond an ancestral range [64]. The two models of range evolution (i.e. DEC and DECj) were implemented in the R package BioGeoBEARS

[65] in two different analytical procedures Lagrange [66] and BayArea-like [64]. The global likelihood of the six biogeographic scenarios were compared using the Akaike Information Criterion (AIC) [67] (Table S2). The model that obtained the lowest AIC values was model 2 with the DEC+J model (M2 – DEC + J), which constrained the dispersal rates between adjacent areas at 0.5 and areas separated by one or more intercalated areas at 0.0001.

Results

DNA sequence alignment and substitution rates

The concatenated alignment of sequence data for 16S rRNA, Cytb, RTN4, Myh6, RAG1 and RAG2 genes resulted in a matrix with 5,816 base pairs (bp), which 2,405 bp were conserved and 3,411 were variable. We do not found evidences for substantial saturation considering that the Iss value was greater than the Iss.c value using the test of Xia [51]. The observed transition/transversion ratio was 2.89, with nucleotide frequencies of 25.0% for adenine, 25.0% for thymine/uracil, 25.0% for cytosine and 25.0% for guanine.

Phylogenetic relationships

Our phylogeny showed strong support for several Hypostominae clades and according with previously molecular hypothesis [36, 37]. Within Hypostominae, our results supported the monophyly of ten monophyletic clades (Fig. 1): *Chaetostoma* clade (BS = 50); *Neblinichthys* clade (BS = 82); *Ancistrini* tribe (BS=75); *Pseudancistrus* clade (BS = 94); *Lithoxus* clade (BS = 57); *Acanthicus* clade (BS = 81); *Hemiancistrus* clade (BS = 74); *Panaque* clade (BS = 96); Hypostomini tribe (BS = 73) and *Peckoltia* clade (BS = <50). However, we found some disagreement as the *Panaque* clade appeared within *Hemiancistrus* clade in Lujan et al. (2015), and *Neblinichthys* clade, composed by *Neblinichthys* species plus *Paulasquama callis*, *Lithoxancistrus orinoco* and *L. yekuana* appeared within *Chaetostoma* clade also in Lujan [36].

Relaxed clock of Hypostominae clades and historical biogeography

The mean substitution rate across all loci estimated using BEAST was 0.00347% per My. The better hypothesis of ancestral range estimation of Hypostominae phylogeny in the seven biogeographic resulted from values of dispersal rate among

adjacent area of 1.00 and no adjacent area of 1.00E-01, according to AIC scores (Table 3).

The origin of Hypostominae was estimated by BEAST to have originated during the Eocene about 40.7 Mya (31.1–50.1 Mya 95% HPD; see Fig. 2 for molecular clock tree including outgroups) and our hypothesis also suggest that the ancestral lineage of this group was widespread through the Region ABCE about 40.7 Mya (31.1-50.1 Mya 95% HPD)(Fig.1) and about 33.4 Mya the ancestor of Hypostominae was extinct from region A and C.

Within Hypostominae, our analysis (Fig. 2, 3, 4, 5) suggest that the ten major clades of this subfamily have been originated at: Region B for *Chaetostoma* clade about 33.4 Mya (26.0–41.2 Mya 95% HPD), Region E for *Neblinichthys* clade about 31.0 Mya (24.0–37.8 Mya 95% HPD), Region B for Ancistrini tribe about 29.6 Mya (23.1–36.1 Mya 95% HPD), Region E for *Pseudancistrus* clade about 28.5 Mya (22.0–34.1 Mya 95% HPD), Region C for *Lithoxus* clade about 27.1 Mya (21.3–32.7 Mya 95% HPD), Region B for *Acanthicus* clade about 23.7 Mya (mean 18.5–28.7 Mya 95% HPD), Region C for *Hemiancistrus* clade about 21.8 Mya (mean 17.0–27.1 Mya 95% HPD), Region C for *Panaque* clade about 21.1 Mya (mean 16.3–26.4 Mya 95% HPD), and Region B for Hypostomini tribe and *Peckoltia* clade about 18.1 Mya (mean 13.8–23.5 Mya 95% HPD).

Discussion

Origin and Diversification of Hypostominae in the Andean and Guyana shield Highlands

We estimated the origin of Hypostominae in the period corresponding to Middle Eocene, about 40.7 Mya. In this period the ancestor lineage of Hypostominae was widespread in the regions A (Pacific Coastal Drainages and Magdalena Basin), B (Amazon Basin), C (Orinoco Basin) and E (Atlantic Guyana drainages), regions that corresponded to the “Paleo-Amazon-Orinoco”, a large hydrographic basin extended to west of the Purus Arch and rivers draining toward the Andean foreland basin that flows to north of the Ocean Caribe [68]. According to Lujan [36] the Hypostominae lineage diversified in rivers draining to highlands of the Guyana shield, however our results suggested that the ancestral lineage of Hypostominae was widespread distributed across the “Paleo-Amazon-Orinoco” basin, and in a subsequent period (33.4 Mya) all

Hypostominae species, except *Chaetostoma* clade, diversified in a highlands of Guyana Shield (see Fig .2), while the *Chaetostoma* clade diversified in the Andean highlands.

The ancestor lineage of Hypostominae were probably extinct from region A and C about 33.4 Mya, in the same time period in the early Oligocene, large parts of the Peruvian and Ecuadorian Andean foreland were occupied by lakes to marginal marine waters “Lago Pozo” [14]. The marine Pozo embayment occupied the western part of present-day Amazonia [68], and this marine incursion may have caused the extinction of ancestral lineage which was in region A and C. The Hypostominae species are rheophilic fishes and do not support lentic environments such as lakes and channels of large rivers [39].

The ancestral lineage of the trans-Andean group composed by *Dolichancistrus*, *Cordylancistrus*, *Leptoancistrus*, *Andeancistrus* and *Transancistrus* from region A (Pacific Coastal drainages and Magdalena basin) is the sister group of the ancestral lineage of the cis-Andean group composed by *Chaetostoma*, *Lipopterichthys* and *Loraxichthys* (region B). These two groups were separated about 31.1 Mya and the trans-Andean ancestor originated in Amazon basin (Region B). Along the phylogeny other lineages colonized the Pacific Coastal drainages and Magdalena basin (region A) from Amazon basin (region B), such as the ancestor of *Hemiancistrus landoni* about 16.8 Mya and ancestor of *Ancistrus clementinae* about 19.8 Mya. This old separation coincides with a mountain uplifted in the Northern Andes mountains (late Oligocene to early Miocene approximately about 23 Mya) [28]. Abe [70] found similar value to separation of *Brycon moorei* (Magdalena river) and the others species of *Brycon* (*B. orthotaenia*, *B. amazonicus*, *B. hilari*, *B. orbygnianus* and *B. golding*) about 21.3 Mya. Furthermore, the second and more intense peaks of northern Andean mountain building occurred from the late middle Miocene (~12 Mya) and early Pliocene (~4.5 Mya), and could be responsible by separate several lineage of Hypostominae (e.g. - *Chaetostoma breve* about 10.7 Mya; *Chaetostoma fischeri* about 12.1 Mya; and *Ancistrus chagresi* about 8.3 Mya in the middle of Miocene; and *Lasiancistrus caucanus* about 2.7 Mya; *Panaque cochliodon* about 5.4 Mya; the ancestral of *Hypostomus rhantos* and *H. robini* about 6.2 Mya, and *Hemiancistrus aspidolepsis* about 4.9 Mya in the early Pliocene).

Moreover, apparently *Chaetostoma vazquezi* and *Chaetostoma jegui* are the unique lineage of *Chaetostoma* that reached the Orinoco and Amazon basin in Guyana shield, all others *Chaetostoma* are restricted in the highland in Andean mountains.

Armbruster [35] and [69] suggested that these species were derived within *Chaetostoma*, however our data showed that this group diverged in a remote time, about 17.2 Mya. [36] found that *Chaetostoma vazquezi* and *C. jegui* also diverged first than other *Chaetostoma* species.

Orinoco/Amazon basin

Our results showed that the *Hypancistrus* genus is composed by two monophyletic groups, one from Orinoco basin (i.e. a clade composed of *Hypancistrus furunculus*, *H. luanorum*, *H. contradens*, *H. debilittera* and *Micracanthicus vandragti*, which must be reallocated in the genus *Hypancistrus*) and other from Amazon basin including four possible new species (i.e. a clade composed of *Hypancistrus* sp. 1, *Hypancistrus* sp. 2, *Hypancistrus* sp. 3 and *Hypancistrus* sp. 4). Furthermore, our time calibrated tree suggested that these two groups diverged about 6.6 Mya. This data is in accordance with the uplift of the Vaupes Arch (late Miocene) [24].

The rise of this arch separated the ancient “Paleo-Amazon-Orinoco” river into two separate drainages, the Orinoco flowing to the northeast, and the Amazon flowing to the east once it had breached the Purus Arch [70]. Moreover, several others ancestral lineages of Hypostominae colonized the Orinoco basin from East Amazon basin approximately in the same time, e.g. - *Leporacanthicus triactis* (8.1 Mya); *Aphanotorulus ammophilus* (6.9 Mya); *Hypostomus plecostomoides* (5.9 Mya), *Lasiancistrus tentaculatus* (6.9 Mya); *Peckoltia vittata* (6.3 Mya), and *Panaqolus maccus* (4.9 Mya). These data are similar to those found by previous published works, as the separation of populations of *Hoplosternum littorale* about 11.7 to 6.0 Mya [71], the separation of *Triportheus orinocensis* from ancestral lineage of *T. venezuelensis*, *T. signatus*, *Triportheus* sp.n, *T. trifurcatus*, *T. angulatus* and *T. pantanensis* about 6.8 Mya [72], and the separation of ancestor of *Brycon falcatus* (Orinoco river) from *B. falcatus* (Negro river) about 4.4 Mya [71].

Moreover, in a more recent period of time several ancestor lineage of Hypostominae dispersed to Orinoco basin from Amazon drainages, as *Ancistrus macrophtalmus* (2.8 Mya), *Pterygoplichthys gibbiceps* (4.3 Mya), *Peckoltia sabaji* (0.6 Mya), *Peckoltia lineola* and *Peckoltia caenosa* (3.3 Mya). These data are consistent with to those found in the separation of *T. brachipomus* (Negro river) and *T. brachipomus* (Orinoco river) about 3.6 Mya [72], the separation of *Brycon falcatus* ancestor (Orinoco river) about 3.9 Mya [73], and the separation of *T. argenteus* ancestor

(Orinoco river) and the lineage ancestral of *T. argenteus* from Paraguay, Parnaíba and Amazon basin about 5.5 Mya [72].

Southern Guiana shield/Northern Brazilian shield

Several lineages of Hypostominae are distributed in rivers of Guyana shield draining to the Atlantic Ocean, and in southern Amazonian drainages to the Brazilian shield. In our phylogeny some of these lineages of northern of Guyana shield form a sister group with lineages from southern of Amazon basin. For example, *Hopliancistrus* sp. 1 and *Hopliancistrus tricornis* (Brazilian shield) is sister group of *Corymbophanes kaieie* (Guiana shield) and this separation occurred about 15.7 Mya. In other groups this also happens, *Pseudancistrus zawadzki* and *Pseudancistrus asurini* (Brazilian shield) separated from *Pseudancistrus corantijiniensis* lineage (Guiana shield) about 9.1 Mya; *Spectracanthicus punctatissimus*, *Spectracanthicus zuanoni*, *Parancistrus nudiventris*, *Spectracanthicus murinus*, *Baryancistrus niveatus*, *Baryancistrus chrysolomus*, *Baryancistrus xanthellus*, *Baryancistrus* sp. 1 and *Baryancistrus* sp. 2 (Brazilian shield) lineages separated from *Hemiancistrus medians* (Guyana shield) about 18.1 Mya; and ancestral lineage of *Ancistrus minutus*, *Ancistrus* sp. 6, *Ancistrus* sp. 7, *Ancistrus* sp. 8, *Ancistrus megalostomus*, *Ancistrus bolivianus*, *Ancistrus* sp. 9, *Ancistrus macrophthalmus*, *Ancistrus* sp. 10, *Ancistrus* sp. 11, *Ancistrus* sp. 12, *Ancistrus* sp. 13, *Ancistrus* sp. 14, *Ancistrus* sp. 15 (Brazilian shield) separated from *Ancistrus leucosticus* (Guyana shield) about 11.4 Mya.

Silva [74] proposed two hypotheses to explain the distribution pattern of *Pseudancistrus* species in Guyana and Brazilian shields. The first hypothesis states that the ancestral lineage of the *Pseudancistrus* was widely distributed through all Guyana Shield rivers and Amazon Brazilian shield rivers. The second hypotheses suggest that the ancestral lineage of *Pseudancistrus* should have reached the Northern Brazilian shield through the mainstream Amazon river. Our results are in accordance with the first hypothesis, whereas all cladogenesis events occurred before the inversion of the Amazon river, that happened about 8 Mya [14]. After this inversion, we could not identify more dispersions among the Southern of Guiana shield and Northern of Brazilian shield (except in some lineage of *Peckoltia* clade and Hypostomini). Possibly the mainstream Amazon river acted as a permeable barrier for endemic taxa on the respective Guiana and Brazilian shields [39] preventing that reophilic species of Hypostominae reached the Northern Brazilian shield. Before the establishment of the

main strain Amazon river in eastern of South America those region was an upland area (Belterra surface) somewhere between the present day areas of Manaus (Purus arch) and Óbidos (Monte Alegre Arch) [14] and through headwater capture events can be allowed the invasion of Northern Brazilian shield from southern Guiana shield by reophilic Hypostominae species.

Modern corridors: Guyana shield

Pseudancistrus nigrescens is a species found in the Essequibo river (region E), while its sister group, *Pseudancistrus* sp.1 is found in the Branco and Negro rivers (region B). These two taxa separated in relative recent time, about 4.9 Mya (see Fig. 2). This data is in accordance with headwater capture events that occurred in the highland portions of the Proto-Berbrice (Rupunini and Essequibo) in the Amazon system at the end of the Pliocene. Other possible cladogenetic event caused by the headwater capture in Proto-Berbrice basin happened between *Chaetostoma vasquezi* (Cuara river) and *Chaetostoma jegui* (Uraricoera) separated about 0.7 Mya. The Cuara and Uraricoara rivers were connected drainages in headwater of the Proto-Berbrice basin [39], and the separation between the Orinoco and Rio Branco basin in recent time also should be separated these two lineages.

Pseudacanthicus leopardus from Takutu river (region B) and *Pseudacanthicus leopardus* from Essequibo river (region E) separated at 0.5 Mya. Our data suggest that this dispersion can be occurred through of the Rupununi Portal. The Rupunini Savanna floods seasonally, creating a lentic corridor between the Essequibo and Takutu rivers. Loricariids of the Essequibo are nearly identical to those of the Takutu, indicating either regular, recent dispersal across the flooded savanna or insufficient time for differentiation since stream capture [39].

We analyzed three samples of *Guyanancistrus brevispinis* from Nickerie, Moroni (both from Atlantic Coastal rivers) and Jari rivers (a south-flowing tributary of the Amazon). Our results suggested that *G. brevispinis* invaded the Amazon basin (region B) from Atlantic Coastal river (region E) about 3.9 Mya, a recent dispersal through headwater capture in a Southern Guiana Shield connection [39]. Cardoso and Montoya [75] suggested that *G. brevispinis* arise in Amazon basin and recently invaded the Atlantic Coastal rivers, however our results suggest that *G. brevispinis* invaded the Amazon basin from Atlantic Coastal rivers and not the opposite.

Southern invasion

The ancestral lineage of *Ancistrus cuiabe* and *Ancistrus multipinnis* reached the Region F (Paraguay and Lower Paraná basin) from Region B (Amazon basin) about 18.3 Mya. Around the same time the ancestral lineage of *Hemiancistrus meizospilos*, *H. maracaiboensis*, *H. aspidolepis*, *H. punctulatus*, *Pterygoplichthys. multiradiatus*, *P. gibbiceps*, *P. disjunctivus*, *H. votouro* and *H. fuliginosus* reached the Region F (Paraguay and Lower Paraná basin) from Region B (Amazon basin) about 13.4 Mya. These two cladogenetic events may be linked to rise of the Chapare Buttress about 30-20 Mya [14] or to the Michiola Arch about 11.8-10.0 Mya [14]. Abe [71] found similar value to separation of *Brycon amazonicus* and *B. hilairei* (12.4 Mya) and of *B. orbygniatius* and *B. gouldingi* (14.4 Mya). More recently others ancestral lineages of Hypostominae found the Region F from Region B, such as the ancestral lineage of *Hypostomus commersoni*, *H. boulengeri* and *Hypostomus* sp.2 at 4.7 Mya and ancestral lineage of *Hypostomus cochliodon* about 3.3 Mya. These recent headwater captures can be explained by reactivation of old faults in this region [76].

Our results are in accordance with hypothesis of Pearson [77] that argued a close resemblance of the fishes of the Paraguay to the diversified fauna of the Amazon indicates their origin from the Amazonia forms. Several works with molecular phylogenies has suggested a colonization of Paraguay from Amazon basin [31,71,70,77]. Carvalho and Albert [77] also reported almost unidirectional manner of fauna dispersal, where the Paraguay basin seems to harbor many groups from the amazon basin than the opposite direction.

The ancestral lineage of *Hypostomus affinis*, *H. piratatu*, *H. derby* and ancestral lineage of *Hypostomus microstomus* colonized the Region F (Paraguay, Lower Paraná and Uruguay basin) from region G (Upper Paraná basin) in recent time (3.2 Mya and 1.6 Mya respectively). Montoya-Burgos [31] pointed that the estuarine connection as in delta of Rio la Plata, due to low sea-level may explain more recent divergence time among Uruguayan species and their closest Paraná system clades. These events of low sea-levels occurred at 6-5 Mya [78,79,80], and coincides with the recent split of the species cited above.

Our results showed that the ancestral lineage of *Hypostomus* reached the Upper Paraná basin from Amazon basin at 7.6 Mya. The ancestor of *Pterygoplichthys multiradiatus* also reached the Upper Paraná basin (region G) from Amazon/Paraguay basin (region B/F) at 7.7 Mya. These results are in accordance with Silva [81] that

found a similar value to invasion of ancestral lineage of *Hypostomus* at the Upper Paraná basin at 12.5 Mya. Roxo [45] found that *Corumbataia tocantinensis* (Amazon basin) separated from *Corumbataia cuستا* (Upper Paraná basin) approximately at 11 Mya (see Fig. 7) while Silva [82] found that *Rhinolekos capetinga* (Amazon basin) separated from *Rhinolekos garavelloii* approximately at 7 Mya (see Fig 2) All these events suggest intense headwater captures between the Upper Paraná and Amazon basin during the late Miocene.

Conclusion

In summary, our results show that the origin and diversification of Hypostominae was close linked to the highlands, several clades originated in the region C (Orinoco basin) and region E (Atlantic Guiana drainages) probably in upland of Andean mountain or Guiana shield. The rheophilic species were confined to uplands of Pacific Coastal drainages and Magdalena basin, Amazon basin, Orinoco basin and Atlantic Guiana drainages, while species more tolerant to lowland as *Hypostomus*, *Ancistrus*, *Pterygoplichthys* and *Hemiancistrus* managed reached and diversified in others regions such as the Paraguay, Lower Paraná, Uruguay (region F), Upper Paraná (region G) and Atlantic Coastal Drainages (region D).

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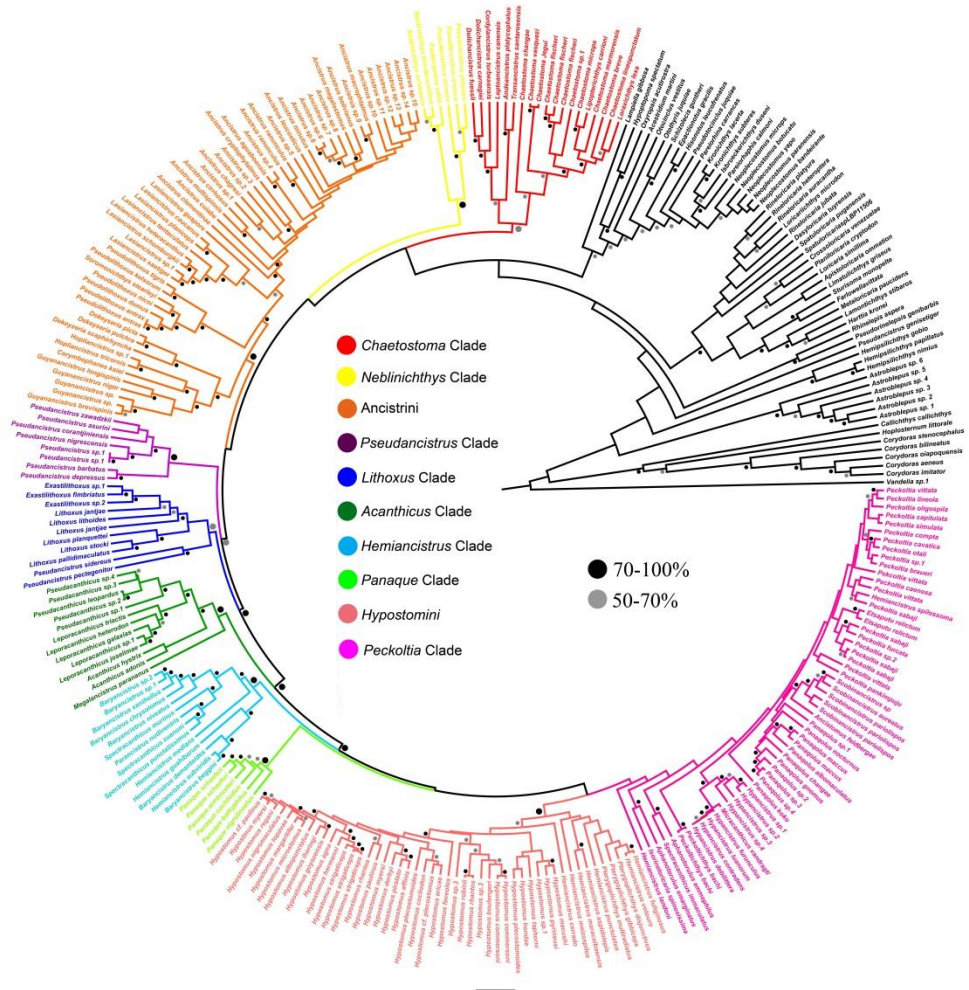


Figure 1. Maximum Likelihood tree showing interrelationships among species of the subfamily Hypostominae.

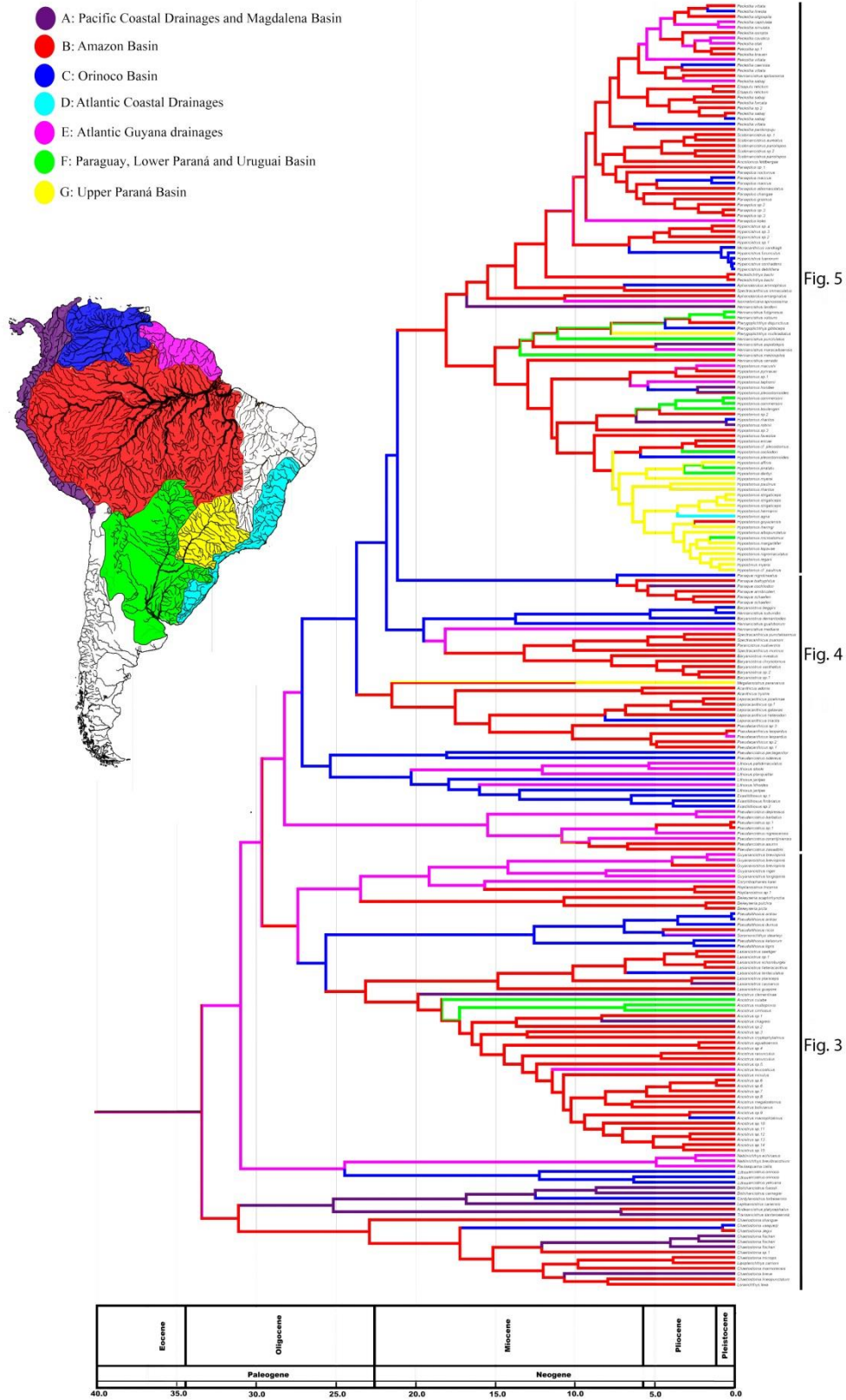


Figure 2. Ancestral range estimation for Hypostominae using DEC+J model. Color changes between squares indicate dispersal and/or extinction events. Different colors represent species present in multiple geographical areas.

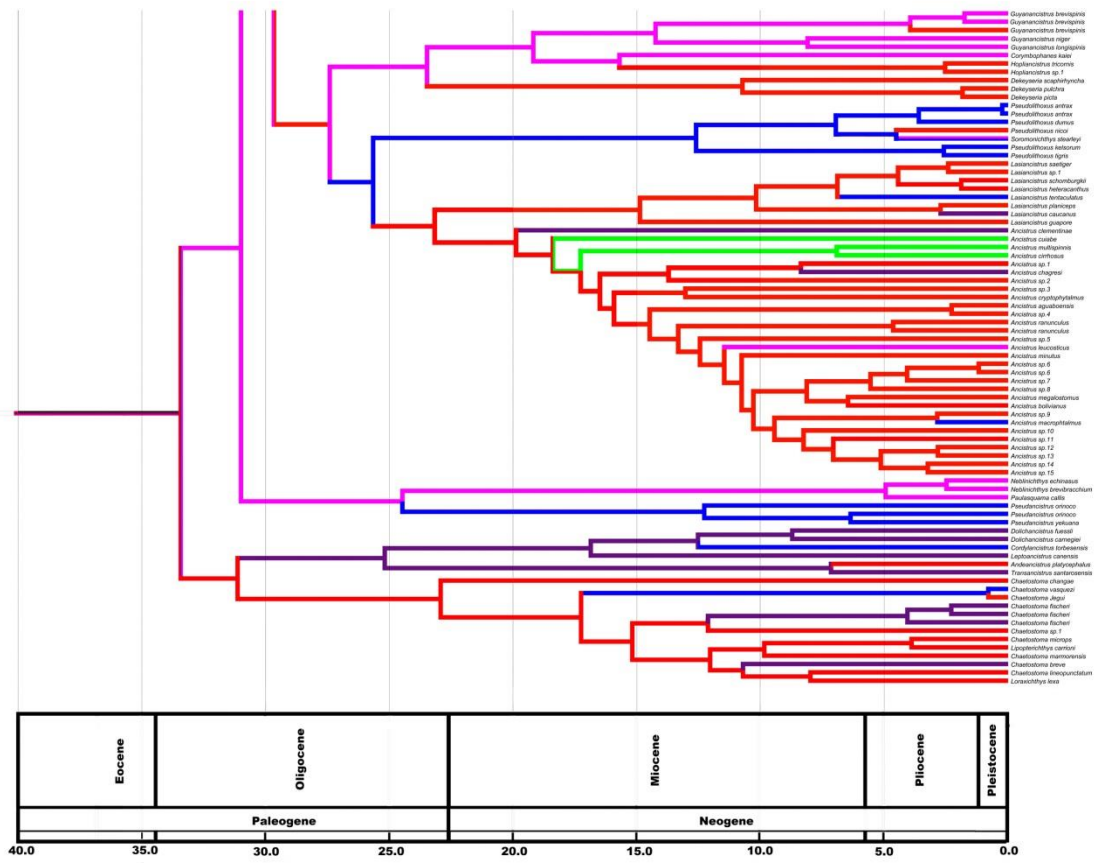


Figure 3. Partial ancestral range estimation for Hypostominae using DEC+J model.

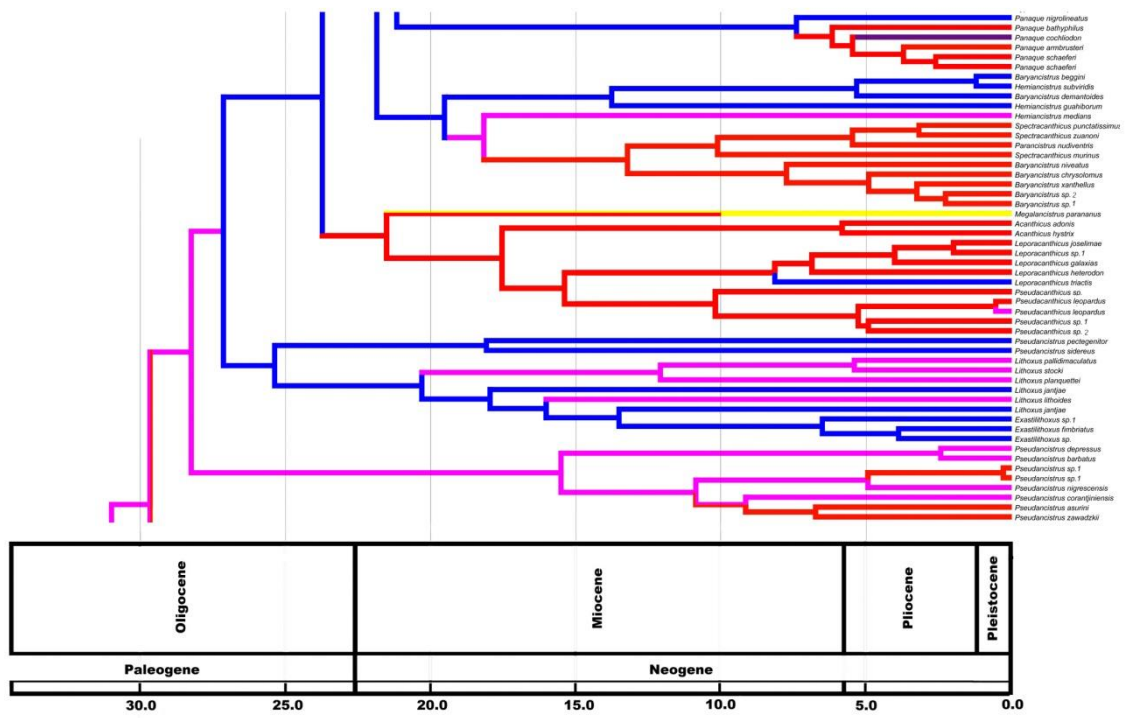


Figure 4. Partial ancestral range estimation for Hypostominae using DEC+J model.

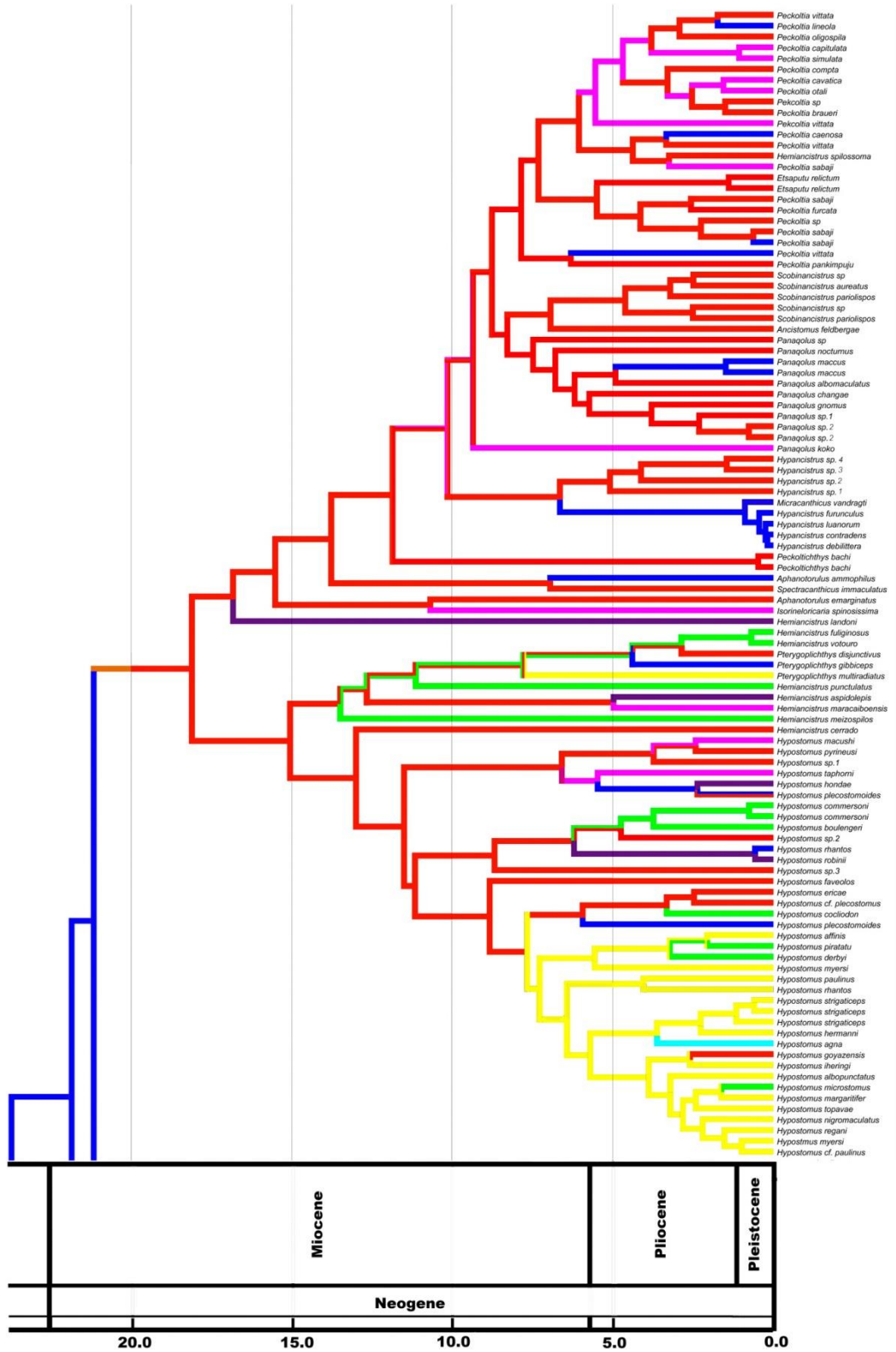


Figure 5. Partial ancestral range estimation for Hypostominae using DEC+J model.

Table 1. Taxa list, collection vouchers, loci sequenced and river drainage for tissue sample analyzed in this present study (n=295).

	Species	Collection No	Fish No	16S	CytB	MyH	Rag1	Rag2	F-ret-4	Location (river system, country)
1	<i>Peckoltia vittata</i>	missing	10514		X	X		X		Madeira river
2	<i>Peckoltia lineola</i>	AUM54033	T09831	X	X	X		X		Venturi river
3	<i>Peckoltia oligospila</i>	MHNG260201	BR98-154						X	Guamá river
4	<i>Peckoltia capitulata</i>	MHNG2011	MUS331						X	Approuague river
5	<i>Peckoltia simulata</i>	MHNG2681	GF06-120						X	Oyapok river
6	<i>Peckoltia compta</i>	ROM91263	T10775	X	X	X	X	X		Xingu river
7	<i>Peckoltia cavatica</i>	LBP 13819	57229						X	Tapajós river
8	<i>Peckoltia otali</i>	ANSP187118	SUR07						X	Litani river
9	<i>Peckoltia</i> sp.1	MCP35628	T14753			X	X			Madeira river
10	<i>Peckoltia braueri</i>	LBP 15368	69540	X	X	X	X	X	X	Takutu river
11	<i>Peckoltia vittata</i>	LBP 16543	64059	X	X				X	Xingu river
12	<i>Peckoltia caenosa</i>	LBP 2186	15501	X					X	Orinoco river
13	<i>Peckoltia vittata</i>	ANSP192078	B2152	X	X	X	X	X		Madeira river
14	<i>Hemiancistrus spilossoma</i>	LBP 4953	11491						X	Araguaia river
15	<i>Peckoltia sabaji</i>	MHNG2651	GY04						X	Rupununi river
16	<i>Etsaputu relictum</i>	MUSM44256	CH157	X	X	X	X	X		Huallaga river
17	<i>Etsaputu relictum</i>	AUM45531	P6099	X	X	X	X	X		Marañón river
18	<i>Peckoltia sabaji</i>	LBP 10625	49510	X	X		X			Guamá river
19	<i>Peckoltia furcata</i>	AUM45593	P6200	X	X	X	X	X		Marañón river
20	<i>Peckoltia</i> sp.2	MCP35628	T14753	X			X			Madeira river
21	<i>Peckoltia sabaji</i>	ANSP199615	B1969	X	X	X	X	X		Xingu river
22	<i>Peckoltia sabaji</i>	ANSP191152	T09602	X	X	X		X		Orinoco river
23	<i>Peckoltia vittata</i>	AUM54314	T09533	X	X	X	X	X		Orinoco river
24	<i>Peckoltia pankimpuju</i>	AUM45594	P6233	X	X		X	X		Marañón river
25	<i>Scobinancistrus</i> sp.1	LBP13814	57217	X					X	Tapajós river
26	<i>Scobinancistrus aureatus</i>	ANSP192094	B2193	X	X					Xingu river
27	<i>Scobinancistrus pariolispos</i>	ANSP193006	B2088	X	X		X	X	X	Xingu river

28	<i>Scobinancistrus</i> sp.2	LBP16549	64042	X	X	X	X		X	Xingu river
29	<i>Scobinancistrus pariolispos</i>	LBP16556	61465		X	X				Xingu river
30	<i>Ancistomus feldbergae</i>	ANSP193012	B2072	X	X	X	X	X		Xingu river
31	<i>Panaqolus</i> sp.1	MHNG2710	PE08900	X	X				X	San Alexandro river
32	<i>Panaqolus nocturnus</i>	AUM45500	P6126	X		X	X	X		Marañon river
33	<i>Panaqolus maccus</i>	AUM53768	T09009	X	X	X	X	X		Guanare river
34	<i>Panaqolus maccus</i>	LBP9948	46698	X	X				X	Manapire river
35	<i>Panaqolus albomaculatus</i>	AUM45502	P6121	X	X	X	X	X		Marañon river
36	<i>Panaqolus changae</i>	ANSP181097	P6218						X	Itaya river
37	<i>Panaqolus gnomus</i>	AUM45501	6128	X	X	X	X	X		Marañon river
38	<i>Panaqolus</i> sp.2	LBP1667	12324	X					X	Aquarium
39	<i>Panaqolus</i> sp.3	AUM44721	G5183		X		X	X		Tacutu river
40	<i>Panaqolus</i> sp.3	LBP15367	69542	X						Tacutu river
41	<i>Panaqolus koko</i>	MHNG2011	GF00115	X	X		X	X	X	Marouini river
42	<i>Hypancistrus</i> sp.4	LBP5355	26994	X					X	Jari river
43	<i>Hypancistrus</i> sp.3	LBP16545	61463	X	X	X	X		X	Xingu river
44	<i>Hypancistrus</i> sp.2	LBP16288	61759	X	X				X	Tapajós river
45	<i>Hypancistrus</i> sp.1	ANSP193084	B2141		X					Xingu river
46	<i>Micracanthicus vandragti</i>	ANSP190780	T09490		X	X	X	X		Ventuari river
47	<i>Hypancistrus furunculus</i>	AUM39225	V028	X	X	X	X	X		Orinoco river
48	<i>Hypancistrus luanorum</i>	ROM92224	T09562	X	X	X	X	X		Ventuari river
49	<i>Hypancistrus contradens</i>	ANSP190815	T09355	X	X	X	X	X		Ventuari river
50	<i>Hypancistrus debilitera</i>	AUM53528	T09279	X	X	X	X	X		Orinoco river
51	<i>Peckoltichthys bachi</i>	AUM45592	P6254	X	X	X	X			Marañon river
52	<i>Peckoltichthys bachi</i>	LBP14809	57727	X		X		X		Amazon river
53	<i>Aphanotorulus ammophilus</i>	Genbank	Genbank	X	X	X	X			Xingu river
54	<i>Spectracanthicus immaculatus</i>	ANSP194670	T1385	X	X	X		X		
55	<i>Aphanotorulus emarginatus</i>	ANSP199645	B2046	X	X					Xingu river
56	<i>Isorineloricaria spinosissima</i>	ROM93722	T13692	X	X	X				Guyanas river
57	<i>Hemiancistrus landoni</i>	AUM93738	T13836	X	X	X	X	X		Clara river

58	<i>Hemiancistrus fuliginosus</i>	MCP40028	T14768	X			X	X	X	Saudade river
59	<i>Hemiancistrus votouro</i>	MCP44181	T14766	X			X		X	Passo Fundo river
60	<i>Pterygoplichthys gibbiceps</i>	AUM42131	P4893		X					Casiquiare river
61	<i>Pterygoplichthys multiradiatus</i>	LBP275	4233	X	X					Rio Paracatu
62	<i>Hemiancistrus punctulatus</i>	MCP40946	T14754		X	X	X	X	X	Carreiro river
63	<i>Hemiancistrus aspidolepis</i>	Genbank	Genbank		X					
64	<i>Hemiancistrus maracaiboensis</i>	Genbank	Genbank		X					
65	<i>Hemiancistrus meizospilos</i>	MCP40168	T14750			X	X	X		Chapecó river
66	<i>Hemiancistrus cerrado</i>	LBP17213	66644	X	X				X	Araguaia river
67	<i>Hypostomus macushi</i>	ROM85939	T07038	X	X	X	X	X		Essequibo river
68	<i>Hypostomus pyrineusi</i>	AUM51394	T10377	X	X	X	X	X		Madre de Dios river
69	<i>Hypostomus</i> sp.1	LBP3510	21309	X	X					Paranapanema river
70	<i>Hypostomus taphorni</i>	ROM86352	T07074	X	X	X	X	X		Essequibo river
71	<i>Hypostomus hondae</i>	Genbank	Genbank		X					
72	<i>Hypostomus plecostomoides</i>	Genbank	Genbank	X	X					
73	<i>Hypostomus commersoni</i>	Genbank	Genbank	X				X		
74	<i>Hypostomus commersoni</i>	LBP3334	20460	X					X	Grande river
75	<i>Hypostomus boulengeri</i>	LBP679	8007	X			X	X	X	Paraguay river
76	<i>Hypostomus</i> sp.2	LBP1681	11262	X	X					Paranapanema river
77	<i>Hypostomus rhanthos</i>	AUM54306	T09530	X	X	X	X	X		Ventuari river
78	<i>Hypostomus robinii</i>	Genbank	Genbank		X					
79	<i>Hypostomus</i> sp.3	ANSP199690	B1475	X				X		Xingu river
80	<i>Hypostomus faveolos</i>	LBP1839	13156	X	X					Araguaia river
81	<i>Hypostomus ericae</i>	LBP2424	16213	X	X				X	Fundo river
82	<i>Hypostomus</i> cf. <i>plecostomus</i>	LBP1827	12943	X	X					Araguaia river
83	<i>Hypostomus cocliodon</i>	NUP	17516	X	X					Manso river
84	<i>Hypostomus plecostomoides</i>	LBP2197	15503	X	X				X	Laguna de Castilho
85	<i>Hypostomus affinis</i>	LBP2371	16087	X	X					Paraiba do Sul river
86	<i>Hypostomus piratatu</i>	LBP2156	15156	X	X				X	Paraguay river
87	<i>Hypostomus derbyi</i>	NUP677	16644	X	X				X	Iguaçu river

88	<i>Hypostomus myersi</i>	NUP680	16637		X						Iguaçu river
89	<i>Hypostomus paulinus</i>	LBP2548	10886						X		Tietê river
90	<i>Hypostomus rhantos</i>	LBP2185	15559	X					X		Cataniapo river
91	<i>Hypostomus strigaticeps</i>	LBP4202	18410	X	X				X		Tietê river
92	<i>Hypostomus strigaticeps</i>	LBP2148	10666	X	X						Tietê river
93	<i>Hypostomus strigaticeps</i>	LBP2543	10914	X	X						Tietê river
94	<i>Hypostomus hermanni</i>	LBP116	3449	X	X				X		Paranema river
95	<i>Hypostomus agna</i>	LBP2360	16031	X	X				X		Cavalo river
96	<i>Hypostomus goyazensis</i>	LBP2150	11482	X	X				X		Amazon river
97	<i>Hypostomus iheringi</i>	LBP2638	17039		X						Tietê river
98	<i>Hypostomus albopunctatus</i>	LBP1671	11272	X	X						Paranapanema river
99	<i>Hypostomus microstomus</i>	NUP 1725	16652	X	X				X		Paraná river
100	<i>Hypostomus margaritifera</i>	LBP 4296	16611		X						Paranaíba river
101	<i>Hypostomus topavae</i>	LBP 3249	18246	X	X						Paranapanema river
102	<i>Hypostomus margaritifera</i>	LBP4296	16621	X	X						Paraná river
103	<i>Hypostomus regani</i>	LBP1672	11261	X	X				X		Parapanema river
104	<i>Hypostomus myersi</i>	NUP680	16637	X	X						Paraná river
105	<i>Hypostomus cf paulinus</i>	LBP2548	10886	X	X						Corumbataí river
106	<i>Panaque nigrelineatus</i>	LBP3033	19170	X					X		Orinoco river
107	<i>Panaque bathyphilus</i>	AUM45503	P6269	X	X	X	X	X			Marañon river
108	<i>Panaque cochliodon</i>	T14628	uncat.	X	X	X	X	X			Magdalena river
109	<i>Panaque armbrusteri</i>	LBP 16546	61477	X	X	X	X		X		Xingu river
110	<i>Panaque schaeferi</i>	LBP14764	57552	X	X		X		X		Marañon river
111	<i>Panaque schaeferi</i>	INHS55408	T9023	X	X	X		X			Solimões river
112	<i>Baryancistrus beggini</i>	AUM54990	T09392	X	X	X	X	X			Orinoco river
113	<i>Hemiancistrus subviridis</i>	AUM54456	T09437	X	X	X	X	X			Ventuari river
114	<i>Baryancistrus demantoides</i>	ROM9339	T09361	X	X	X	X	X			Ventuari river
115	<i>Hemiancistrus guahiborum</i>	AUM39239	V096	X	X	X	X				Ventuari river
116	<i>Hemiancistrus medians</i>	ANSP187122	6948	X	X	X	X	X	X		Maroni river
117	<i>Spectracanthicus punctatissimus</i>	ANSP199539	B1496	X	X	X	X				Xingu river

118	<i>Spectracanthicus zuanoni</i>			X						
119	<i>Parancistrus nudiventris</i>					X	X	X		
120	<i>Spectracanthicus murinus</i>	LBP13821	57231		X					Tapajós river
121	<i>Baryancistrus niveatus</i>	missing	HLF1288	X	X	X	X	X		Iriri rivers
122	<i>Baryancistrus chrysolomus</i>	LBP16552	61466	X	X	X	X		X	Xingu river
123	<i>Baryancistrus xanthellus</i>	LBP16559	61488	X	X	X	X	X	X	Xingu river
124	<i>Baryancistrus</i> sp.2	LBP5252	26473		X				X	Jari river
125	<i>Baryancistrus</i> sp.1	LBP13839	57286	X					X	Tapajós river
126	<i>Megalancistrus parananus</i>	LBP1668	12132		X	X	X	X	X	Paranapanema river
127	<i>Acanthicus adônis</i>	LBP14755	57520	X	X	X		X		Amazon river
128	<i>Acanthicus hystrix</i>	LBP15014	-	X	X	X	X	X	X	Amazon river
129	<i>Leporacanthicus joselimae</i>	LBP 13835	57273	X	X				X	Tapajós river
130	<i>Leporacanthicus</i> sp.1	LBP 16285	61752	X	X		X		X	Tapajós river
131	<i>Leporacanthicus galáxias</i>	LBP9155	42512	X					X	Guamá river
132	<i>Leporacanthicus heterodon</i>	LBP 16553	64007	X	X	X	X	X	X	Xingu river
133	<i>Leporacanthicus triactis</i>	AUM54030	T09826	X	X	X	X	X		Ventuari river
134	<i>Pseudacanthicus</i> sp.3	LBP16548	64046	X	X	X	X		X	Xingu basin
135	<i>Pseudacanthicus leopardos</i>	LBP15369	69545		X		X		X	Tacutu river
136	<i>Pseudacanthicus leopardos</i>	AUM54030	T09826	X	X	X		X		Essequibo river
137	<i>Pseudacanthicus</i> sp.2	LBP15369	69544	X	X				X	Rio Branco river
138	<i>Pseudacanthicus</i> sp.1	LBP 17284	66572	X					X	Tapajós river
139	<i>Pseudancistrus pectegenitor</i>	AUM 42202	V5363	X	X	X	X		X	Casiquiare river
140	<i>Pseudancistrus sidereus</i>	AUM 43443	P4871	X	X	X	X	X	X	Casiquiare river
141	<i>Lithoxus pallidimaculatus</i>	MHNG2621	SU01-096	X	X	X	X	X	X	Suriname river
142	<i>Lithoxus stocki</i>	ANSP189135	6909	X	X	X	X	X		Ventuari river
143	<i>Lithoxus planquettei</i>	missing	T9040	X	X			X	X	Oyapock river
144	<i>Lithoxus jantjæ</i>	AUM39475	T9020			X	X			Ventuari river
145	<i>Lithoxus lithoides</i>	AUM37922	T412	X		X	X		X	Essequibo river
146	<i>Lithoxus jantjæ</i>	MHNG2651	GY04136		X			X		Rupununi river
147	<i>Exastilithoxus</i> sp.1	AUM56685	T09165	X		X	X	X		Cuaó river

148	<i>Exastilithoxus fimbriatus</i>	AUM36632	V049	X	X	X	X	X		Caroni river
149	<i>Exastilithoxus</i> sp.2	AUM43875	V5536	X	X	X	X	X		Soromoni river
150	<i>Pseudancistrus depressus</i>	MHNG261069	JMB2	X	X	X	X	X	X	Essequibo river
151	<i>Pseudancistrus barbatus</i>	MHNG265305	85.1	X	X	X	X	X	X	Maroni river
152	<i>Pseudancistrus</i> sp.1	MCP46144	T14760		X	X	X	X		Negro river
153	<i>Pseudancistrus</i> sp.1	MCP46103	T14764		X	X	X	X		Branco river
154	<i>Pseudancistrus nigrescensis</i>	MHNG265106	85.3	X	X		X	X	X	Essequibo river
155	<i>Pseudancistrus corantjiniensis</i>	MHNG267209	JMB1	X	X		X	X	X	Corentyne river
156	<i>Pseudancistrus asurini</i>	LBP16551	61473	X	X	X	X	X	X	Xingu river
157	<i>Pseudancistrus zawadzki</i>	LBP15045	61628		X				X	Tapajós river
158	<i>Guyanancistrus brevispinnis</i>	MHNG272509	86.1	X	X	X	X	X	X	Maroni river
159	<i>Guyanancistrus brevispinnis</i>	MHNG267909	MUS300	X		X			X	Nassau river
160	<i>Guyanancistrus brevispinnis</i>	LBP5253	26475						X	Jari river
161	<i>Guyanancistrus niger</i>	MHNG2722	85.6	X	X	X	X	X	X	Oyapock river
162	<i>Guyanancistrus longispinis</i>	MHNG2725	85.7	X		X	X	X	X	Oyapock river
163	<i>Corymbophanes kaiei</i>	ROM89856	T12637	X	X	X	X	X		Potaro river
164	<i>Hopliancistrus tricornis</i>	AUM39853	T9017	X	X	X	X	X	X	Aquarium species
165	<i>Hopliancistrus</i> sp.1	ANSP193087	B2167	X	X	X	X	X		Xingu river
166	<i>Dekeyseria scaphirhyncha</i>	AUM54309	T09540	X	X	X	X	X	X	Ventuari river
167	<i>Dekeyseria pulchra</i>	AUM44110	V5296	X	X	X	X	X		Atabapo river
168	<i>Dekeyseria picta</i>	MHNG258804	MUS162	X					X	Negro river
169	<i>Pseudolithoxus antrax</i>	AUM53557	T09934			X	X	X		Caura river
170	<i>Pseudolithoxus antrax</i>	AUM53520	T09282	X	X	X	X	X		Orinoco river
171	<i>Pseudolithoxus dumus</i>	ANSP190757	T09512	X		X	X	X	X	Ventuari river
172	<i>Pseudolithoxus nicoi</i>	AUM43726	P4647	X	X	X	X	X		Cassiquiare river
173	<i>Soromonichthys stearleyi</i>	AUM43872	V5533	X	X	X				Soromoni river
174	<i>Pseudolithoxus kelsorum</i>	AUM51644	T09895	X	X	X	X	X	X	Orinoco river
175	<i>Pseudolithoxus tigres</i>	AUM57674	T09376	X	X	X	X	X	X	Orinoco river
176	<i>Lasiancistrus saetiger</i>	MHNG260201	BR98-148	X					X	Guamá river
177	<i>Lasiancistrus</i> sp.1	LBP14229	59403	X					X	Tapajós river

178	<i>Lasiancistrus schomburgkii</i>	MHNG265100	PE08-719	X	X	X	X	X	X	Cushabatay river
179	<i>Lasiancistrus heteracanthus</i>	MHNG261303	CA013	X					X	Pauya river
180	<i>Lasiancistrus tentaculatus</i>	MHNG uncat	MUS 573	X	X	X	X	X	X	Orinoco river
181	<i>Lasiancistrus planiceps</i>	MHNG258604	MUS 118						X	Cesar river
182	<i>Lasiancistrus caucanus</i>	STRI-01805	Stri 3526	X			X		X	Cesar river
183	<i>Lasiancistrus guapore</i>	MCP35652	T14769			X	X	X		Purús river
184	<i>Ancistrus clementinae</i>	ROM93737	T13829	X		X	X	X		Guayas river
185	<i>Ancistrus cuiabe</i>	LBP 7676	36492	X	X				X	Coxipó river
186	<i>Ancistrus multispinnis</i>	LBP 794	7988	X					X	Itapucu river
187	<i>Ancistrus cirrhosus</i>	MHNG	MUS202	X					X	Uruguay river
188	<i>Ancistrus sp.1</i>	LBP 191	4103	X					X	Madeira river
189	<i>Ancistrus chagresi</i>	LBP 2756	18534		X		X		X	Liano river
190	<i>Ancistrus sp.2</i>	LBP3160	19302	X	X					Negro river
191	<i>Ancistrus sp.3</i>	LBP12129	51825	X	X					Madeira river
192	<i>Ancistrus cryptophthalmus</i>	LBP15299	63274		X				X	Tocantins river
193	<i>Ancistrus aguaboensis</i>	LBP17146	66676						X	Tocantins river
194	<i>Ancistrus sp.4</i>	LBP17118	66660						X	Tocantins river
195	<i>Ancistrus ranunculus</i>	LBP16547	64047		X				X	Xingu river
196	<i>Ancistrus ranunculus</i>	ANSP199525	B1500	X	X	X	X	X		Xingu river
197	<i>Ancistrus sp.5</i>	LBP6949	33369						X	Negro river
198	<i>Ancistrus leucosticus</i>	ROM88561	T08143	X	X		X	X		Essequibo river
199	<i>Ancistrus minutus</i>	LBP17164	66668	X					X	Tocantins river
200	<i>Ancistrus sp.6</i>	LBP191	4105	X					X	Madeira river
201	<i>Ancistrus sp.6</i>	LBP10595	49354		X				X	Madeira river
202	<i>Ancistrus sp.7</i>	LBP12129	51825	X	X				X	Madeira river
203	<i>Ancistrus sp.8</i>	LBP12129	51521	X	X					Madeira river
204	<i>Ancistrus megalostomus</i>	AUM51165	T10092	X	X	X	X	X		Araza river
205	<i>Ancistrus bolivianus</i>	ROM90368	T12872	X	X	X	X	X		Mamoré river
206	<i>Ancistrus sp.9</i>	ANSP199611	B1988	X		X		X		Xingu river
207	<i>Ancistrus macropthalmus</i>	AUM54994	T09397	X	X	X	X	X		Orinoco river

208	<i>Ancistrus</i> sp.10	LBP15981	66178						X	Xingu river
209	<i>Ancistrus</i> sp.11	LBP12553	53597	X						Marañon river
210	<i>Ancistrus</i> sp.12	LBP16407	67354	X						Tapajós river
211	<i>Ancistrus</i> sp.13	LBP5408	27105	X					X	Jari river
212	<i>Ancistrus</i> sp.14	LBP5712	27643	X					X	Araguaia river
213	<i>Ancistrus</i> sp.15	LBP15981	66178		X					Xingu river
214	<i>Neblinichthys echinatus</i>	ROM83692	T06066		X			X		Mazaruni river
215	<i>Neblinichthys brevibracchium</i>	ROM83692	T06068				X	X		Mazaruni river
216	<i>Paulasquama callis</i>	ROM83784	T06189		X		X	X	X	Mazaruni river
217	<i>Lithoxancistrus orinoco</i>	AUM54439	T09663				X	X		Ventuari river
218	<i>Lithoxancistrus orinoco</i>	AUM43725	V5246		X					Casiquiari river
219	<i>Lithoxancistrus yekuana</i>	AUM39473	T9004	X	X	X	X			Ventuari river
220	<i>Dolichancistrus fuessli</i>	ROM94484	T14621	X	X	X			X	Guaviare river
221	<i>Dolichancistrus carnegiei</i>	ANSP189598	6647		X	X	X	X		Magdalena river
222	<i>Cordylancistrus torbesensis</i>	INHS55478	T674	X	X	X	X	X		Torbes river
223	<i>Leptoancistrus canensis</i>	STRI11580	T9033	X	X	X	X	X		Tuira river
224	<i>Andenacistrus platycephalus</i>	ROM93847	T14019	X	X	X			X	Santiago river
225	<i>Transancistrus santaroensis</i>	ROM93798	T13980	X	X	X	X	X		Santa Rosa river
226	<i>Chaetostoma changae</i>	LBP1347	11442	X	X				X	Aquarium trade
227	<i>Chaetostoma vasquezi</i>	AUM53812	T09945	X	X					Caura river
228	<i>Chaetostoma jegui</i>	LBP15478	63784	X	X				X	Amazon basin
229	<i>Chaetostoma fischeri</i>	LBP2758	18540	X	X				X	Llano river
230	<i>Chaetostoma fischeri</i>	STRI11581	T9034	X	X	X	X	X		Tuira river
231	<i>Chaetostoma fischeri</i>	STRI7604	T9026	X	X	X	X	X		Chagres river
232	<i>Chaetostoma</i> sp.1	ROM94925	T12930		X				X	Meta river
233	<i>Chaetostoma micros</i>	ROM93895	T14125	X	X		X			Santiago river
234	<i>Lipopterichthys carrioni</i>	ROM93845	T14016	X	X	X	X	X		Santiago river
235	<i>Chaetostoma marmorensis</i>	MUSM44898	CH198	X	X	X	X	X		Huallaga river
236	<i>Chaetostoma breve</i>	AUM46515	P6292	X	X	X	X	X		Marañon river
237	<i>Chaetostoma lineopunctatum</i>	MHNG2712	PE08047	X	X	X	X	X		Ucauali river

238	<i>Loraxichthys lexa</i>	MHNG2712	PE08591	X	X	X	X	X		Haallaga river
239	<i>Lampiella gibbosa</i>	LBP2652	17407	X	X			X		Carombé river
240	<i>Hypoptopoma inexpectatum</i>	LBP4042	22905				X	X		Moa river
241	<i>Oxyropsis acutirostra</i>	LBP4300	23945				X	X		Negro river
242	<i>Acestridium Martini</i>	LBP7204	35332	X			X	X		Negro river
243	<i>Otocinclus vestitus</i>	Genbank	Genbank		X		X	X		
244	<i>Otothyris juquiae</i>	Genbank	Genbank				X			
245	<i>Schizolecis guntheri</i>	LBP2514	13846				X	X		Sagrado river
246	<i>Epactionotus gracilis</i>	MCP23606	EU37100				X	X		Morto river
247	<i>Hisonotus leucofrenatus</i>	LBP7407	35655				X	X		Batatau river
248	<i>Pseudotocinclus juquiae</i>	LBP616	7564	X	X			X		Juquiá river
249	<i>Pareiorhina carrancas</i>	LBP10256	10256	X	X		X			Sapateiro river
250	<i>Kronichthys lacerta</i>	LBP795	8304	X	X					Morumbi river
251	<i>Kronichthys subterres</i>	LBP515	6334	X	X		X			Betari river
252	<i>Isbrueckerichthys duceni</i>	LBP2650	17402	X	X		X	X		Pulador river
253	<i>Pareiorhaphis calmoni</i>	Genbank	Genbank	X	X					
254	<i>Neoplecostomus microps</i>	LBP645	7593	X	X		X	X		Paraíba do Sul river
255	<i>Neoplecostomus botucatu</i>	LBP7525	34832	X					X	Paranapanema river
256	<i>Neoplecostomus yapo</i>	NUP3560	9701	X					X	Paranapanema river
257	<i>Neoplecostomus paranensis</i>	LBP2732	17444	X					X	Grande river
258	<i>Neoplecostomus bandeirantes</i>	LBP2861	18616	X	X					Tietê basin
259	<i>Rineloricaria platyura</i>	MHNG2651		X	X					
260	<i>Rineloricaria heteroptera</i>	LBP1731	12864	X	X					Negro river
261	<i>Rineloricaria auracantha</i>	Genbank	Genbank	X	X					
262	<i>Loricariichthys microdon</i>	MHNG2650		X	X					
263	<i>Rineloricaria jubata</i>	ROM93680	T13597	X	X					Esmeralda rivers
264	<i>Dasylicaria pujanensis</i>	MHNG2674		X	X					
265	<i>Spatuloricaria sp</i>	Genbank	Genbank		X					
266	<i>Crossoloricaria venezuelae</i>	INHS35467		X	X					
267	<i>Planiloricaria cryptodon</i>	MHNG2677		X	X					

268	<i>Loricaria simlíma</i>	Genbank	Genbank	X	X					
269	<i>Apistoloricaria ommation</i>	Genbank	Genbank							
270	<i>Limatulichthys griséus</i>	G5066	AUM444	X	X			X		Essequibo river
271	<i>Sturissoma monopelte</i>	T06853	ROM862	X	X					
272	<i>Farlowella vittata</i>	V5314	AUM422	X	X		X	X		Orinoco river
273	<i>Metaloricaria paucidens</i>	MHNG2677		X	X					
274	<i>Lamontichthys stibaros</i>	AUM57480	T10365	X	X	X	X	X		Madre de Dios river
275	<i>Harttia kronei</i>	Genbank	Genbank					X		
276	<i>Rhinelepis áspera</i>	Genbank	Genbank	X	X		X	X	X	Paraná river
277	<i>Pseudorhinelepis genibarbis</i>	Genbank	Genbank	X	X				X	
278	<i>Pseudancistrus genisetiger</i>	MHNG2593	86.2	X	X					São Francisco river
279	<i>Hemipsilichthys gobio</i>	MCP42452	T14765	X	X	X	X		X	Pirapetinga river
280	<i>Hemipsilichthys papillatus</i>	LBP4956	10241		X	X			X	Paraíba do Sul river
281	<i>Hemipsilichthys nimius</i>	MCP30671	T14761		X	X	X	X		Perequê-Açu river
282	<i>Astroblepus sp.6</i>	STRI3803	T9038	X	X	X				Azucar river
283	<i>Astroblepus sp.5</i>	LBP3284	20010	X	X	X				Chorobamba river
284	<i>Astroblepus sp.4</i>	STRI3967	T9028	X			X	X		Zamora river
285	<i>Astroblepus sp.3</i>	MUSM44239	CH169				X	X		Huallaga river
286	<i>Astroblepus sp.2</i>	missing	CH161				X	X		Huallaga river
287	<i>Astroblepus sp.1</i>	MUSM44237	CH173		X	X	X	X		Huallaha river
288	<i>Callichthys callichthys</i>	LBP485	6040		X		X	X		Pombo river
289	<i>Hoplosternum littorale</i>	LBP210	4134	X	X					Amazon river
290	<i>Corydora stenocephalus</i>	ROM90345	T12839	X	X	X	X	X		Mamoré river
291	<i>Corydoras bilineatus</i>	ROM90344	T12840	X	X			X		Mamoré river
292	<i>Corydoras oiapoquensis</i>	LBP2809	18894	X	X				X	Coastal river Guiana
293	<i>Corydoras aeneus</i>	ROM90346	T12836	X	X		X	X		Mamoré river
294	<i>Corydoras imitator</i>	LBP6268	32502	X	X				X	Negro river
295	<i>Vandellia sp.1</i>	AUM90346	V5509	X	X	X	X			Orinoco river

Table 2. Primers used in the present study to amplify partial sequences of F-reticulon 4, 16S rRNA, cytochrome B (CytB), MyH6, RAG1 and RAG2.

Region and Fragment Length	Name	References	Primer Sequence
	Freticul4-D		5'-AGG CTA ACT CGC TYT SGG CTT TG-3'
	Freticul4-R		5'-GGC AVA GRG CRA ART CCA TCT C-3'
F-reticulon 4	Freticul4 D2	Chiachio et al. 2008	5'-CTT TGG TTC GGA ATG GAA AC-3'
	Freticul4 R2		5'-AAR TCC ATC TCA CGC AGG A-3'
	Freticul4 iR		5'-AGG CTC TGC AGT TTC TCT AG-3'
16S rRNA	16Sar	Kocher et al. 1989	5'-ACG CCT GTT TAT CAA AAA CAT-3'
	16Sbr		5'-CCG GTC TGA ACT CAG ATC ACG T-3'
CytB	L14841	Oliveira et al. 2011	5'-CCA TCC AAC ATC TCA GCA TGA TGA AA-3'
	H15915b		5'-AAC CTC CGA TCT TCG GAT TAC AAG AC-3'
MyH6	Myh6_F459	Li et al. 2007	5'-CATMTTYTCCATCTCAGATAATGG
	Myh6_F507		5'-GGAGAATCARTCKGTGCTCATCA-3'
	Myh6_R1325		5'-ATTCTCACCACCATCCAGTTGAA-3'
	Myh6_R1322		5'-CTCACACCATCCAGTTGAACAT-3'

RAG1	RAG1Fa	Lujan et al. 2105	5'-CCTGGTTTTTCATGCATTTGAGTGGCA-3'
	RAG1R1186		5'-AGCGCTCTTCTGARGGAACTA-3'
	RAG1Ra		5'-AGGGCATCTAATGTGGGCTGTGT
RAG2	RAG2 Fc	Lujan et al. 2015	5'-ATGGAGGCCGAACACCCAACA-3'
	RAG2R9661		5'-CGCTGCTGWACTCCATTT

Table 3. DEC models tested to estimate distribution ranges inherited by the descending lineages at each node of the tree.

		Dispersal rate between adjacent areas	Dispersal rate between no adjacents areas	LnL	K	d	μ	J	AIC
M0	DEC	1.00	1.00	-546.55	2	0.0041	0.00752	0	1097.1
	DEC+J	1.00	1.00	-435.83	3	0.00061	1.00E-12	0.03066	877.668
	BAYAREALIKE	1.00	1.00	-616.27	2	0.00375	0.07515	0	1236.54
	BAYAREALIKE+J	1.00	1.00	-444.56	3	0.00049	1.00E-07	0.03225	895.111
M1	DEC	1.00	1.00E-20	-513.99	2	0.00786	0.00855	0	1031.98
	DEC+J	1.00	1.00E-20	-424.18	3	0.00161	0.00138	0.05957	854.366
	BAYAREALIKE	1.00	1.00E-20	-592.73	2	0.00751	0.07444	0	1189.46
	BAYAREALIKE+J	1.00	1.00E-20	-432.35	3	0.00124	0.00168	0.06224	870.71

M2	DEC	1.00	5.00E-01	-531.14	2	0.00536	0.00747	0	1066.28
	DEC+J	1.00	5.00E-01	-421.28	3	0.00085	1.00E-12	0.03246	848.556
	BAYAREALIKE	1.00	5.00E-01	-606.7	2	0.00502	0.07476	0	1217.39
	BAYAREALIKE+J	1.00	5.00E-01	-432.58	3	0.00055	1.00E-07	0.06032	871.16
M3	DEC	0.50	1.00E-04	-531.99	2	0.01572	8.57E-03	0	1031.98
	DEC+J	0.50	1.00E-04	-420.41	3	0.00242	0.00023	0.12303	854.723
	BAYAREALIKE	0.5	1.00E-04	-592.74	2	0.015	7.44E-02	0	1189.48
	BAYAREALIKE+J	0.5	1.00E-04	-428.76	3	0.00203	0.00048	0.12035	866,117
M4	DEC	1.00	1.00E-04	-513.99	2	0.00786	0.00857	0	1031.98
	DEC+J	1.00	1.00E-04	-421.86	3	0.00137	0.00066	0.05977	849.723
	BAYAREALIKE	1.00	1.00E-04	-592.74	2	0.00751	0.07449	0	1189.48

BAYAREALIKE+J	1.00E-04	1.00E-04	-430.06	3	0.00106	0.00074	0.06541	866.117
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Capítulo 2

**Shift from slow- to fast-water habitats accelerates lineage and
phenotype evolution in a clade of Neotropical suckermouth catfishes
(Loricariidae: Hypoptopomatinae)**

Target jornal: Molecular Ecology

**Shift from slow- to fast-water habitats accelerates lineage and
phenotype evolution in a clade of Neotropical suckermouth catfishes
(Loricariidae: Hypoptopomatinae)**

*Fábio F. Roxo**, *Nathan K. Lujan[‡]*, *Victor A. Tagliacollo^Ω*, *Brandon T. Waltz[§]*, *Gabriel S. C. Silva**,
*Claudio Oliveira**, & *James S. Albert**

**Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, Universidade Estadual Paulista, UNESP, Botucatu, SP, Brazil; [‡]Department of Biology, University of Toronto Scarborough, Toronto, ON M1C1A4, Canada; ^ΩPrograma Ciências do Ambiente (CIAMB), Universidade Federal do Tocantins, UFT, Palmas, TO, Brazil; [§]Department of Biology, University of Louisiana at Lafayette, Lafayette, LA 70504–2451, USA*

Correspondence: Fábio F. Roxo, Fax: + 14 38116264 120;

E-mail: roxoff@hotmail.com.br

Abstract

Identifying habitat characteristics that accelerate organismal evolution is essential to understanding both the origins of life on Earth and the ecosystem properties that are most critical to maintaining life into the future. Searching for these characteristics on a large scale has only recently become possible via advances in phylogenetic reconstruction, time-calibration, and comparative analyses. In this study, we combine these tools with habitat and phenotype data for 105 species in a clade of Neotropical suckermouth catfishes commonly known as cascudinhos. Our goal was to determine whether riverine mesohabitats defined by different flow rates (i.e., pools vs. rapids) may have affected rates of cascudinho cladogenesis and morphological diversification. In contrast to predictions based on general theory related to life in fast-flowing riverine habitats, cascudinho lineages associated with these habitats exhibited increased body size, head shape diversity, and lineage and phenotype diversification rates. These findings are consistent with a growing understanding of river rapids as incubators of biological diversification and specialization. They also support ongoing efforts to conserve rapids habitats in the face of ongoing hydroelectric powerplant construction throughout the major tropical rivers of the world.

Keywords: Allometry; character evolution, evolutionary rates, Neotropical biodiversity; macroevolution; rheophily; size evolution

Running head: Fast waters accelerate evolution

Introduction

A growing body of research indicates that colonization of novel habitats can dramatically alter rates of evolutionary diversification. Habitat-mediated shifts in rates of speciation and morphological diversification have been documented in a wide range of organisms. Examples include aquatic amphipods (Hou *et al.* 2011), dragon lizards (Collar *et al.* 2010), and various fishes (e.g. wrasses, Price *et al.* 2011; sea catfishes, Betancur *et al.* 2013; pufferfishes, Santini *et al.* 2013; silversides, Bloom *et al.* 2013; minnows, Hollingsworth *et al.* 2013). Habitats implicated in these rate shifts span broad spatial scales, from biomes (e.g. marine vs. freshwater; Hou *et al.* 2011; Betancur *et al.* 2013; Santini *et al.* 2013; Bloom *et al.* 2013) and ecosystems (e.g. open ocean vs. coral reefs; Santini *et al.* 2013), to habitats that are regularly interspersed at relatively small spatial scales (e.g., benthic vs. pelagic stream habitats, Hollingsworth *et al.* 2013; arboreal vs. terrestrial habitats, Collar *et al.* 2010).

Natural examples in which clades diversify significantly more quickly or slowly after colonizing new environments are rarely observed. These instances are of broad significance, though, because they yield insights into both the drivers of evolutionary disparity and the fundamental ecological differences between habitats occupied by descendent lineages. Habitats that are novel to an evolutionary lineage and correlate with accelerated diversification, for example, may be inferred to have had reduced competition and/or greater ecological opportunity than the lineage's ancestral habitats at the time of colonization (Hou *et al.* 2011; Betancur *et al.* 2013; Bloom *et al.* 2013). Conversely, habitats that correlate with reduced diversification may be inferred to have had ecological niches that were already saturated at the time of colonization (*ibid.*). The robustness of such ecological inferences depends on both the number of parallel habitat transitions within a given lineage, and the degree to which the historical ecological state of each habitat can be corroborated by additional data (e.g. modern

species richness; Betancur *et al.* 2013). Although the competitive context and resource diversity of a habitat are the principal or only driving factors invoked by many studies of habitat-mediated evolutionary rate shifts, other possible drivers of diversification include habitat patchiness and connectivity over various spatial scales (Losos & Ricklefs 2009; Brown *et al.* 2013), and the complexity and selective strength of alternative adaptive landscapes (Rieseberg *et al.* 2002; Wellborn & Broughton 2008; Gavrillets & Losos 2009). Moreover, different lineages at different times can exhibit different evolutionary responses to transitions among habitats, yielding alternative inferences about the competitive context and ecological opportunities of those habitats (e.g. freshwater vs. marine ecosystems, Hou *et al.* 2011; Betancur *et al.* 2013; Santini *et al.* 2013; Bloom *et al.* 2013). Thus, ambiguity regarding mechanisms, as well as stochastic effects (Losos 2010) can inhibit robust generalizations about a habitat's influence on lineage diversification.

Despite these caveats, studies of habitat-mediated evolutionary rate shifts provide valuable historical tests of eco-evolutionary hypotheses. Such examples are becoming increasingly difficult to test in modern settings as habitats become globally more degraded. As human impacts rise, and as conservationists increasingly rely on phylogenetic patterns to prioritize habitat protections (López-Osorio & Miranda-Esquivel 2010), historical comparative analyses grow more important to a wider range of audiences. In this study, we examine the differential effects of water velocity on rates of diversification in a clade of Neotropical river fishes commonly known as cascudinhos (Loricariidae: Hypoptopomatinae), which vary in their preference for fast- vs. slow-flowing riverine habitats.

Fast-flowing waters in streams and rivers exhibit phylogenetically diverse fish assemblages with remarkable morphological convergences on a similar array of

phenotypic specializations, such as dorsoventral depression, attachment organs, surface-scraping jaws (Conway *et al.* 2012; Lujan & Conway 2015). Unfortunately, fast-flowing aquatic habitats and their resident biotas are also increasingly threatened worldwide by hydroelectric dams and other human impacts (Anderson & Maldonado-Ocampo 2011; Lujan & Conway 2015). In the Amazon alone, over 400 hydroelectric dams are now operational or under construction and over 300 more are planned (Winemiller *et al.* 2016), with a majority of these targeting high-gradient river habitats. Large tropical rapids habitats supporting exceptionally diverse, specialized and endemic fish assemblages are being especially impacted (Roberts 1993; Sabaj-Pérez 2015). There is therefore an urgent need to better understand the distinctive contribution that river rapids make to Earth's biological diversification.

Although morphological convergence clearly illustrates the evolutionary specializations that frequently occur in rapids habitats (Lujan & Conway 2015), the large-scale effect that these habitats can have on evolutionary diversification, particularly compared with adjacent slower-flowing habitats, remains poorly known. We hypothesize that, because of the apparently similar and strong selection pressures that drive morphological convergence in rapids habitats, lineages specializing in these habitats would exhibit a reduction in both overall morphological diversity and diversification rates. In contrast, the deeper, more three-dimensional and complexly-structured habitat of slow-flowing habitats should correlate with higher overall morphological diversity (e.g. Montaña & Winemiller 2010) and diversification rates.

Swiftly-flowing lotic riverine habitats are also associated with smaller body sizes (Schlosser 1987; Lavin & McPhail 1993; Lujan & Conway 2015) and greater philopatry (i.e., smaller geographic range sizes) in fishes – the latter putatively because of the greater risk that long-distance movement might lead to dislodgement and

exportation from a fast-flowing environment (Markert *et al.* 2010). Smaller body sizes and geographic range sizes are also both expected to correlate with increased rates of speciation (Markert *et al.* 2010; Wollenberg *et al.* 2011; Albert & Johnson 2012). We test these hypotheses by generating a densely sampled, time-calibrated molecular phylogeny for the cascudinho subfamily Hypoptopomatinae, which comprises three strongly monophyletic tribes that vary in habitat preference: the riffle- and pool-dwelling Hypoptopomatini and Otothyriini, and the rapids-dwelling Neoplecostomini (Roxo *et al.* 2014). We then use this phylogeny in combination with habitat and phenotype data to test for correlation between a lineage's occupation of fast- vs. slow-flowing habitats and its rates of speciation and diversification in head shape and maximum body size (MBS).

Material and Methods

Taxon sampling and phylogenetic inference

All comparative analyses were performed using the time-calibrated phylogenetic hypothesis of Roxo *et al.* (2014). This phylogeny was inferred from partial DNA sequences of three mitochondrial loci (16S rRNA, COI, Cytb) and one nuclear locus (F-*reticulon 4*; 4,500 base pairs total), and included 105 species representing approximately 50% of all Hypoptopomatinae species, with every tribe, genus and major lineage represented by at least one species (see Table S1 for identities, localities and catalog numbers of all tissue vouchers). Throughout this paper, we follow the taxonomic scheme of Lujan *et al.* (2015) in which Hypoptopomatinae is a monophyletic subfamily containing the respectively monophyletic tribes Hypoptopomatini, Otothyriini, and Neoplecostomini (vs. the Roxo *et al.* 2014 taxonomic framework in which these last three clades were treated as subfamilies).

Time calibration

We calibrated the phylogeny of Roxo *et al.* (2014) using an uncorrelated relaxed molecular clock in the program BEAST v1.6.2 (Drummond & Rambaut 2007). Partitioning scheme and nucleotide substitution models for our BEAST analysis were determined using the software PartitionFinder v1.1.1 (Lanfear *et al.* 2012; Tables S3 and S4 in Roxo *et al.* 2014). Two fossil calibrations were used to constrain divergence times for all clades of the phylogenetic tree: The first calibration was implemented as a normally distributed prior offset to 125 million years ago (Mya) with a standard deviation of 15 My, which matches current estimates of a Lower Cretaceous (145–100 Mya) origin of the catfish order Siluriformes (Lundberg 1993; Sullivan *et al.* 2006; Lundberg *et al.* 2007). We used a birth–death model for speciation likelihood, which specifically models extinction throughout the phylogeny, and used a starting tree optimized according to maximum likelihood (ML). The BEAST analysis was run for 100 million generations with tree space sampled every 1,000th generation. Stationarity and sufficient mixing of parameters (ESS>200) were evaluated using Tracer v1.5 (Rambaut & Drummond 2007a), and a consensus tree was assembled using TreeAnnotator v1.6.2 (Rambaut & Drummond 2007b).

Ancestral habitat estimation

Habitat data were obtained from original descriptions of each species included in our analysis and from the personal field experience of the authors FFR and VAT, who collected most of the specimens examined in this study (Table S2). We estimated historical rates of habitat evolution using the `make.simmap` function in the R package `phytools` v0.3-10 (Revell 2012) and the software SIMMAP v1.5 (Bollback 2006). For

the SIMMAP analysis, we generated a presence/absence matrix consisting of all species assigned to one or more of four habitat types: rapids, lakes, pools, and riffles (classifications follow Crampton 2011). The resulting matrix was run through 1,000 stochastic character map simulations. We also evaluated the fit of equal-rates (ER), symmetric (SYM), all-rates-different (ARD), and meristic macroevolutionary models to our data using the fitDiscrete function for discrete data in the R package geiger (Harmon *et al.* 2008), which ranks models according to the Akaike information criterion (AICc; Sugihara 1978).

Testing effect of habitat on speciation rate

We examined the effect of each habitat category on the speciation rate of each Hypoptopomatinae tribe using the implementations BiSSE (Binary State Speciation and Extinction; Maddison 2007) and MuSSE (Multi-State Speciation and Extinction; FitzJohn 2012) in the R package diversitree v0.9-8 (FitzJohn 2012). BiSSE was used to test the effect of each habitat individually while MuSSE was used to test the effect of all habitat categories combined.

Speciation rate estimation

To minimize incomplete sampling biases, we accounted for all known missing species in all speciation analyses (see Table S2 for a list of missing species and their lineage assignments). To estimate rates of speciation and extinction across the subfamily Hypoptopomatinae, we used BAMM v2.1.0 (Rabosky 2014). BAMM assumes that speciation and extinction are heterogeneously distributed throughout a phylogeny and uses a reversible jump Markov chain Monte Carlo algorithm to explore the universe of candidate cladogenesis models (Rabosky *et al.* 2013; Rabosky 2014; Rabosky *et al.*

2014a). The analysis was conducted with two chains running simultaneously for a total of five million generations. We sampled tree space every 1,000th generation and checked for MCMC convergence by plotting the log-likelihood trace using the R package BAMMtools (Rabosky *et al.* 2014b), with burnin set to 0.5 (i.e. first half of all samples discarded). To account for the effects of phylogenetic uncertainty on our analyses, we conducted BAMM analyses of species diversification across 2,500 trees sampled from the posterior distribution of topologies and branch lengths. To visualize the evolutionary rate dynamics from BAMM output results we also used BAMMtools.

To specifically test the hypothesis that Neoplecostomini underwent an increase in speciation rate relative to other Hypoptopomatinae lineages, we used the MEDUSA implementation (Alfaro *et al.* 2009) in the R package Geiger (Harmon *et al.* 2008). For this analysis, the entire Hypoptopomatinae phylogeny was divided into 24 lineages to which a total number of species for each lineage (including missing species) were assigned.

Morphometric data selection, collection and size correction

Both body size and head shape are important correlates of trophic ecological, locomotory and life history characteristics in fishes (Winemiller *et al.* 1995; López-Fernández *et al.* 2013; Steele & López-Fernández 2014). We therefore measured variation in these traits among Hypoptopomatinae species to provide a morphological index from which to infer ecological diversity.

Maximum standard length (SL in cm) is a useful estimate of body size and associated life-history traits in most fish species (Albert & Johnson 2012). Maximum standard lengths (or maximum body size: MBS) were compiled for all species from original species descriptions, the Check List of the Freshwater Fishes of South and

Central America (Reis *et al.* 2003), and validated museum records (Table S2). To quantify head shape, we made 14 point-to-point measurements between external, putatively homologous landmarks using a digital caliper. A total of 291 specimens from 105 species and 22 genera distributed throughout Hypoptopomatinae were measured (Table S4). Landmarks and interlandmark distances were a subsample of those originally proposed by Armbruster (2003), and were only measured from adult specimens (>18.0 mm SL for Otothyriini, >30 mm SL for Hypoptopomatini, and >50 mm SL for Neoplecostomini).

To minimize allometric (body size) influence on morphometric data, we followed the method of Dryden & Mardia (1998) by using the program Past v1.28 (Hammer *et al.* 2004) to conduct a principal component analysis (PCA), normalize the first two coordinate dimensions, divide all coordinate values by the centroid size for each specimen and conduct a Procrustes superimposition of the left half to a mirrored version of the right half. We then used Past to conduct a PCA on the covariance matrix of phylogenetically corrected residuals.

Estimating phenotypic diversification rates

To estimate rates of continuous evolution in body size and head shape, we used BAMM v2.1.0 (Rabosky 2014; see BAMM input values in Table S2). BAMM was programmed to use two chains running simultaneously for a total of 50 million generations, and to sample tree space every 5,000th generation. MCMC convergence was checked by plotting the output log-likelihood trace using the R package BAMMtools (Rabosky *et al.* 2014b) with burn-in set to 0.5. Final BAMM analyses of phenotypic evolution examined 5,000 trees sampled from the posterior distribution. Evolutionary rate dynamics were visualized from BAMM output using BAMMtools.

Ancestral body size estimation

To estimate rates of body size evolution, we used the `contMap` function in the R package `phytools` v0.3-10 (Revell 2012). This function maps a continuous character on to a phylogenetic tree by estimating ancestral states at each nodes using `fastAnc` (fast estimation of ML ancestral states). Macroevolutionary models were evaluated using the `fitContinuous` function in the R package `geiger` (Harmon *et al.* 2008), which ranks models according to AICc scores.

Phylomorphospace analysis

To visualize the distribution of modern species and ancestral lineages in morphospace (Sidlauskas 2008), we generated phylomorphospace biplots using the `phylomorphospace` function in the R package `phytools` v0.3-10 (Revell 2012). The first principal component of size-corrected head shape for each taxonomic grouping was plotted against log maximum body size (MBS). Species were plotted using colors that correspond to their habitat classification.

Results

Time-calibrated phylogenetic analysis

We obtained a time-calibrated phylogeny that is the most taxonomically comprehensive to date for catfishes of the subfamily Hypoptopomatinae (Fig. S1, *sensu* Lujan *et al.* 2015) with a tree topology that parallels earlier studies (i.e. Chiachio *et al.* 2008; Roxo *et al.* 2014). The subfamily Hypoptopomatinae is estimated to have originated during the Paleocene, approximately 58.4 Mya (40.8–79.7 Mya 95% highest posterior density, HPD), the tribe Hypoptopomatini and clade composed of Neoplecostomini + Otothyriini

during the Lower Eocene, approximately 49.9 Mya (33.6–67.4 Mya 95% HPD), and the tribes Neoplecostomini and Otothyryni during the Lower Eocene, approximately 45.9 Mya (31.0–62.2 Mya 95% HPD). Mean substitution rate for the entire dataset was estimated to be 0.27% substitutions per million years.

Ancestral habitat estimation

Our SIMMAP analysis indicated that the most recent common ancestor (MRCA) of both the subfamily Hypoptopomatinae and the tribe Otothyryni occupied mostly lake, pool and riffle habitats, whereas the Hypoptopomatini MRCA occupied lake and pool habitats almost exclusively (Fig. 1). In contrast, the Neoplecostomini MRCA and almost all descendent lineages were found to have occupied mostly fast-flowing riffle and rapids habitats (Fig. 1). The equal-rates (ER) model best fit our habitat data and was used in the SIMMAP analysis of habitat (Table 2).

Effect of habitat on speciation rate

Our MuSSE analysis found that all four habitats had a combined effect on speciation in Hypoptopomatinae (Table 3), whereas our separate BiSSE analyses of each habitat found that only pool and rapids habitats affected speciation (lake and pool habitats did not have an effect).

Speciation rate analyses

For the subfamily Hypoptopomatinae as a whole, speciation rate increased from the root until approximately 40 Mya, then decreased from that time to the present (Fig. 2).

Within Hypoptopomatinae, our BAMM analysis indicated an overall deceleration in speciation rate starting with the most recent common ancestor of the tribes

Hypoptopomatini and Otothyrini, whereas Neoplecostomini exhibited a constant overall speciation rate, with a strong increase along the branch leading to the rapids-dwelling genus *Pareiorhaphis* (Fig. 2; Fig. 3a). Speciation rate-through-time dynamics for tribes Hypoptopomatini and Otothyrini are also consistent with speciation decreasing from the oldest node to the present, whereas Neoplecostomini showed a small increase in speciation rate near the present (Fig. 2).

Our specific test of a speciation rate shift using MEDUSA indicated that speciation rate significantly increased along the branch giving rise to the relatively large-bodied clade comprising the genera *Neoplecostomus*, *Isbrueckerichthys*, *Kronichthys*, *Pareiorhaphis* and *Pareiorhina* (Fig. S2).

Rates of diversification in body size and head shape

Our BAMM analysis indicated an acceleration in the rate of MBS evolution in the Neoplecostomini, which was greatest in the genus *Pareiorhaphis* (Fig. 4). In contrast, MBS diversification was relatively constant in the Hypoptopomatini and decelerated in the Otothyrini (Fig. 4). We also observed an acceleration in head shape diversification along the branch leading to the tribe Neoplecostomini (Fig. 5), and evidence of a shift in the rate of head-shape diversification at the node giving rise to Neoplecostomini (Fig. 5).

Estimating ancestral body size

Our ContMAP analysis revealed an evolutionary trend toward larger body sizes in the Neoplecostomini and an overall decrease in body sizes in Otothyrini (Fig. 1). Some clades nested within Neoplecostomini showed very fast rates of evolution to both larger (*Kronichthys subteres* and *Pareiorhaphis cameroni*) and smaller (*K. lacerta* and *P.*

eurycephalus) body sizes. The Kappa model best fit our body size data and was used for our ContMAP analysis (Table 1).

Phylomorphospace analysis

The first principal component (PC1) axis of our phylomorphospace analysis explained 52.4% of variation in head shape for all Hypoptopomatinae species. Characters that loaded strongly on PC1 were head–eye length, orbit diameter, snout length, internares width, interorbital width, mouth width, and barbel length (Table S4). Species of the highly rheophilic tribe Neoplecostomini occupied a distinct region of head shape/body size morphospace (Fig. 6), largely exclusive of the morphospace occupied by slower-water dwelling members of the Hypoptopomatini and Otothyryni.

Discussion

Our analyses indicate that the most recent common ancestor of the cascudinho catfish subfamily Hypoptopomatinae had a relatively small body size (~6 cm SL) and occupied riffle and pool habitats approximately 58.4 Mya (40.8–79.7 Mya 95% HPD; Figs. S1 and 1). The tribes Hypoptopomatini and Otothyryni – two of three major cascudinho subclades – remained largely restricted to small body sizes and similar habitats (Fig. 1), and exhibited mostly decreasing or constant rates of speciation (Fig. 2) and morphological diversification (Figs. 4 and 5). In stark contrast, the primarily riffle- and rapids-dwelling tribe Neoplecostomini exhibited constant or increasing rates of speciation and morphological diversification, with a significant shift toward faster speciation in the lineage leading to exclusively rapids-dwelling species (Fig. S2). With maximum body sizes ranging from 3.9 to 17.0 cm SL, modern neoplecostomin species

exhibit ~120% greater size range than the Hypoptopomatini (3.3–14.3 cm SL) and ~300% greater size range than the Otothyriini (2.1–6.6 cm SL; Table S2). Moreover, neoplecostomin species span a broader range of head shape diversity than either of the two other clades combined (Fig. 6). Of the four habitat categories occupied by cascudinho catfishes, only two – rapids and pools – appear to have significantly influenced net speciation rates throughout Hypoptopomatinae (Table 3), with the former accelerating and the latter decelerating speciation.

Ecological opportunity is often invoked to explain accelerations in evolutionary diversification following a habitat shift, due to reduced competition associated with the availability of more resources in new habitats (Hou *et al.* 2011; Betancur *et al.* 2013; Bloom *et al.* 2013). However, the specific role of ecological opportunity is difficult to disentangle from other influences associated with habitat shifts. Such influences may include reduced dispersal ability, the spatial scale of gene flow, and effective population sizes (Losos & Ricklefs 2009; Markert *et al.* 2010; Brown *et al.* 2013), or increases in the complexity or selective strength of adaptive landscapes (Rieseberg *et al.* 2002; Wellborn & Broughton 2008; Gavrillets & Losos 2009; Lujan & Conway 2015). Contrary to the predictions of ecological opportunity, we predicted that rapids-dwelling Hypoptopomatinae lineages would exhibit accelerated speciation and decelerated morphological diversification. We based these predictions on prior observations or inferences of reductions in body size, dispersal ability, and the spatial scale of gene flow (Lavin & McPhail 1993; Markert *et al.* 2010), and morphological trends suggesting increased stabilizing selection (Lujan & Conway 2015) in fast- vs. slow-water specialized fish lineages.

Empirical patterns, however, differed from our predictions. As predicted, speciation did increase in Neoplecostomini, but this acceleration was accompanied by

larger, not smaller, body sizes. Also, diversification of both body size and head shape significantly increased in neoptocostomini lineages occupying rapids habitats exclusively. These differences between predicted and observed patterns highlight major gaps in our understanding of the evolutionary influence that rapids habitats can have on rheophilic organisms. Questions raised by our study include whether the distinctive and diverse head shapes observed in Neoptocostomini correspond to as yet unrecognized adaptive ecological peaks – such as distinctive benthic food resources (e.g., Lujan *et al.* 2011, 2012) – that might only be present in rapids habitats. If this were known, and these niches were underexploited prior to the neoptocostomini invasion of these habitats, then the otherwise poorly supported hypothesis of ecological opportunity would become increasingly plausible. Likewise, it would be valuable to know if the distinctive hydrodynamic environment of rapids habitats selects for a narrow range of optimal body sizes. If such a size optimum were larger than the Hypoptopomatinae ancestor, but smaller than ancestors of other fish lineages that have shrunk upon invasion of fast-water habitats (e.g., Gasterosteidae: *Gasterosteus*, Lavin & McPhail 1993; Cichlidae: *Teleocichla*, Steele & López-Fernández 2014; Percidae: Etheostomatinae, Stepián & Haponski 2015), the ecological process driving divergent body size shifts could be understood more clearly.

Unfortunately, geographic ranges and the spatial scale of gene flow in these species and habitats are unknown, making the job of disentangling evolutionary mechanisms all the more difficult. Studies of relatively recent, intraspecific differentiation between populations or individuals associated with slow- vs. fast-water habitats provide strong evidence that the adaptive landscape of these habitats are distinct, but applications of these studies to the Hypoptopomatinae system are limited. For example, Langerhans *et al.* (2003) investigated the effects of different flow rates on

body shape in adjacent populations of a mid-water and a near-bottom dwelling fish species. They observed shifts toward a more fusiform body, a higher aspect ratio caudal fin, and respectively upturned (mid-water species) or downturned (near-bottom species) mouths. Likewise, in studies of lake vs. stream populations of three-spine stickleback (*Gasterosteus aculeatus*; Berner *et al.* 2008; Sharpe *et al.* 2008), lotic populations were found to have deeper bodies and caudal peduncles. In a review of flow regime studies on fishes, Langerhans (2008) found that fishes adapted for life in high-flow environments share several specialized physiological and biomechanical traits related to swimming, including relatively more red muscle, stiffer bodies, higher steady swimming performance, and lower unsteady swimming performance.

Hypoptomatinae species, however, are entirely benthic, have dorsoventrally depressed bodies, entirely ventral mouths and oral disks, and rarely swim freely in the water column, preferring instead to make short movements between substrate attachment sites (all authors pers. obs.). Indeed, the oral disk of all members of the family Loricariidae seems to facilitate attachment and station-holding in fast-water habitats without otherwise specialized body morphologies or enhanced swimming performance (Geerinckx *et al.* 2007). Similar morphological specializations have repeatedly evolved in a wide range of other fast-water specialized fish lineages throughout the world (Lujan & Conway 2015).

Despite the elusiveness of a single compelling narrative to explain the observed diversification rate shifts in fast- vs. slow-water specialized Hypoptomatinae lineages, results of this study should serve as robust support for the further investigation and conservation of riverine rapids habitats. These habitats can be challenging for fishes to occupy, and also to investigate from a real-time, ecological perspective because of the strong hydraulic forces that define them. Macroevolutionary studies such as this

provide an alternative to working directly in the habitat by focusing instead on specimens that have been removed from it. There is an urgent need, however, for studies such as this to be complemented with detailed ecological and population genetic data so that a more complete understanding of eco-evolutionary dynamics in these distinctive but threatened ecosystems can be achieved. Given the intensity and global scale of especially tropical river development (Winemiller *et al.* 2016), such data should be gathered soon.

Acknowledgments

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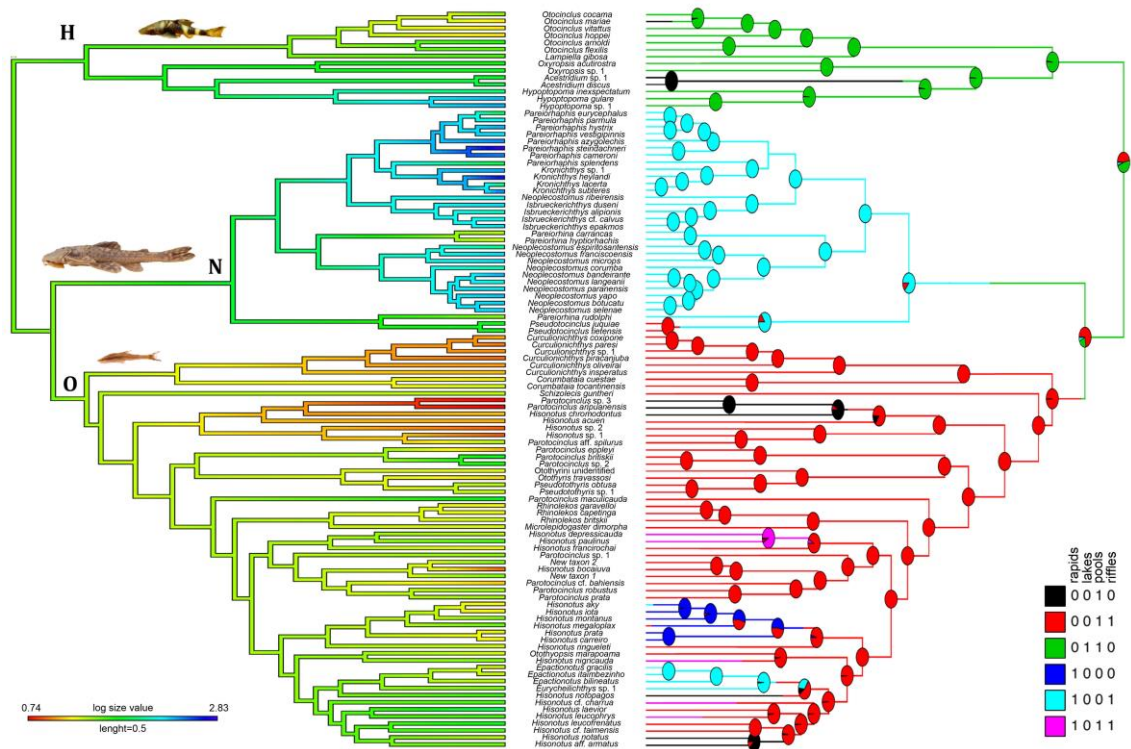


Fig. 1. Left: ContMap cladogram illustrating estimated ancestral maximum body size (MBS) constructed using a time calibrated phylogeny of Hypoptopomatinae, including the tribes Hypoptopomatini (H), Neoplecostomini (N) and Otothyriini (O). Branch colors correspond to estimates of \ln MBS (i.e., maximum standard body length measured from tip of snout to base of caudal fin), with red indicating smaller and green indicating larger MBS. Right: SIMMAP cladogram illustrating ancestral habitat estimates for the subfamily Hypoptopomatinae. See Table S2 for habitat classifications of each species.

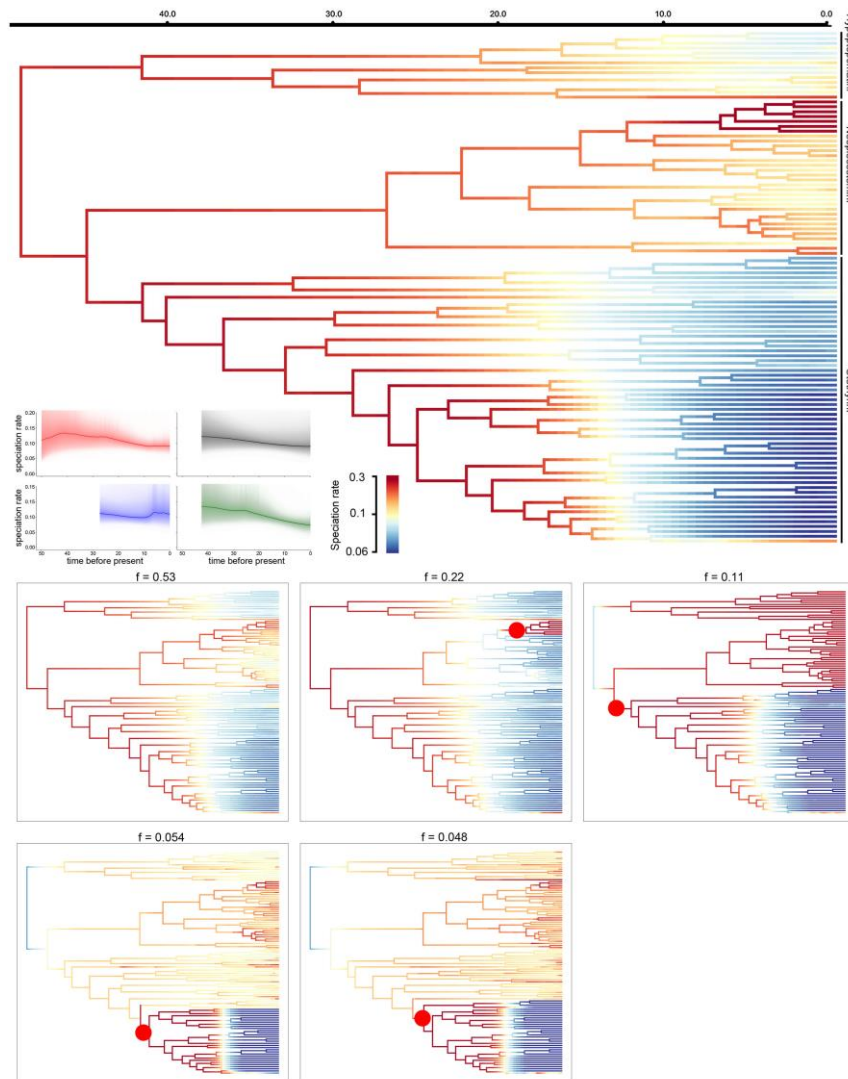


Fig. 2. Phylorate plots showing speciation rates for the subfamily Hypoptopomatinae. Branch colors denote instantaneous rates (cool colors = slow, warm = fast). The large cladogram at top depicts mean Phylorate, with colors indicating the mean evolutionary rate across all shift configurations sampled during simulation. Smaller cladograms at bottom show the five distinct shift configurations having the highest posterior probabilities. For each distinct shift configuration, the locations of rate increases are shown as red circles. Text labels denote the posterior probability of each shift configuration. The small Rate-Through-Time plots at left display the cumulative speciation rate from the root of the tree to the present computed from the joint posterior density in BAMM for the entire subfamily (red) and for each tribe (black = Hypoptopomatini; blue = Neoplecostomini; green = Otothyriini). The order of terminal taxa follows that in Fig. 1.

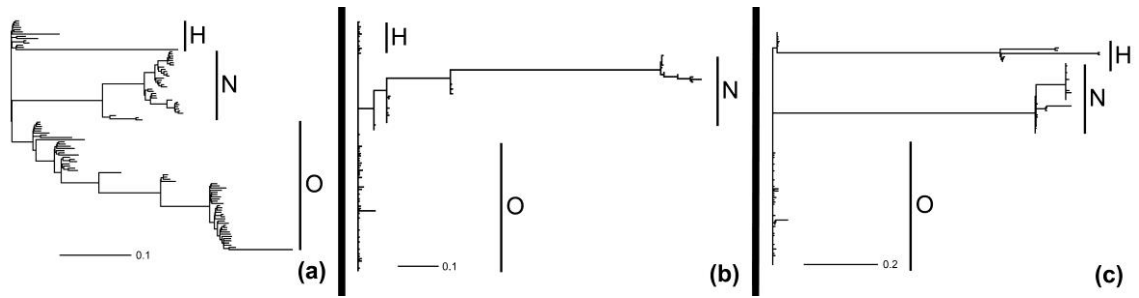


Fig. 3. Marginal probabilities of rate shifts for speciation (a), MBS (b), and head shape (c) in the Hypoptopomatinae tribes Hypoptopomatini (H), Neoplecostomini (N) and Otothyriini (O). Branch lengths are scaled by the probability that they contain a shift event.

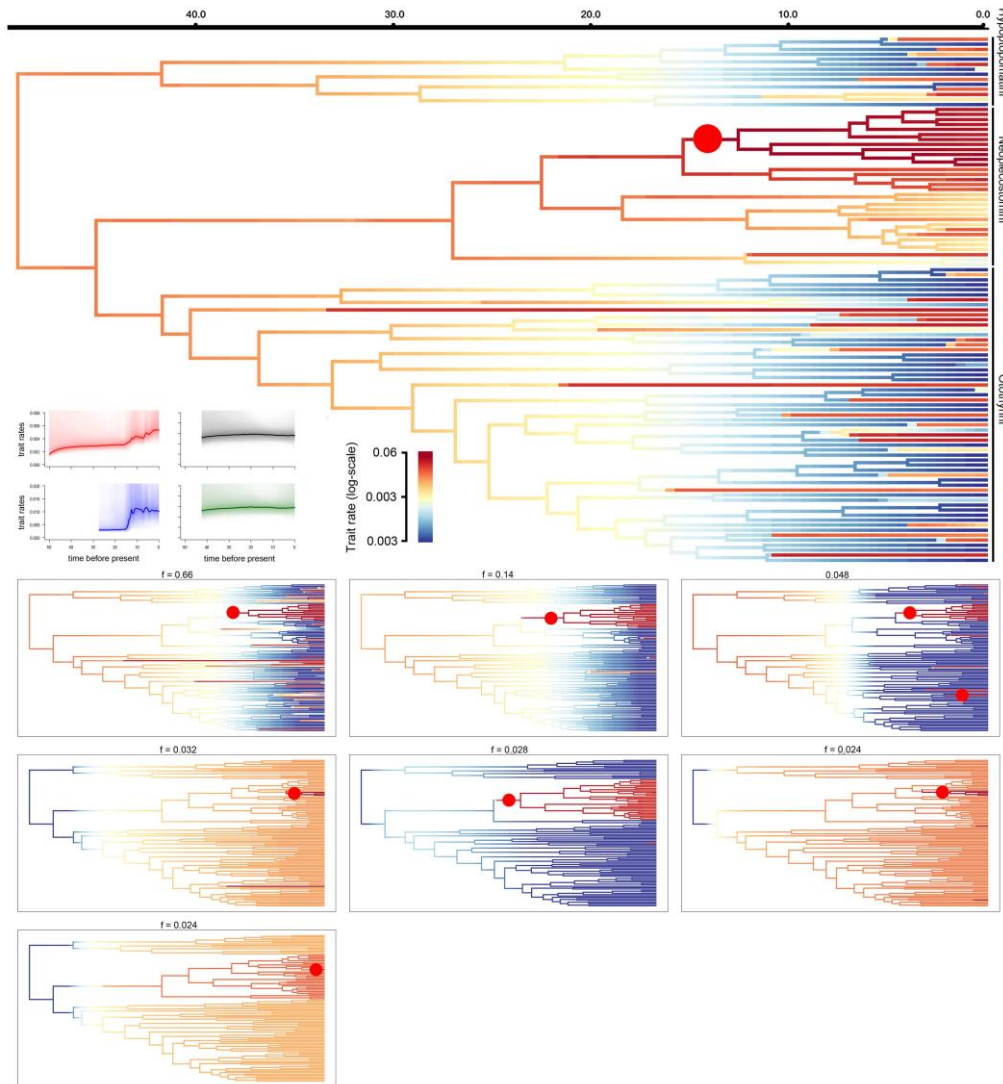


Fig. 4. Phylorate plots showing rates of maximum body size (MBS) evolution throughout the subfamily Hypoptopomatinae. Branch colors denote instantaneous rates (cool colors = slow, warm = fast). The large cladogram at top depicts mean Phylorate, with red circles indicating the most frequent rate increases along all sampled trees of the Bayesian analysis. Smaller cladograms at bottom show the seven distinct shift configurations having the highest posterior probabilities. For each distinct shift configuration, the locations of rate increases are shown as red circles. Text labels denote the posterior probability of each shift configuration. The small Rate-Through-Time plots at left display cumulative MBS rates from the root to the present computed from the joint posterior density in BAMM for the subfamily Hypoptopomatinae (red) and each tribe (black = Hypoptopomatini; blue = Neoplecostomini; green = Otothyri). The order of terminal taxa follows that in Fig. 1.

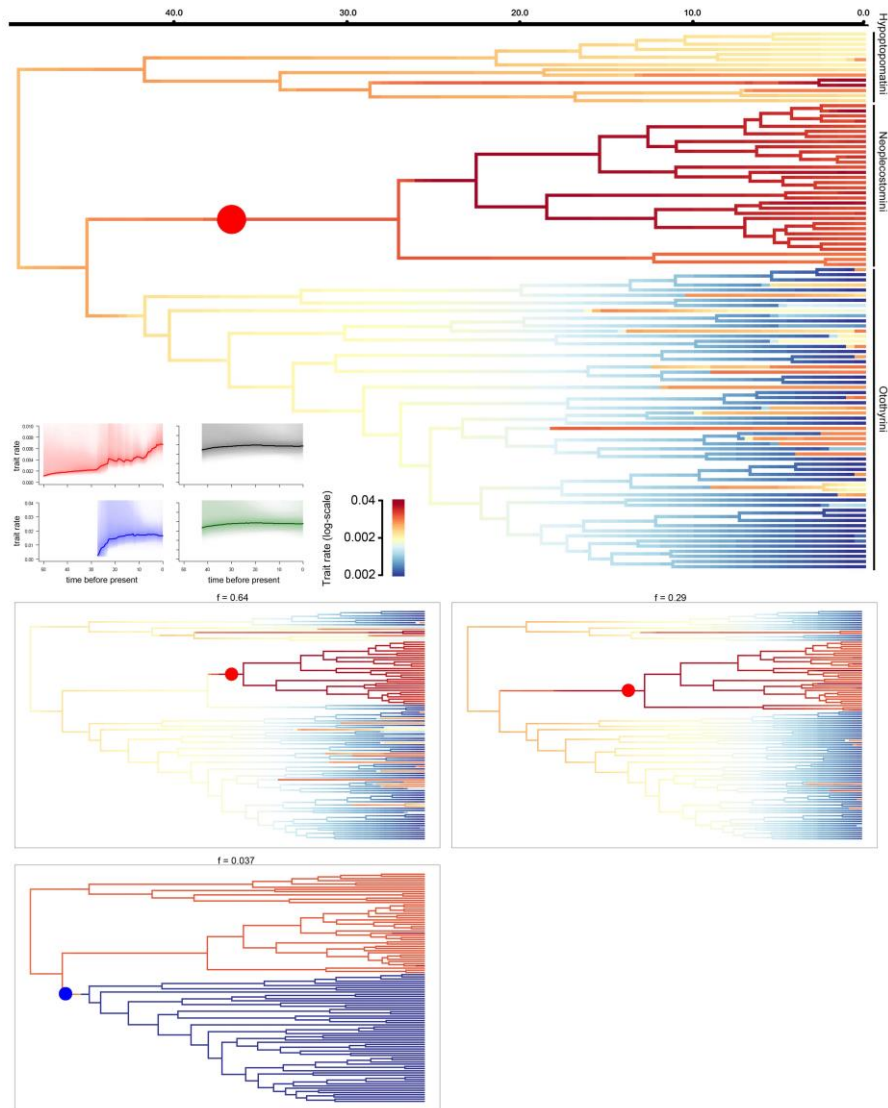


Fig. 5. Phylorate plots showing head-shape diversification rates for the subfamily Hypoptopomatinae. Branch colors denote instantaneous rates (cool colors = slow, warm = fast). The large cladogram at top depicts the mean Phylorate with the red circle indicating the most frequent rate increase along all sampled trees of the Bayesian analysis. Smaller cladograms at bottom show the three distinct shift configurations having the highest posterior probabilities for head shape evolutionary rate. For each distinct shift configuration, the locations of rate increases are shown as red circles. Text labels denote the posterior probability of each shift configuration. The small Rate-Through-Time plots at left display the cumulative head-shape diversification rate from the root of the tree to the present computed from the joint posterior density in BAMM for the subfamily Hypoptopomatinae (red plot) and each tribe (black = Hypoptopomatini; blue = Neoplecostomini; green = Ootothyri). The order of terminal taxa follows that in Fig. 1.

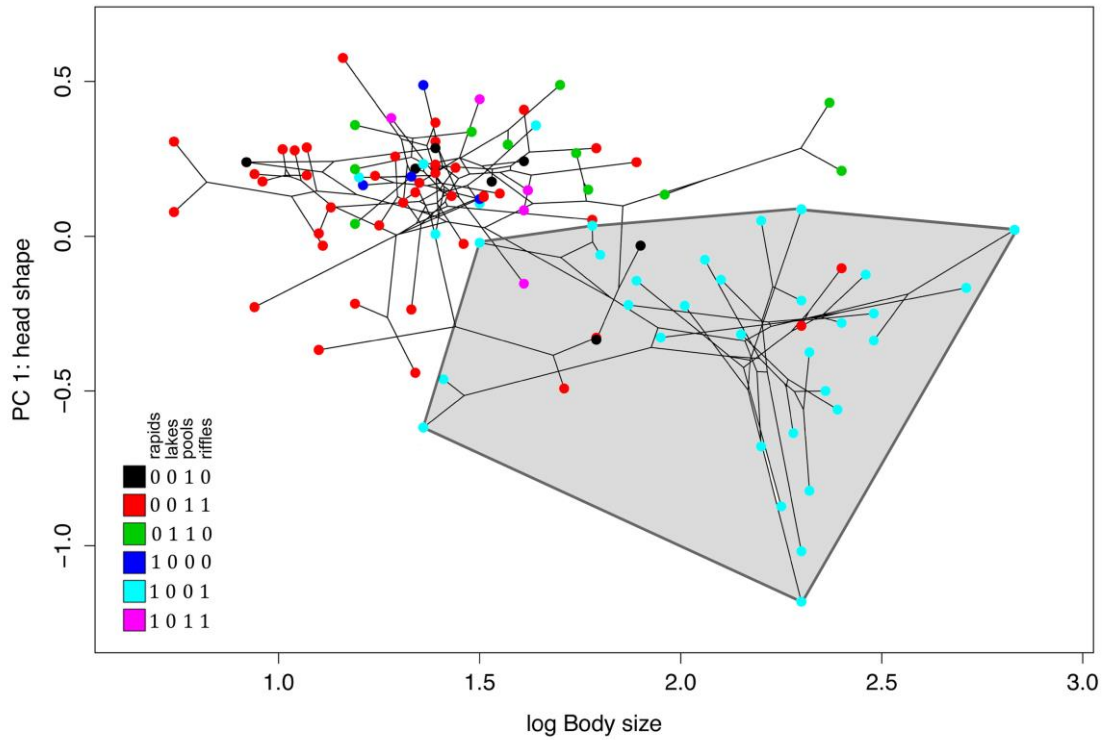


Fig. 6. Phylomorphospace plot for the subfamily Hypoptopomatinae in which terminal colors correspond to habitat and the shaded convex hull encloses all examined species of the tribe Neoplecostomini. The y-axis is the first principal component (PC) from a PC analysis of log-normalized linear distances between 14 external landmarks on the head (following head landmarks originally proposed by Armbruster 2003) and the x-axis is log maximum body size (MBS).

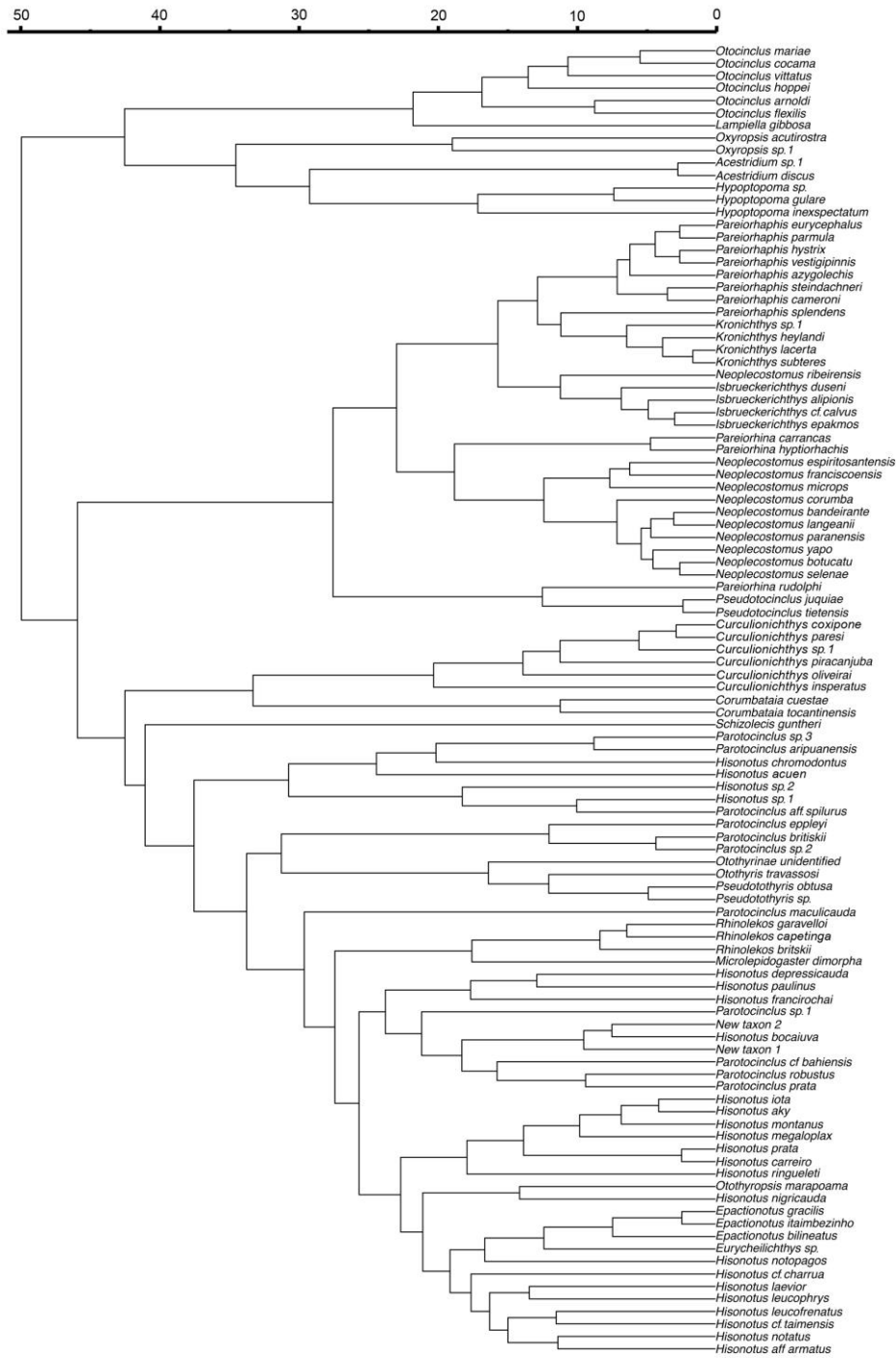


Fig. S1. Time calibrated tree of Hypoptopomatinae from Roxo *et al.* (2014) used in the present study. All nodes have a Bayesian posterior probability higher than 0.95. Duplicate terminals were deleted from the original time calibrated tree of Roxo *et al.* (2014). See Table S1 for all taxa information.

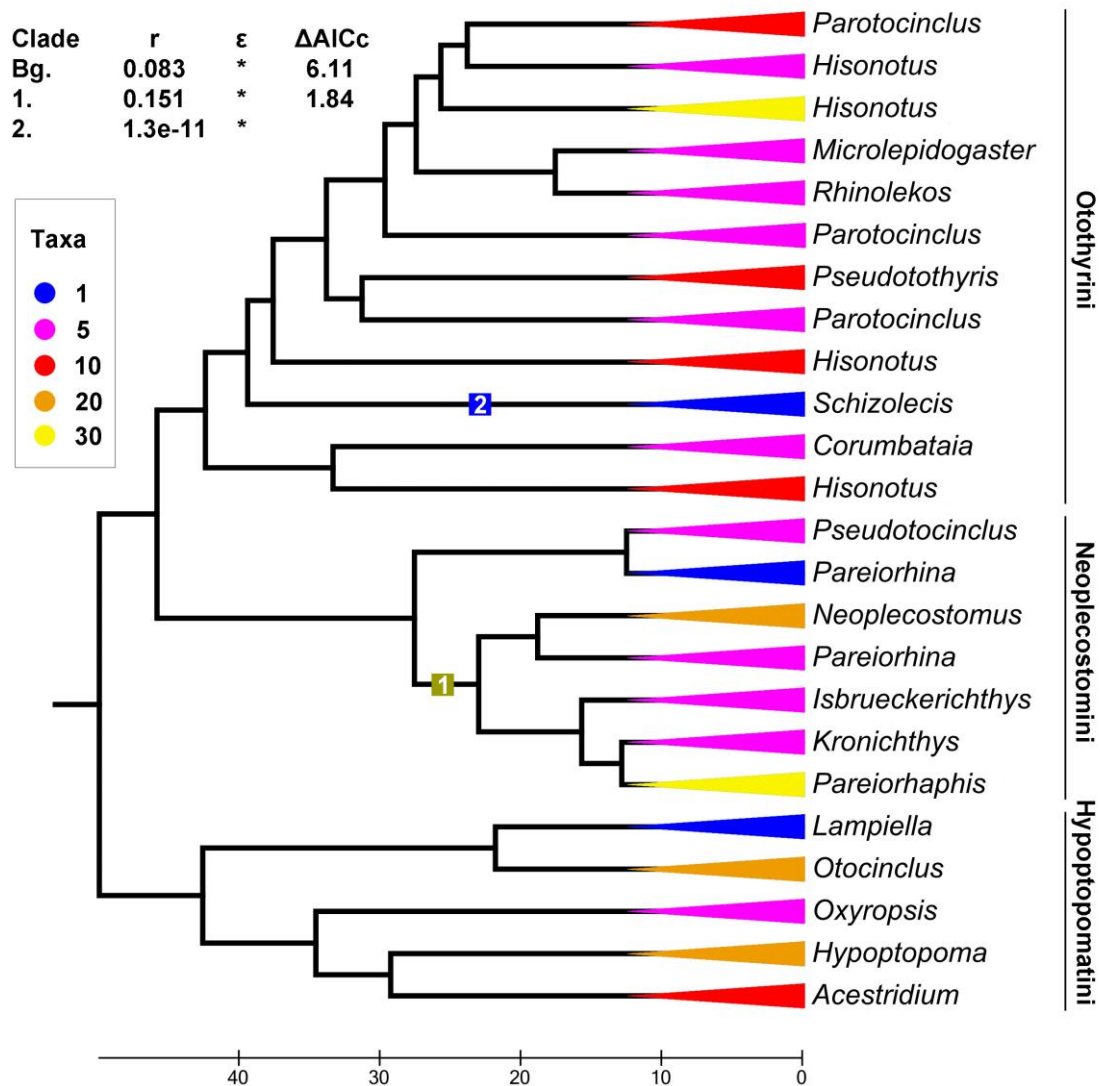


Fig. S2. Diversity tree from our MEDUSA analysis of lineage diversification in the subfamily Hypoptopomatinae. Clades are collapsed to represent stem lineages and colored by extant species diversity. Clades with unusual diversification rates are denoted with numbers: 1 (yellow) denotes a significant lineage diversification rate increase compared with the background (Bg) in large-bodied species of the tribe Neoplecostomini, and 2 (blue) indicates a significant lineage diversification rate decrease in the lineage leading to the genus *Schizolecis*. Estimates for net diversification rate (r) and relative extinction rate (ϵ) are included in the upper left table. See Table S4 for taxonomic divisions and species richnesses.

Table 1. Results of model fitting tests combining likelihoods across all clades for maximum body size (MBS) continuous character evolution. Lower AIC values indicate better model support; asterisk denotes the best-supported model. BM = Brownian Motion, OU = Ornstein Uhlenbeck, EB = Early Burst.

	BM			OU			EB			Trend			Lambda			Kappa*		
	lnL	AICc	ΔAICc	lnL	AICc	ΔAICc	lnL	AICc	ΔAICc	lnL	AICc	ΔAICc	lnL	AICc	ΔAICc	lnL	AICc	ΔAICc
MBS	-12.3	28.8	16.7	-10.5	27.3	15.1	-12.3	30.9	18.8	-11.0	28.3	16.2	-5.2	16.7	4.5	-2.9	12.1*	0.0

Table 2. Results of model fitting tests combining likelihoods across discrete habitat continuous character evolution. Lower AIC values indicate better model support; asterisk denotes the best-supported model.

	ER*			SYM			ARD		
	lnL	AICc	Δ AICc	lnL	AICc	Δ AICc	lnL	AICc	Δ AICc
All habitats	-78.2	158.5	0	-71.5	178.5	20.0	-60.5	206.2	47.7

Table 3. BiSSE (Binary State Speciation and Extinction) and MuSSE (Multi-State Speciation and Extinction) models showing evidence for the habitat categories “Rapids” and “Pools” affecting speciation rates.

BiSSE model					
	LnLik	AIC	ΔAIC	ChiSq	Pr(>Chi)
Rapids					
Full	-412.2	836.5			
Constrained	-420.5	849.1	12.6	16.5	0.0002*
Lakes					
Full	-392.5	797.1			
Constrained	-393.5	795.0	2.1	1.9	0.3842
Riffles					
Full	-411.0	834.0			
Constrained	-412.7	833.5	0.5	3.4	0.1786
Pools					
Full	-400.4	812.8			
Constrained	-405.7	819.5	6.7	10.6	0.0047*
MUSSE model					
	LnLik	AIC	ΔAIC	ChiSq	Pr(>Chi)
All Habitats					
Full	-469.6	967.3			
Constrained	-478.0	976.0	8.7	16.6	0.002*

Supplementary Table 1. Species included in the present study. ANSP = Academy of Natural Sciences of Drexel University, Philadelphia; AUM = Auburn University Natural History Museum; LBP = Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista; MCP = Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul; MNRJ = Museu Nacional da Universidade Federal do Rio de Janeiro; NUP = Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura, Universidade Estadual de Maringá; MHNG = Museum of Natural History of the City of Geneva.

Number	Collection No	Fish No	GenBank (Reticulon/16S/COI/CytB)	Species	Location (river, city, state, country)
1	LBP 6037	29054	KM205118/KM104327/JN998540/KM104515	<i>Hisonotus</i> aff. <i>armatus</i>	Rio Maquiné/Osório/RS/Brazil
2	LBP 3472	20258	KM205120/KM104329/JN998579/KM104517	<i>Hisonotus notatus</i>	Afluente Rio Aduelas/Macaé/RJ/Brazil
3	LBP 4765	25554	KM205122/KM104331/KM104433/-	<i>Hisonotus</i> cf. <i>taimensis</i>	Rio Guaíba/Barra do Ribeiro/RS/Brazil
4	LBP 7407	35655	-/KM104333/KM104435/-	<i>Hisonotus leucofrenatus</i>	Rio Batatau/Itapeúna/SP/Brazil
5	MCP 41351		KM205124/KM104335/JN998546/KM104521	<i>Hisonotus leucophrys</i>	Rio Ariranhas/Xavantina/SC/Brazil
6	LBP 3376	21246	KM205128/KM104339/JN998536/KM104525	<i>Hisonotus laevior</i>	Arroio dos Corrientes/Pelotas/RS/Brazil
7	LBP 4720	24941	KM205130/KM104341/JN998531/-	<i>Hisonotus</i> cf. <i>charrua</i>	Arroio Cuaró Grande/Artigas/Uruguay
8	MCP 40762		-/KM104342/KM104436/KM104526	<i>Hisonotus notopagos</i>	Arroio Mantiqueira/Lavras do Sul/RS/Brazil
9	LBP 4723	24951	KM205131/KM104343/EU817529/KM104527	<i>Eurycheilichthys</i> sp. 1	Arroio Jaboticaba/Veranópolis/RS/Brazil
10	LBP 4871	24919	KM205132/KM104344/EU817532/KM104528	<i>Epactionotus bilineatus</i>	Arroio Carvalho/Itati/RS/Brazil
11	MCP 23683		-/EU371004/-	<i>Epactionotus itaimbezinho</i>	Rio Mangue/Morrinho do Sul/RS/Brazil
12	MCP 23606		-/EU371005/-	<i>Epactionotus gracilis</i>	Rio Morto/Meleiro/SC/Brazil
13	LBP 3335	20465	-/KM104345/JN998575/KM104529	<i>Hisonotus nigricauda</i>	Arroio sem nome/Rio Grande/RS/Brazil
14	LBP 5528	25343	KM205135/KM104348/EU817534/KM104531	<i>Otothyropsis marapoama</i>	Ribeirão Cubatão/Marapoama/SP/Brazil
15	LBP 4863	24930	KM205136/KM104349/KM104438/KM104532	<i>Hisonotus ringueleti</i>	Arroio Cuaró Grande/Artigas/Uruguay
16	MCP 40943		-/KM104350/KM104439/-	<i>Hisonotus carreiro</i>	Arroio Guabiju/Guabiju/RS/Brazil
17	MCP 40942		-/KM104351/KM104440/KM104533	<i>Hisonotus prata</i>	Rio da Prata/Nova Prata/RS/Brazil
18	MCP 41352		KM205137/KM104352/JN998543/KM104534	<i>Hisonotus megaloplax</i>	Arroio Cragoata/Passo Fundo/RS/Brazil
19	LBP 13055	51035	-/KM104353/KM104441/-	<i>Hisonotus montanus</i>	Rio Canoas/Vargem/SC/Uruguay
20	MCP 41474		-/KM104354/JN998533/KM104535	<i>Hisonotus aky</i>	Rio Forquilha/Paim Filho/RS/Brazil
21	MCP 40029		KM205139/KM104356/JN998544/KM104536	<i>Hisonotus iota</i>	Rio Chapeco/Coronel Freitas/SC/Brazil

22	LBP 8249	38464	-/KM104357/KM104442/KM104537	<i>Parotocinclus prata</i>	Rio Santo Antônio/Luizlândia/MG/Brazil
23	LBP 8258	38298	-/KM104443/KM104538	<i>Parotocinclus robustus</i>	Córrego Cachoeira/Bocaiúva/MG/Brazil
24	LBP 7182	34694	KM205140/KM104359/KM104444/KM104539	<i>Parotocinclus cf. bahiensis</i>	Rio Coité/Lençóis/BA/Brazil
25	LBP 5867	28345	KM205141/KM104360/KM104445/-	<i>New taxon 1</i>	Rio Cural das Éguas/Três Marias/MG/Brazil
26	LBP 17402	67143	-/KM104361/KM104446/KM104540	<i>Hisonotus bocaiuva</i>	Córrego Cachoeira/Bocaiuva/MG/Brazil
27	LBP 7244	33302	KM205142/KM104362/KM104447/KM104541	<i>New taxon 2</i>	Afluente Rio Araguari/Perdizes/MG/Brazil
28	LBP 3457	20293	-/KM104363/KM104448/KM104542	<i>Parotocinclus sp. 1</i>	Rio da Conceição/Guarapari/ES/Brazil
29	LBP 13923	58218	KM205143/KM104364/KM104449/KM104543	<i>Hisonotus francirochai</i>	Córrego sem nome/Capitinga/MG/Brazil
30	LBP 3932	22661	KM205145/KM104366/KM104451/KM104545	<i>Hisonotus paulinus</i>	Rio Paraitinguinha/Salesópolis/SP/Brazil
31	LBP 2910	18756	-/KM104371/KM104456/KM104550	<i>Hisonotus depressicauda</i>	Ribeirão da Quinta/Botucatu/SP/Brazil
32	LBP 4854	25626	EU817533/KM104372/KM104457/-	<i>Microlepidogaster dimorpha</i>	Riacho Grotão/Araxá/MG/Brazil
33	LBP 7245	34405	KM205148/KM104374/KM104459/KM104551	<i>Rhinolekos britskii</i>	Afluente Rio Arapuca/Bela Vista de Goiás/GO/Brazil
34	LBP 5848	28365	KM205149/KM104375/KM104460/-	<i>Rhinolekos sp. 1</i>	Riacho sem nome/Águas Fria de Goiás/GO/Brazil
35	LBP 7246	33305	KM205151/KM104377/KM104462/-	<i>Rhinolekos garavelloi</i>	Córrego Fazenda Lageado/Caldas Novas/GO/Brazil
36	LBP 2869	18571	EU817527/KM104378/KM104463/KM104552	<i>Parotocinclus maculicauda</i>	Rio Fau/Miracatu/SP/Brazil
37	LBP 4722	24946	EU817525/KM104379/KM104464/-	<i>Pseudotothyris sp. 1</i>	Lago Acaraí/São Francisco do Sul/SC/Brazil
38	LBP 2094	13851	KM205152/KM104380/KM104465/-	<i>Pseudotothyris obtusa</i>	Riacho Descoberto/Guaratuba/PR/Brazil
39	LBP 1971	13685	EU817526/KM104381/KM104466/KM104553	<i>Otothyris travassosi</i>	Riacho Rosário/Canavieiras/BA/Brazil
40	MHNG 2586.95	BR1200	EU817552/-/-/-	<i>Otothyrinae unidentified</i>	Rio Taiaçupeba/-/SP/Brazil
41	LBP 6950	35328	KM205153/KM104382/KM104467/-	<i>Parotocinclus sp. 2</i>	Igarapé Nouba Uba/São Gabriel da Cachoeira/AM/Brazil
42	ANSP 179131		-/GQ225426/-	<i>Parotocinclus britskii</i>	-/-/-/
43	AUM 43947		-/EU359455/-	<i>Parotocinclus eppleyi</i>	-/-/-/
44	LBP 5624	35327	KM205154/KM104384/KM104469/-	<i>Parotocinclus aff. spilurus</i>	Rio Maravilha/Balsas/MA/Brazil
45	LBP 334	4276	KM205156/KM104386/KM104471/KM104555	<i>Hisonotus sp. 1</i>	Ribeirão Santo Inácio/Moema/MG/Brazil
46	LBP 8276	38487	KM205157/KM104387/KM104472/KM104556	<i>Hisonotus sp. 2</i>	Rio Verde Grande/Jaíba/MG/Brazil
47	LBP 16277	61771	KM205158/KM104388/KM104473/KM104557	<i>Hisonotus sp. 3</i>	Rio Feio/Querência/MT/Brazil
48	LBP 7948	35749	KM205160/KM104390/KM104475/-	<i>Hisonotus chromodontus</i>	Rio dos Patos/Nova Mutum/MT/Brazil

49	LBP 10981	50459	-/KM104391/KM104476/KM104558	<i>Parotocinclus aripuanensis</i>	Rio Lajeado/Guajará Mirim/RO/Brazil
50	LBP 2414	16276	KM205161/KM104393/KM104478/KM104560	<i>Parotocinclus</i> sp. 3	Córrego Fundo/Barra do Garça/MT/Brazil
51	LBP 2988	19646	EU817537/KM104398/KM104483/KM104561	<i>Schizolecis guntheri</i>	Rio Indaiá/Ubatuba/SP/Brazil
52	LBP 1653	11477	EU817520/KM104399/KM104484/-	<i>Corumbataia tocantinensis</i>	Rio Vermelho/Goiás/GO/Brazil
53	LBP 2001	12191	EU817521/KM104400/FJ965499/FJ965509	<i>Corumbataia cuestae</i>	Rio Alambari/Botucatu/SP/Brazil
54	LBP 5529	25381	EU817518/KM104401/KM104485/KM104562	<i>Hisonotus insperatus</i>	Ribeirão Cubatão/ Marapoama/SP/Brazil
55	LBP 1325	11238	KM205162/KM104402/KM104486/KM104563	<i>Hisonotus oliveirai</i>	Ribeirão Keller/Marialva/PR/Brazil
56	LBP 17256	66655	KM205164/KM104404/KM104488/KM104565	<i>Hisonotus piracanjuba</i>	Córrego sem nome/Morrinhos/GO/Brazil
57	MNRJ 29502	490	-/KM104405/KM104489/KM104566	<i>Hisonotus</i> sp. 4	-/Cotriguaçu/MT/Brazil
58	LBP 17532	68706	-/KM104406/KM104490/KM104567	<i>Hisonotus paresi</i>	Rio Maracanã/Tangará da Serra/MT/Brazil
59	LBP 5062	26057	KM205165/KM104407/KM104491/-	<i>Hisonotus</i> sp. 5	Afluente Rio Aricá Mirim/Chapada dos Guimarães/MT/Brazil
60	LBP 2931	18994	JN689285/JN089823/JN089799/JN089774	<i>Pseudotocinclus tietensis</i>	Rio Paraitinga/Salesópolis/SP/Rio Tietê Basin
61	LBP 616	7564	JN689286/FJ625810/FJ625819/FJ965511	<i>Pseudotocinclus juquiae</i>	Rio Juquiá/Juquitiba/SP/Rio Ribeira do Iguape Basin
62	LBP 4391	24189	JN689280/ FJ434517/FJ434540/FJ965510	<i>Pareiorhina rudolphi</i>	Ribeirão Guaxinduva/Jundiá/SP/Rio Tietê Basin
63	LBP 7383	34843	JN689310/ FJ965496/FJ965507/FJ965517	<i>Neoplecostomus selenae</i>	Ribeirão das Batéias/Riacho Grande/SP/Rio Paranapanema Basin
64	LBP 7525	34832	-/JN089843/JN089818/JN089794	<i>Neoplecostomus botucatu</i>	Cachoeira Véu da Noiva/Botucatu/SP/Rio Paranapanema Basin
65	NUP 3560	9701	JN689293/ FJ434506/FJ434529/FJ965516	<i>Neoplecostomus yapo</i>	Rio Tibagi/Fortaleza/PR/Rio Paranapanema Basin
66	LBP 2732	17444	JN689303/FJ965495/FJ965505/FJ965515	<i>Neoplecostomus paranensis</i>	Córrego Mocoquinha/Cajuru/SP/Rio Grande Basin
67	LBP 5901	27990	JN689304/JN089837/JN089812/JN089788	<i>Neoplecostomus langeanii</i>	Córrego São Domingos/Muzambinho/MG/Rio Grande Basin
68	LBP 2861	18616	JN689298/JN089833/JN089808/JN089784	<i>Neoplecostomus bandeirante</i>	Rio Paraitinga/Salesópolis/SP/Rio Tietê Basin
69	NUP 2528	9423	JN689295/FJ434520/FJ434543/FJ965513	<i>Neoplecostomus corumba</i>	Rio Corumbá/GO/Rio Paranaíba Basin/Upper Rio Paraná Basin
70	LBP 645	7593	JN689311/FJ434508/FJ434531/FJ965518	<i>Neoplecostomus microps</i>	Ribeirão Cajarana/Pindamonhangaba/SP/Rio Paraíba do Sul Basin
71	LBP 6537	31681	JN689313/FJ965494/FJ965504/FJ965519	<i>Neoplecostomus franciscoensis</i>	Rio das Velhas/Brumadinho/MG/Rio São Francisco Basin
72	LBP 2551	15243	JN689312/FJ434507/FJ434530/ FJ965512	<i>Neoplecostomus espiritosantensis</i>	Rio Jucu/Domingos Martins/ES/Oriental Coastal Basin

73	LBP 8380	37559	JN689288/JN089826/JN689277/JN089777	<i>Pareiorhina hyptiorhachis</i>	Rio Pomba/Santa Barbara do Tugúrio/MG/Rio Paraíba do Sul Basin
74	LBP 8368	37565	JN689291/JN089829/JN089804/JN089780	<i>Pareiorhina carrancas</i>	Córrego Beijinho/Carrancas/MG/Rio Grande Basin
75	LBP7385	34852	JN689324/FJ965491/FJ965502/FJ965526	<i>Isbrueckerichthys epakmos</i>	Rio Água Doce/Tapiraí/SP/Rio Ribeira do Iguape Basin
76	LBP 6389	29765	JN689326/FJ965489/FJ965503/FJ965529	<i>Isbrueckerichthys cf. calvus</i>	Rio Taquará/California/PR/Rio Paranapanema Basin
77	LBP 7373	34853	JN689297/FJ965490/FJ965506/FJ965525	<i>Isbrueckerichthys alipionis</i>	Rio Betari/Iporanga/SP/Rio Ribeira do Iguape Basin
78	LBP 2650	17402	JN689325/FJ625812/FJ625821/FJ965528	<i>Isbrueckerichthys duseni</i>	Rio Pulador/Campinhos/PR/Rio Ribeira de Iguape Basin
79	LBP 7384	34837	JN689323/JN089845/JN089820/JN089796	<i>Neoplecostomus ribeirensis</i>	Rio Água Doce/Tapiraí/SP/Rio Ribeira do Iguape Basin
80	LBP 515	6334	JN689316/FJ965492/FJ965500/FJ965523	<i>Kronichthys subteres</i>	Rio Betari/Iporanga/SP/Rio Ribeira do Iguape Basin
81	LBP 795	8304	JN689315/FJ434503/FJ434526/FJ965522	<i>Kronichthys lacerta</i>	Rio Marumbi/Morretes/PR/Oriental Coastal Basin
82	LBP 2122	15096	JN689314/FJ434502/FJ434525/FJ965520	<i>Kronichthys heylandi</i>	Rio Parati-Mirim/Parati/RJ/Oriental Coastal Basin
83	LBP 1766	12886	JN689317/FJ965493/FJ965501/FJ965521	<i>Kronichthys</i> sp. 1	Rio Sítio do Meio/Mongaguá/SP/Oriental Coastal Basin
84	LBP 748	8257	JN689318/FJ625811/FJ625820/FJ965524	<i>Pareiorhaphis splendens</i>	Rio São João/Guaruva/PR/Oriental Coastal Basin
85	MCP 41275		-/-/EU359438/-	<i>Pareiorhaphis cameroni</i>	Rio Cubatão/Águas Mornas/SC/Brazil
86	LBP 902	7989	JN689319/FJ434514/FJ434537/FJ965532	<i>Pareiorhaphis steindachneri</i>	Rio Itapucu/Jaraguá do Sul/SC/Oriental Coastal Basin
87	MCP 41909		-/-/EU359437/-	<i>Pareiorhaphis azygolechis</i>	Rio Araraquara/Guaratuba/PR/Brazil
88	LBP 1161	8935	JN689320/FJ434512/FJ434535/FJ965530	<i>Pareiorhaphis vestigipinnis</i>	Rio Caveiras/Painel/SC/Rio Uruguai Basin
89	LBP 701	7363	JN689321/FJ434513/FJ434536/FJ965531	<i>Pareiorhaphis hystrix</i>	Rio Tainhas/Tainhas/RS/Oriental Coastal Basin
90	MCP 41747		EU359443/-/-/-	<i>Pareiorhaphis parmula</i>	Rio dos Patos/Lapa/PR/Brazil
91	MCP 41458		EU359439/-/-/-	<i>Pareiorhaphis eurycephalus</i>	Rio Rufino/Rio Rufino/SC/Brazil
92	LBP 4042	22905	KM205167/KM104409/KM104493/-	<i>Hypoptopoma inexpectatum</i>	Rio Moa/Cruzeiro do Sul/AC/Brazil
93	LBP 3081	19713	EU817541/KM104411/KM104495/-	<i>Hypoptopoma gulare</i>	Rio Orinoco/Caicara del Orinoco/Bolivar/Venezuela
94	MHNG 2709.024	MUS 388	EU817554/KM104412/KM104496/-	<i>Hypoptopoma</i> sp. 1	Export Iquitos, Upper Amazon basin, Peru
95	LBP 3165	19315	EU817543/KM104413/KM104497/KM104569	<i>Acestridium discus</i>	Rio Preto da Eva/Rio Preto da Eva/AM/Brazil
96	LBP 7204	35332	KM205169/KM104414/KM104498/-	<i>Acestridium</i> sp. 1	Igarapé Ya-Mirim/São Gabriel da Cachoeira/AM/Brazil
97	LBP 6973	35324	KM205170/KM104415/KM104499/KM104570	<i>Oxyropsis</i> sp. 1	Igarapé Demuriari/São Gabriel da Cachoeira/AM/Brazil

98	LBP 4300	23945	EU817542/KM104416/KM104500/KM104571	<i>Oxyropsis acutirostra</i>	Igarapé Zamula/Barcelos/AM/Brazil
99	LBP 2652	17407	EU817545/KM104417/KM104501/-	<i>Lampiella gibbosa</i>	Rio Carombé/Campinhos/PR/Brazil
100	LBP 877	8564	EU817546/KM104418/KM104502/KM104572	<i>Otocinclus flexilis</i>	-/Santo Antônio da Patrulha/RS/Brazil
101	MCP 25234		-/-EU370983/-	<i>Otocinclus arnoldi</i>	Rio Inhacunda/São Francisco de Assis/RS/Brazil
102	LBP 5310	26831	KM205171/KM104419/KM104503/-	<i>Otocinclus hoppei</i>	Igarapé Uiratapura/Laranjal do Jari/AP/Brazil
103	LBP 5132	26233	EU817544/KM104421/KM104505/KM104573	<i>Otocinclus vittatus</i>	Lagoa Bairro Caiçara/Cáceres/MT/Brazil
104	MCP 34842		-/-EU359432/-	<i>Otocinclus cocama</i>	Afluente do Cano da Concha Supay/Jenaro Herrera/Loreto/Peru
105	MHNG no number	SU07- 350	EU817558/KM104422/KM104506/-	<i>Otocinclus mariae</i>	Witoto Ecu creek/Sipaliwini/Suriname
106	LBP 3510	21309	JN689283/FJ625809/FJ625818/FJ965533	<i>Hypostomus nigromaculatus</i>	Córrego Hortelã/Botucatu/SP/Rio Paranapanema Basin
107	NUP 1725	16652	KM205172/KM104424/KM104508/-	<i>Hypostomus microstomus</i>	Rio Paraná/Guaíra/PR/Brazil
108	LBP 2544	10887	KM205173/KM104425/KM104509/-	<i>Hypostomus ancistroides</i>	Rio Corumbataí/Corumbataí/SP/Brazil
109	LBP 1557	11505	KM205174/KM104426/KM104510/-	<i>Rineloricaria lanceolata</i>	Ribeirão Ínsula/Barra do Garça/MT/Brazil
110	LBP 5049	11506	KM205175/KM104427/KM104511/-	<i>Spatuloricaria</i> sp. 1	Ribeirão Ínsula/Barra do Garça/MT/Brazil
111	MCP 31467		-/AY307290/-/-	<i>Delturus parahybae</i>	-/Laranjal/MG/Brazil
112	LBP 2368	15363	JN689278/FJ434499/FJ434524/FJ965535	<i>Hemipsilichthys gobio</i>	Rio Macaquinho/Bairro dos Macacos/SP/Rio Paraíba do Sul Basin
113	LBP 4956	10241	JN689279/FJ625808/FJ625817/FJ965534	<i>Hemipsilichthys papillatus</i>	Ribeirão da Jacutinga/Bom Jardim de Minas/MG/Rio Paraíba do Sul Basin
114	LBP 1352	11454	-/KM104428/KM104512/-	<i>Astroblepus</i> sp. 1	Rio Jequetepeque/Magdalena/Cajamarca/Peru
115	LBP 3284	20010	KM205176/KM104429/KM104513/-	<i>Astroblepus</i> sp. 2	Rio Chorobamba/Huancabamba/Pasco/Peru
116	LBP 485	6040	-/KM104430/KM104514/-	<i>Callichthys callichthys</i>	Córrego do Pombo/Marília/SP/Brazil
117	LBP 210	4134	-/GU210868/-/-	<i>Hoplosternum littorale</i>	Igarapé São Francisco/Rio Branco/AC/Brazil
118	LBP 2809	18894	GU210997/-/-/-	<i>Corydoras oiapoquensis</i>	Guyana coastal rivers/-/-/Guyana
119	LBP 6862	32502	-/GU210613/-/-	<i>Corydoras imitator</i>	Igarapé Puranga/São Gabriel da Cachoeira/AM/Brazil
120	LBP 449	5815	-/KM104431/EU179801/-	<i>Diplomystes mesembrinus</i>	Rio Chubut/Los Altares/Chubut/Argentina

Supplementary Table 2. Species of the ingroup included in the present study with information about habitat, maximum body size (MBS), genera division used in BAMB and head shape values also used in BAMB. Abbreviation in the table are: Rap – rapids; Str – stream; Rif – riffles; MBS – maximum body size values used in BAMB analysis; Gen-Div – Genera division used in BAMB; Perc – percent of sampled species of the lineage in BAMB; HS – head shape values used in BAMB analysis. The habitat classification follows Crampton (2011).

Number	Collection No	Fish No	Species	Rap	Lake	Str-Rif	Str-Pool	Size (cm)	MBS	Gen-Div	Perc	HS
1	LBP 6037	29054	<i>Hisonotus</i> aff. <i>armatus</i>	0	0	0	1	4.60	1.53	1	0.72	0.17648
4	LBP 3472	20258	<i>Hisonotus notatus</i>	0	0	0	1	4.00	1.39	1	0.72	0.22732
6	LBP 4765	25554	<i>Hisonotus</i> cf. <i>taimensis</i>	0	0	1	1	5.00	1.61	1	0.72	0.40867
8	LBP 7407	35655	<i>Hisonotus leucofrenatus</i>	0	0	1	1	6.00	1.79	1	0.72	0.28438
10	MCP 41351		<i>Hisonotus leucophrys</i>	1	0	1	1	5.00	1.61	1	0.72	0.083766
14	LBP 3376	21246	<i>Hisonotus laevior</i>	0	0	1	1	6.60	1.89	1	0.72	0.23925
16	LBP 4720	24941	<i>Hisonotus</i> cf. <i>charrua</i>	1	0	1	1	5.05	1.62	1	0.72	0.14842
17	MCP 40762		<i>Hisonotus notopagos</i>	0	0	0	1	5.17	1.64	1	0.72	0.35781
18	LBP 4723	24951	<i>Eurycheilichthys</i> sp. 1	1	0	1	0	4.50	1.50	1	0.72	0.10581
19	LBP 4871	24919	<i>Epactionotus bilineatus</i>	1	0	1	0	4.00	1.39	1	0.72	0.0067382
20	MCP 23683		<i>Epactionotus itaimbezinho</i>	1	0	1	0	3.80	1.34	1	0.72	0.21832
21	MCP 23606		<i>Epactionotus gracilis</i>	1	0	1	0	3.90	1.36	1	0.72	0.23299
22	LBP 3335	20465	<i>Hisonotus nigricauda</i>	1	0	1	1	5.00	1.50	1	0.72	0.44262
25	LBP 5528	25343	<i>Otothyropsis marapoama</i>	0	0	1	1	3.85	1.35	1	0.72	0.17244
26	LBP 4863	24930	<i>Hisonotus ringueleti</i>	0	0	1	1	3.90	1.36	1	0.72	0.48822
27	MCP 40943		<i>Hisonotus carreiro</i>	1	0	0	0	3.77	1.33	1	0.72	0.19254
28	MCP 40942		<i>Hisonotus prata</i>	1	0	0	0	3.32	1.20	1	0.72	0.19027
29	MCP 41352		<i>Hisonotus megaloplax</i>	0	0	1	1	4.71	1.55	1	0.72	0.13791
30	LBP 13055	51035	<i>Hisonotus montanus</i>	1	0	0	0	4.50	1.50	1	0.72	0.12027
31	MCP 41474		<i>Hisonotus aky</i>	1	0	1	0	3.45	1.24	1	0.72	0.19518
33	MCP 40029		<i>Hisonotus iota</i>	1	0	0	0	3.34	1.21	1	0.72	0.16487
34	LBP 8249	38464	<i>Parotocinclus prata</i>	0	0	1	1	4.19	1.43	2	1.00	0.12992

36	LBP 8258	38298	<i>Parotocinclus robustus</i>	0	0	1	1	4.20	1.44	2	1.00	0.2216
37	LBP 7182	34694	<i>Parotocinclus cf. bahiensis</i>	0	0	1	1	3.10	1.13	2	1.00	0.093163
38	LBP 5867	28345	<i>New taxon 1</i>	0	0	1	1	5.00	1.51	2	1.00	0.12996
39	LBP 17402	67143	<i>Hisonotus bocaiuva</i>	0	0	1	1	2.56	0.94	2	1.00	-0.22919
40	LBP 7244	33302	<i>New taxon 2</i>	0	0	1	1	5.00	1.51	2	1.00	0.12666
41	LBP 3457	20293	<i>Parotocinclus sp. 1</i>	0	0	1	1	4.30	1.46	2	1.00	-0.024727
42	LBP 13923	58218	<i>Hisonotus francirochai</i>	0	0	1	1	3.60	1.28	2	1.00	0.38179
44	LBP 3932	22661	<i>Hisonotus paulinus</i>	1	0	1	1	4.00	1.39	2	1.00	0.30589
49	LBP 2910	18756	<i>Hisonotus depressicauda</i>	1	0	1	1	5.00	1.61	2	1.00	-0.15324
50	LBP 4854	25626	<i>Microlepidogaster dimorpha</i>	0	0	1	1	3.77	1.33	3	0.44	-0.2367
52	LBP 7245	34405	<i>Rhinolekos britskii</i>	0	0	1	1	3.83	1.34	3	0.44	0.14186
53	LBP 5848	28365	<i>Rhinolekos sp. 1</i>	0	0	1	1	3.50	1.25	3	0.44	0.034833
55	LBP 7246	33305	<i>Rhinolekos garavelloii</i>	0	0	1	1	3.62	1.29	3	0.44	0.2575
56	LBP 2869	18571	<i>Parotocinclus maculicauda</i>	0	0	1	1	6.00	1.78	4	0.33	0.053122
57	LBP 4722	24946	<i>Pseudotothyris sp. 1</i>	0	0	1	1	4.00	1.39	5	0.57	0.20421
58	LBP 2094	13851	<i>Pseudotothyris obtusa</i>	0	0	1	1	4.00	1.39	5	0.57	0.367
59	LBP 1971	13685	<i>Otothyris travassosi</i>	0	0	1	1	3.20	1.16	5	0.57	0.57609
60	MHNG 2586.95	BR1200	<i>Otothyrinae unidentified</i>	0	0	1	1	5.00	1.31	5	0.57	0.10852
61	LBP 6950	35328	<i>Parotocinclus sp. 2</i>	0	0	1	1	6.10	1.71	6	0.50	-0.49205
62	ANSP 179131		<i>Parotocinclus britskii</i>	0	0	1	1	6.00	1.79	6	0.50	-0.32776
64	AUM 43947		<i>Parotocinclus eppleyi</i>	0	0	1	1	3.00	1.10	6	0.50	-0.36725
65	LBP 5624	35327	<i>Parotocinclus aff. spilurus</i>	0	0	1	1	4.00	1.39	7	0.58	0.28468
67	LBP 334	4276	<i>Hisonotus sp. 1</i>	0	0	1	1	2.51	0.92	7	0.58	0.2392
68	LBP 8276	38487	<i>Hisonotus sp. 2</i>	0	0	1	1	2.51	0.92	7	0.58	0.2392
69	LBP 16277	61771	<i>Hisonotus sp. 3</i>	0	0	1	1	2.91	1.07	7	0.58	0.28676
71	LBP 7948	35749	<i>Hisonotus chromodontus</i>	0	0	0	1	3.03	1.11	7	0.58	-0.030663

72	LBP 10981	50459	<i>Parotocinclus aripuanensis</i>	0	0	0	1	2.10	0.74	7	0.58	0.078385
74	LBP 2414	16276	<i>Parotocinclus</i> sp. 3	0	0	0	1	2.10	0.74	7	0.58	0.30574
79	LBP 2988	19646	<i>Schizolecis guntheri</i>	0	0	1	1	4.00	1.39	8	1.00	0.23107
80	LBP 1653	11477	<i>Corumbataia tocantinensis</i>	0	0	1	1	3.80	1.34	9	0.80	-0.44116
81	LBP 2001	12191	<i>Corumbataia cuetae</i>	0	0	1	1	3.30	1.19	9	0.80	-0.21798
82	LBP 5529	25381	<i>Hisonotus insperatus</i>	0	0	1	1	3.00	1.10	9	0.80	0.0090896
83	LBP 1325	11238	<i>Hisonotus oliveirai</i>	0	0	1	1	2.84	1.04	9	0.80	0.27738
85	LBP 17256	66655	<i>Hisonotus piracanjuba</i>	0	0	1	1	2.56	0.94	9	0.80	0.20075
86	MNRJ 29502	490	<i>Hisonotus</i> sp. 4	0	0	1	1	2.75	1.01	9	0.80	0.28103
87	LBP 17532	68706	<i>Hisonotus parsi</i>	0	0	1	1	2.62	0.96	9	0.80	0.17691
88	LBP 5062	26057	<i>Hisonotus</i> sp. 5	0	0	1	1	2.91	1.07	9	0.80	0.19673
90	LBP 2931	18994	<i>Pseudotocinclus tietensis</i>	0	0	1	1	6.00	1.80	10	0.75	-0.060191
91	LBP 616	7564	<i>Pseudotocinclus juquiae</i>	0	0	1	1	5.87	1.78	10	0.75	0.033934
92	LBP 4391	24189	<i>Pareiorhina rudolphi</i>	0	0	1	0	4.50	1.50	10	0.75	-0.021072
93	LBP 7383	34843	<i>Neoplecostomus selenae</i>	1	0	1	0	10.17	2.32	11	0.76	-0.82235
94	LBP 7525	34832	<i>Neoplecostomus botucatu</i>	1	0	1	0	10.20	2.32	11	0.76	-0.37478
95	NUP 3560	9701	<i>Neoplecostomus yapo</i>	1	0	1	0	10.60	2.36	11	0.76	-0.49997
96	LBP 2732	17444	<i>Neoplecostomus paranensis</i>	1	0	1	0	9.82	2.28	11	0.76	-0.63604
97	LBP 5901	27990	<i>Neoplecostomus langeanii</i>	1	0	1	0	8.55	2.15	11	0.76	-0.31678
98	LBP 2861	18616	<i>Neoplecostomus bandeirante</i>	1	0	1	0	10.90	2.39	11	0.76	-0.56024
99	NUP 2528	9423	<i>Neoplecostomus corumba</i>	1	0	1	0	7.83	2.06	11	0.76	-0.076065
100	LBP 645	7593	<i>Neoplecostomus microps</i>	1	0	1	0	10.00	2.30	11	0.76	0.086719
101	LBP 6537	31681	<i>Neoplecostomus franciscoensis</i>	1	0	1	0	7.50	2.01	11	0.76	-0.22454
102	LBP 2551	15243	<i>Neoplecostomus espiritosantensis</i>	1	0	1	0	10.00	2.30	11	0.76	-1.1807
103	LBP 8380	37559	<i>Pareiorhina hyptiorhachis</i>	0	0	1	0	3.88	1.36	12	0.50	-0.61813
105	LBP 8368	37565	<i>Pareiorhina carrancas</i>	0	0	1	0	4.10	1.41	12	0.50	-0.46219

106	LBP7385	34852	<i>Isbrueckerichthys epakmos</i>	1	0	1	0	10.00	2.30	13	1.00	-0.20789
107	LBP 6389	29765	<i>Isbrueckerichthys cf. calvus</i>	1	0	1	0	9.00	2.20	14	1.00	0.050339
108	LBP 7373	34853	<i>Isbrueckerichthys alipionis</i>	1	0	1	0	8.20	2.10	14	1.00	-0.14043
109	LBP 2650	17402	<i>Isbrueckerichthys duseni</i>	1	0	1	0	10.00	2.30	14	1.00	-1.018
110	LBP 7384	34837	<i>Neoplecostomus ribeirensis</i>	1	0	1	0	9.00	2.20	14	1.00	-0.67915
111	LBP 515	6334	<i>Kronichthys subteres</i>	0	0	1	0	12.00	2.48	15	1.00	-0.24954
112	LBP 795	8304	<i>Kronichthys lacerta</i>	0	0	1	0	7.00	1.95	15	1.00	-0.32732
113	LBP 2122	15096	<i>Kronichthys heylandi</i>	0	0	1	0	15.00	2.71	15	1.00	-0.16689
114	LBP 1766	12886	<i>Kronichthys sp. 1</i>	0	0	1	0	11.00	2.40	15	1.00	-0.27992
115	LBP 748	8257	<i>Pareiorhaphis splendens</i>	1	0	1	0	6.50	1.87	15	1.00	-0.22233
116	MCP 41275		<i>Pareiorhaphis cameroni</i>	1	0	1	0	17.00	2.83	16	0.33	0.020547
117	LBP 902	7989	<i>Pareiorhaphis steindachneri</i>	1	0	1	0	12.00	2.48	16	0.33	-0.3372
118	MCP 41909		<i>Pareiorhaphis azygolechis</i>	0	0	1	0	11.70	2.46	16	0.33	-0.12389
119	LBP 1161	8935	<i>Pareiorhaphis vestigipinnis</i>	1	0	1	0	10.00	2.30	16	0.33	-0.28948
120	LBP 701	7363	<i>Pareiorhaphis hystrix</i>	1	0	1	0	11.00	2.40	16	0.33	-0.10346
121	MCP 41747		<i>Pareiorhaphis parmula</i>	0	0	1	0	9.45	2.25	16	0.33	-0.8728
122	MCP 41458		<i>Pareiorhaphis eurycephalus</i>	0	0	1	0	6.30	1.89	16	0.33	-0.14376
123	LBP 4042	22905	<i>Hypoptopoma inexpectatum</i>	0	1	0	1	14.30	1.96	17	0.20	0.13446
126	LBP 3081	19713	<i>Hypoptopoma gulare</i>	0	1	0	1	11.00	2.40	17	0.20	0.21128
127	MHNG 2709.024	MUS 388	<i>Hypoptopoma sp. 1</i>	0	1	0	1	10.70	2.37	17	0.20	0.43124
128	LBP 3165	19315	<i>Acestridium discus</i>	0	0	0	1	6.70	1.90	18	0.28	-0.030523
129	LBP 7204	35332	<i>Acestridium sp. 1</i>	0	0	0	1	6.00	1.79	18	0.28	-0.33458
130	LBP 6973	35324	<i>Oxyropsis sp. 1</i>	0	1	0	1	5.87	1.77	19	0.66	0.15033
131	LBP 4300	23945	<i>Oxyropsis acutirostra</i>	0	1	0	1	5.70	1.74	19	0.66	0.26852
132	LBP 2652	17407	<i>Lampiella gibbosa</i>	0	1	0	1	5.00	1.61	20	0.36	0.24239
133	LBP 877	8564	<i>Otocinclus flexilis</i>	0	1	0	1	5.50	1.70	20	0.36	0.4887

134	MCP 25234		<i>Otocinclus arnoldi</i>	0	1	0	1	4.80	1.57	20	0.36	0.29607
135	LBP 5310	26831	<i>Otocinclus hoppei</i>	0	1	0	1	3.30	1.19	20	0.36	0.039753
137	LBP 5132	26233	<i>Otocinclus vittatus</i>	0	1	0	1	3.30	1.19	20	0.36	0.21689
138	MCP 34842		<i>Otocinclus cocama</i>	0	1	0	1	4.40	1.48	20	0.36	0.33721
139	MHNG no number	SU07-350	<i>Otocinclus mariae</i>	0	0	0	1	3.30	1.19	–	–	0.35918

Supplementary Table 4. Variable loadings in the first Principal Component Analysis (PCA 1) for head shape of combined samples of Hypoptopomatinae tribes.

		PCA1 (head shape)
1	Head length	0.01858
2	Cleithral width	-0.01287
3	Head-pectoral length	-0.00124
4	Head-eye length	0.1134
5	Orbit diameter	0.2724
6	Snout length	-0.1068
7	Internares width	0.1457
8	Interorbital width	0.1658
9	Head depth	0.0423
10	Mouth length	-0.03791
11	Mouth width	-0.2039
12	Barbel length	0.89
13	Dentary tooth cup length	-0.0781
14	Premaxillary tooth cup length	-0.09653

Supplementary Table 4. Subdivision of lineages of Hypoptopomatinae and number of species included in each line used for MEDUSA analysis. The order of taxa follows the same present in Fig. S2.

		Taxonomical richness for each lineage
1	<i>Parotocinclus</i>	7
2	<i>Hisonotus</i>	3
3	<i>Hisonotus</i>	29
4	<i>Microlepidogaster</i>	5
5	<i>Rhinolekos</i>	3
6	<i>Parotocinclus</i>	3
7	<i>Pseudotothyris</i>	6
8	<i>Parotocinclus</i>	3
9	<i>Hisonotus</i>	7
10	<i>Schizolecis</i>	1
11	<i>Corumbataia</i>	4
12	<i>Hisonotus</i>	6
13	<i>Pseudotocinclus</i>	3
14	<i>Pareiorhina</i>	1
15	<i>Neoplecostomus</i>	13
16	<i>Pareiorhina</i>	4
17	<i>Isbrueckerichthys</i>	5
18	<i>Kronichthys</i>	3
19	<i>Pareiorhaphis</i>	21
20	<i>Lampiella</i>	1
21	<i>Otocinclus</i>	18
22	<i>Oxyropsis</i>	3

23	<i>Hypoptopoma</i>	15
24	<i>Acestridium</i>	7

Capítulo 3

New species of *Curculionichthys* (Siluriformes: Loricariidae) from the eastern Guiana Shield

Target jornal: Zootaxa

New species of *Curculionichthys* (Siluriformes: Loricariidae) from the eastern Guiana Shield

GABRIEL S. C. SILVA¹, FÁBIO F. ROXO¹, BRUNO F. MELO^{1,2} & CLAUDIO OLIVEIRA¹

¹ *Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, R Prof. Dr. Antonio C. W. Zanin, s/n, Rubião Jr, 18618–689, Botucatu SP, Brazil*

² *Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington DC, USA*

Running head: New species of *Curculionichthys*

Corresponding author: Fábio F. Roxo (roxoff@hotmail.com.br)

Abstract

We describe a new species of *Curculionichthys* from northern Brazil. The new species is known from the rio Cassiporé, an Atlantic coastal river and an unnamed affluent of the rio Jari, left tributary of the Amazon river. It can be distinguished from congeners by (1) the irregular concentration of chromatophores that cover the anal-fin origin and adjacent region, and distal portions of the first unbranched anal-fin ray, (2) lack of dark-brown spots scattered over the body, (3) lack of contrasting dark spots over the anterodorsal region of the body, (4) presence of papillae randomly distributed across the lower lip, (5) small, inconspicuous odontodes that form rows over the head and trunk,

(6) anterior profile of the head pointed, (7) higher number of premaxillary and dentary teeth, (8) lack of unpaired platelet on the dorsal portion of caudal peduncle, (9) lower number of vertebrae, and (10) higher number of lateral abdomen plates. A discussion on the morphological variation of diagnostic features within *Curculionichthys* is also provided.

Key words: Amazon basin, Cascudinhos, Freshwater fishes, Otothyridi, Taxonomy

Introduction

The Neotropical catfish genus *Curculionichthys* Roxo, Silva, Ochoa & Oliveira, 2015, was recently described in the current tribe Otothyridi (*sensu* Lujan et al., 2015) based on the recognition of a monophyletic assemblage returned from a phylogenetic analysis of molecular data (Roxo *et al.* 2014a). Morphological characters supporting *Curculionichthys* include the (1) presence of a pair of rostral plates at the snout tip, (2) two large pre-nasal plates right posterior to the rostral plates, (3) supra-opercular plate receiving the laterosensory canal from the compound pterotic before preopercle, (4) a well-developed membrane at anal opening in females, and (5) a V-shaped spinelet (Roxo *et al.* 2015). *Curculionichthys* includes five species previously assigned to *Hisonotus* Eigenmann & Eigenmann, 1889, plus three new species described therein (Roxo *et al.* 2015). These are distributed mostly throughout the Brazilian Shield drainages: *C. insperatus*, *C. oliveirai* and *C. piracanjuba* in the upper rio Paraná; *C. coxipone* and *C. parsi* in the upper rio Paraguay; *C. sagarana* in the rio São Francisco; *C. luteofrenatus* in the upper rio Tapajós; and *C. sabaji* in the rio Xingu (Roxo *et al.* 2015).

Recent expeditions in Amapá and Pará, northern Brazil, particularly in the rio Jari and Atlantic coastal versants, have revealed some new records of species for Brazil (Melo *et al.*, in press) and also discovered an undescribed species of *Curculionichthys* that is formally described herein. This represents a range extension of the genus to northern rivers draining the eastern Guiana Shield.

Material and Methods

Measurements and counts were taken from the left side of specimens and were made from point to point to the nearest 0.01 mm with a digital caliper. Nomenclature of body plates and osteology follow Schaefer (1997). Measurements and abbreviation follow Carvalho & Reis (2009). Morphometrics are given as percentages of standard length (SL), except for subunits of head region expressed as percentages of head length (HL). Specimens were cleared and double stained (c&s) according to Taylor & Van Dyke (1985). Vertebral counts include the five vertebrae of the Weberian apparatus and the compound caudal centrum (PU1+U1) as one element. Counts of dorsal-fin rays include the spinelet as the first unbranched ray. Institutional abbreviations follow Fricke & Eschmeyer (2015). Specimens are deposited at the Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista, Botucatu, Brazil (LBP) and Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP). Zoological nomenclature follows the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 1999).

Results

***Curculionichthys karipuna*, new species**

Fig. 1, Table 1

Holotype. MZUSP 120190, female, 23.7 mm SL, Brazil, Amapá, Oiapoque, rio Cassiporé, Atlantic coastal river, small stream under the bridge of the road BR-156 at Campo Alegre farm, 3°04'49.7"N 51°28'50.7"W, 01 Dec 2015, B. F. Melo & C. Oliveira.

Paratypes. All from Brazil. LBP 20434, 3 females (19.7–21.3 mm SL), 2 males (21.2–21.4 mm SL) (tissues 80723–80727), Pará, Monte Dourado-Almeirim, Amazon basin, rio Jari, small stream under the bridge, road to UHE Santo Antônio Jari, 0°45'07.3"S 52°34'30.6"W, 24 Sep 2015, B. F. Melo & C. Oliveira. LBP 21106, 1 male (18.3 mm SL), 4 females (19.4–20.0 mm SL), 1 c&s undetermined sex, Pará, Monte Dourado-Almeirim, Amazon basin, rio Jari, small stream under the bridge, road to UHE Santo Antônio Jari, 0°49'03.9"S 52°35'08.2"W, 25 Nov 2015, B. F. Melo & C. Oliveira. LBP 21166, 15 females (19.9–21.5 mm SL), 25 males (19.4–21.8 mm SL), 2 c&s undetermined sex (tissues 83032–83036), same data as holotype. NUP uncat., 4 females (19.8–21.9 mm SL), 4 males (20.7–22.3 mm SL), same data as holotype.

Diagnosis. *Curculionichthys karipuna* differs from all congeners, except *C. sabaji* and *C. paresi*, by the presence of an irregular concentration of chromatophores that entirely cover the anal-fin origin and adjacent region, and distal portions of the first unbranched anal-fin ray (Fig. 2a) (*vs.* absence of such pigmentation pattern, Fig. 2b). *Curculionichthys karipuna* differs from *C. sabaji* by the absence of dark-brown spots scattered over the body (*vs.* presence); it differs from *C. paresi* by the lack of contrasting dark spots at the anterodorsal region of body (*vs.* presence of such spots). In addition, the new species differs from *C. insperatus*, *C. sabaji*, *C. paresi* and *C. coxipone* by having all papillae randomly distributed throughout lower lip (*vs.* papillae aligned in series that extends from the distal portion of lower lip to dentary); it differs

from *C. insperatus* and *C. oliveirai* by the presence of small, inconspicuous odontodes forming rows on head and trunk (*vs.* presence of large, conspicuous odontodes). *Curculionichthys karipuna* differs from *C. coxipone* and *C. oliveirai* by having a pointed profile of the anterior portion of the head (*vs.* rounded profile); it differs from *C. parsi* by having 10–16 premaxillary teeth (*vs.* 6–10) and 10–14 dentary teeth (*vs.* 4–7); it differs from *C. sagarana* by the absence of an unpaired platelet on dorsal portion of caudal peduncle (*vs.* presence). The new species also differs from *C. coxipone* by having 27–28 vertebrae (*vs.* 29–30). Finally, *C. karipuna* differs from *C. oliveirai* by the presence of 7–8 lateral abdomen plates (*vs.* 4–5).

Description. Morphometric and meristic data are summarized on Table 1. Small-size loricariid; maximum standard length reaches 23.9 mm. Dorsal profile of body slightly convex from snout tip to interorbital region; slightly convex from this point to dorsal-fin origin and then slightly concave to origin of anteriormost caudal procurrent ray. Ventral surface of body convex from tip of snout to first anal-fin ray; slightly concave from this point to first anteriormost caudal procurrent ray. Greatest body depth at dorsal-fin origin. Greatest body width at opercular region; progressively narrowing towards snout and caudal fin. Trunk and caudal peduncle almost ellipsoid; rounded laterally and almost flat dorsally and ventrally.

Head elliptical in dorsal view; snout long, slightly pointed with a rounded tip and flat between orbits. Dorsal and ventral series of odontodes completely covering anterior margin of snout; odontodes of snout larger in size than remaining ones found on head. Odontodes on head and trunk well defined and arranged in longitudinal rows but not necessarily forming parallel series. Eye relatively small and round, situated dorsolaterally at midpoint of head. Iris operculum present, but poorly developed. No ridge between eyes and nares. Nostril small. Supraoccipital process not elevated and

without tuft of odontodes in specimens of all sizes. Mouth wide; oral disk roundish with papillae randomly distributed. Lower lip larger than upper, not reaching cleithrum region; its border strongly fringed. Maxillary barbel short, slender and free distally. Teeth slender and bicuspidate. Cusps symmetrical; central cusp larger than lateral cusps. Premaxillary teeth 11–17 (15). Dentary teeth 10–15 (13).

Dorsal fin rays ii,7. Dorsal-fin origin slightly posterior through pelvic-fin origin; distal margin of dorsal fin slightly convex. Tip of adpressed dorsal-fin rays surpassing terminus of anal-fin base. Dorsal fin spinelet short and V-shaped; lock mechanism functional. Pectoral-fin rays i,6; its tip reaching beyond pelvic-fin insertion when depressed. Presence of pectoral axillary slit between pectoral-fin insertion and lateral process of cleithrum. Pelvic-fin rays i,5; distal margin slightly convex; tip of adpressed pelvic fin reaching anal-fin origin in males, but not in females. Adipose fin absent. Anal-fin rays i,4; distal margin slightly convex. Caudal fin rays i,7–i,7; slightly emarginated; unbranched rays of same size. Adpressed rays of all fins covered with pointed odontodes. Total vertebrae 27–28 (2 c&s).

Body completely covered by bony plates, except on ventral part of head, around pectoral- and pelvic-fin origins and dorsal-fin base. Abdomen entirely covered by plates. Six to seven elongate and large plates reaching anal shield. Lateral surface of body entirely covered by plates; middorsal plate series poorly developed, almost reaching terminus of dorsal-fin base; median plate series uninterrupted in median portion of body and perforated by lateral line; mid-ventral plates almost reaching middle of caudal peduncle. Cleithrum and coracoid totally exposed. Arrector fossae partially enclosed by ventral lamina of coracoids.

Bone plates of dorsal head presented in Fig. 3. Snout tip formed by one or two rostral square-shaped plates (r); nasal (n) almost rectangular forming anterior medial

nostril margin in contact posteriorly with frontals (f) and anteriorly and laterally with pre-nasals (pn) and second infra-orbital (io2). Pre-nasals (pn) positioned posteriorly of rostral plates (r) formed by two large and three small triangular-shaped plates between nares. Top of head composed by compound pterotic (cpt), parieto supraoccipital (soc) and frontal (f), largest bones of head, and prefrontal (pf) and sphenotic (sp). Compound pterotic (cpt) fenestrate randomly distributed. Posterior rostrum plates pr1-pr2 small, and rectangular shaped; pr4-pr3 largest, and rectangular shaped. Infraorbital plate series complete (io1-io5), present just above posterior rostrum series, all covered by the laterosensory canal system; io2 largest and io5 smallest; io3, io4 and io5 forming inferior orbital margin of eyes; preopercle (pop) elongated and rectangular, covered by laterosensory canal; pre-opercle present under io4 and io5, and upper cp1, cp2. Supra-opercular plate (spop) situated just above preopercle, covered by latero-sensory canal. Subocular cheek plates (cp1–cp2) and opercle (op) form posterior lateral margin of head.

Color in alcohol. Ground color of dorsal region of head and trunk brown. Four dark saddle along dorsal portion of body: first at dorsal-fin origin, second at the end of dorsal-fin base, third at the middle of caudal peduncle, and fourth reaching anteriormost caudal procurrent ray. Unpigmented portion of snout appears as two hyaline parallel stripes from rostral plate to nares. Midlateral dark stripe extending from posterior margin of orbit to caudal peduncle. Ventral portion of the body entirely whitish, except the concentration of dark chromatophores at the anal-fin origin and adjacent region. Dorsal, pectoral, and pelvic fins with dark, irregularly distributed chromatophores. Caudal fin hyaline with dark stripe extending from caudal-peduncle base onto base of median caudal fin rays; dark chromatophores forming one irregularly diffuse band.

Sexual dimorphism. Adult males possess a papilla covering urogenital opening, a long pelvic fin that extends beyond anal-fin origin, and the unbranched pelvic-fin ray supporting a dermal flap along its dorsal surface, features absent in all analyzed females.

Distribution and habitat. *Curculionichthys karipuna* is known from two localities: a small stream of the rio Cassiporé, an Atlantic coastal river of northern Amapá and a small stream reaching the rio Jari (Fig. 4). These localities are composed of shallow, fast-flowing stream bordered with marginal vegetation (Fig. 5). Portions of the stream of rio Cassiporé (Fig. 5a) contain large rocks and do not have preserved vegetation resulting in an intense penetration of light. The stream of the rio Jari (Fig. 5b) is composed of 1-meter deep water in which *C. karipuna* lives associated to aquatic roots stuck on the border.

Etymology. The specific name “*karipuna*” is a reference to the Karipuna indigenous people that inhabit the region of the Oiapoque river, northern Amapá, Brazil. A noun in apposition.

Remarks. Le Bail *et al.* (2000) catalogued a “gen. nov. aff. *Parotocinclus*” in the Marowijne river basin. Although that specimen, based on their photography, strongly resembles *Curculionichthys karipuna*, examination of that material is still required to confirm the identification.

Discussion. *Curculionichthys karipuna* is included within the recently described genus *Curculionichthys* by possessing four of five diagnostic features (see Introduction). However, *C. karipuna* does not possess the well-developed membrane at anal opening in females and present variable morphology related to rostral plates in some specimens. Yet, one counterstained specimen has a single rostral plate at the snout tip (Fig. 3). *Curculionichthys insperatus*, type species of the genus, similarly presents

intraspecific variability in the rostral plates (see details in Roxo *et al.* 2014b). Though, such character is useful to diagnose *Curculionichthys* from other Ootothyriini genera. In addition to the five proposed diagnostic characters for *Curculionichthys* (Roxo *et al.* 2015), we found two additional features related to external morphology that facilitates its identification: (1) presence of a dark stripe extending from caudal-peduncle base to median caudal-fin rays and (2) absence of hypertrophied odontodes covering snout tip.

Despite species of Ootothyriini present a wide variation in the color pattern of the caudal fin, only species of *Curculionichthys* have a hyaline caudal fin with a dark stripe. *Curculionichthys insperatus* and *C. sagarana* have a dark stripe that extends to the caudal-peduncle base but does not reach median caudal-fin rays, whereas all other congeners (including *C. karipuna*) possess a dark stripe that covers the caudal-peduncle base and extends to median caudal-fin rays.

Schaefer (1998) proposed that the presence of enlarged odontodes covering the snout tip can diagnose species of *Hisonotus*. Recently, Silva *et al.* (2016) found that such character is exclusive to species related to the type species, *H. notatus* (*e.g.*, *H. armatus*, *H. laevior*, *H. leucofrenatus*, *H. nigricauda*). Conversely, species not related to *H. notatus*, such as *H. acuen* and *H. chromodontus* (Roxo *et al.* 2014a; Silva *et al.* 2016) do not present such hypertrophied odontodes. Similarly, species of *Curculionichthys* previously included in *Hisonotus* (*C. insperatus*, *C. luteofrenatus*, *C. oliveirai*, *C. parsi* and *C. piracanjuba*) and those recently described (*C. coxipone*, *C. sabaji*, *C. sagarana* and also *C. karipuna*) do not present hypertrophied odontodes, which confirms the variable nature of this character within Ootothyriini.

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Comparative material:

Curculionichthys coxipone Roxo, Silva, Ochoa & Oliveira, 2015: Brazil, Mato Grosso Cuiabá, tributary of rio Aricá-Mirim, upper Paraguay basin: MZUSP 117380, holotype, 29.0 mm SL; LBP 5061, 5, paratypes, 21.7–30.0 mm SL; LBP 5062, 3, paratypes, 22.5–28.7 mm SL; LBP 5069, 13, paratypes, 22.5–29.6 mm SL; LBP 5646, 18, paratypes, 21.8–28.8 mm SL.

Curculionichthys insperatus (Britski & Garavello, 2003): All from Brazil, upper Paraná: MZUSP 78957, holotype, 29.6 mm SL, São Paulo, Botucatu, rio Capivara; LBP 4945, 7, 27.3–29.9 mm SL, São Paulo, Botucatu, rio Araquá; LBP 6770, 8, 20.2–28.2 mm SL, São Paulo, Marapoama, ribeirão Cubatão; MZUSP 22826, 1, paratype, 25.4 mm SL, Mato Grosso do Sul, Três Lagoas, córrego Água Tirada; MZUSP 24832, 1, paratype, 23.8 mm SL, São Paulo, Corumbataí, rio Corumbataí; MZUSP 78960, 31, paratypes, 12.6–26.0 mm SL, 5 c&s, 22.7–24.7 mm SL, São Paulo, Botucatu, rio Pardo; MZUSP 78968, 5, paratypes, 24.1–27.3 mm SL, São Paulo, Lins, córrego da Figueira.

Curculionichthys luteofrenatus (Britski & Garavello, 2007): All from Brazil, Mato Grosso, Amazon basin, upper rio Tapajós, rio Teles Pires: MZUSP 62593, holotype, 28.6 mm SL, Cláudia, córrego Loanda; MZUSP 62594, 8, paratypes, 22.4–30.5 mm

SL, Sinop, riacho Selma; MZUSP 87144, 8, paratypes, 16.8–27.9 mm SL, Cláudia, córrego Loanda.

Curculionichthys oliveirai (Roxo, Zawadzki & Troy, 2014): All from Brazil, Paraná, upper rio Paraná: MZUSP 115061, holotype, 26.4 mm SL, Cambira, ribeirão Cambira.; LBP 13332, paratypes, 1 male, 23.2 mm SL, 1 undetermined sex c&s, 23.7 mm SL, Campo Mourão, rio Mourão; LBP 17578, 5, paratypes, 25.4–30.4 mm SL, between Engenheiro Beltrão and Quinta do Sol, rio Mourão; NUP 3578, 15, paratypes, 24.7–28.1 mm SL, 2 c&s, 25.5–27.6 mm SL, Maria Helena, ribeirão Salto Grande.

Curculionichthys parsi (Roxo, Zawadzki & Troy, 2014): All from Brazil, Mato Grosso, Santo Afonso, upper rio Paraguay, rio Sepotuba, riacho Águas Claras: MZUSP 115062, holotype, 26.2 mm SL; LPB 13351, 9, paratypes, 14.7–24.3 mm SL; LBP 13352, 1, paratype, 23.7 mm SL; NUP 10928, paratypes, 2 males, 23.2–24.2 mm SL, 2 c&s, 23.6–24.2 mm SL.

Curculionichthys piracanjuba (Martins & Langeani, 2012): All from Brazil, Goiás, upper rio Paraná: LBP 17256, 9, 17.2–26.3 mm SL, 1, c&s 27.1 mm SL, Morrinhos, córrego sem nome; NUP 5059, 1, 24.7 mm SL, Anápolis, córrego Posse; MZUSP 110491, 3, paratypes, 17.5–24.4 mm SL, Morrinhos, rio Quente; NUP 10979, 3, 21.4–21.8 mm SL, Piracanjuba, ribeirão Bocaina.

Curculionichthys sabaji Roxo, Silva, Ochoa & Oliveira, 2015: All from Brazil, Amazon basin, rio Xingu: MZUSP 117379, holotype, 23.3 mm SL, Pará, Altamira, rio 13 de Maio; LBP 19763, 1, paratype, 23.4 mm SL, Pará, Altamira, rio Curuá; MZUSP 95711, 5, paratypes, 16.3–20.0 mm SL, 2 c&s, 18.7–19.9 mm SL, Mato Grosso, Gaúcha do Norte, rio Coronel Vanick; MZUSP 96959, 2, paratypes, 19.1–20.7 mm SL, Pará, Altamira, rio 13 de Maio.

Curculionichthys sagarana Roxo, Silva, Ochoa & Oliveira, 2015: All from Brazil, Minas Gerais, rio São Francisco basin, Rio das Velhas: MZUSP 117381, holotype, 23.7 mm SL, Santo Hipólito, rio Pardo Grande; LBP 19983, 1, paratype, 21.9 mm SL, Santo Hipólito, rio Pardo Grande; NUP 9714, 1, paratype, 24.4 mm SL, Augusto de Lima, rio Curimataí; NUP 9715, paratypes, 2, 17.5–18.4 mm SL, 1 c&s, 23.3 mm SL, Santo Hipólito, rio Pardo Grande.

Curculionichthys sp. 1 sp. n.: LBP 17485, 7, 19.0–24.1 mm SL, Brazil, Pará, Itaituba, Amazon basin, rio Tapajós, Igarapé Imambuaí.

Curculionichthys sp. 2 sp. n.: LBP 1856, 2, 21.0–23.2 mm SL, Brazil, Mato Grosso, Barra do Garças, Amazon basin, rio Araguaia, rio Insula.

Curculionichthys sp. 3 sp. n.: All from Brazil, Maranhão: MZUSP 87452, 3, 22.4–24.5 mm SL, Estreito, unknown river; MZUSP 87553, 3, 21.7–24.3 mm SL, Feira Nova do Maranhão, unknown river.

Table 1. Morphometric and meristic data for *Curculionichthys karipuna*. SD = standard deviation.

	Holotype	<i>C. karipuna</i> , n = 36				<i>C. karipuna</i> , n = 25 (Cassiporé)				<i>C. karipuna</i> , n = 11 (Jari)			
		Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Standard length	23.7	18.3	23.9	20.8	1.15	19.9	23.9	21.8	1.04	18.3	21.4	20.0	1.00
Percents of standard length													
Head length	38.6	36.4	44.1	39.1	1.27	37.7	41.6	38.9	0.8	36.4	44.1	39.6	1.9
Predorsal length	47.5	47.0	52.6	48.9	1.30	47.0	51.2	49.1	1.0	47.0	52.6	48.7	1.7
Dorsal-fin spine length	22.0	17.2	26.3	23.5	1.67	17.2	26.3	23.8	1.9	21.5	24.1	23.0	0.7
Anal-fin unbranched ray length	16.7	14.5	18.7	17.1	0.95	14.5	18.4	16.9	0.9	16.5	18.7	17.5	0.8
Pectoral-fin spine length	25.7	17.5	27.8	18.1	1.85	17.5	27.8	25.4	2.1	24.1	27.5	25.5	1.1
Pelvic-fin unbranched ray length	18.2	15.3	22.0	18.1	1.19	15.3	19.7	17.9	1.0	16.8	22.0	18.4	1.5
Cleithral width	22.3	21.8	24.4	22.8	0.61	22.0	24.4	22.9	0.5	21.8	24.0	22.7	0.7
Thoracic length	16.8	14.3	18.4	16.5	1.13	14.7	18.4	16.7	1.1	14.3	17.3	15.9	0.9
Abdominal length	18.3	15.8	19.6	17.5	0.74	15.8	18.5	17.4	0.6	16.5	19.6	17.8	0.9
Body depth at dorsal-fin origin	17.6	15.8	18.1	16.5	0.58	15.8	18.1	16.8	0.6	16.1	16.8	16.4	0.6
Caudal-peduncle length	23.3	22.9	28.1	26.0	1.37	22.9	28.1	26.0	1.4	24.0	27.4	26.1	1.2
Caudal-peduncle depth	8.7	8.4	10.0	9.15	0.37	8.4	10.0	9.0	0.3	9.0	9.7	9.4	0.2
Percents of head length													
Snout length	56.6	51.0	57.0	53.7	1.76	51.0	57.0	54.1	1.7	51.0	54.6	52.7	1.4
Orbital diameter	12.7	10.8	16.2	13.9	1.17	10.8	16.2	14.0	1.2	12.1	15.5	13.7	0.9
Interorbital width	31.3	28.8	36.3	32.4	1.65	29.4	35.5	32.6	1.4	28.8	36.3	31.9	2.0
Head depth	42.1	38.8	49.6	42.6	2.00	38.8	45.4	42.5	1.6	40.0	49.6	42.7	2.8
Suborbital depth	20.5	13.3	22.9	18.1	2.03	15.8	22.9	18.3	1.9	13.3	21.1	17.7	2.22
Mandibular ramus	8.7	4.7	10.7	8.5	1.29	6.2	10.4	8.7	0.9	4.7	10.7	8.0	1.8
Meristics													
Left premaxillary teeth	14	9	17	15	–	11	17	14	–	9	16	12	–
Left dentary teeth	13	10	15	13	–	10	14	12	–	10	15	13	–



Figure 1. *Curculionichthys karipuna*, MZUSP 120190, holotype, female, 23.7 mm SL, Brazil, Amapá, Oiapoque, rio Cassiporé, stream at road BR-156 at Campo Alegre farm.

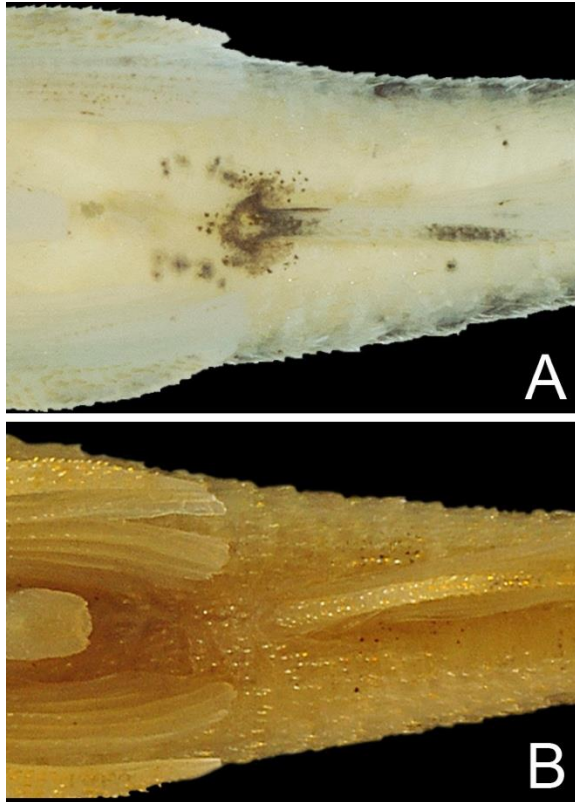


Figure 2. **A)** *Curculionichthys karipuna*, MZUSP 120190, holotype, showing the diagnostic character, irregular concentration of chromatophores covering the anal-fin origin and adjacent region along with the distal portions of the first unbranched anal-fin ray. **B)** *Curculionichthys sagarana*, MZUSP 117381, holotype, evidencing the absence of that pigmentation on the anal-fin origin.

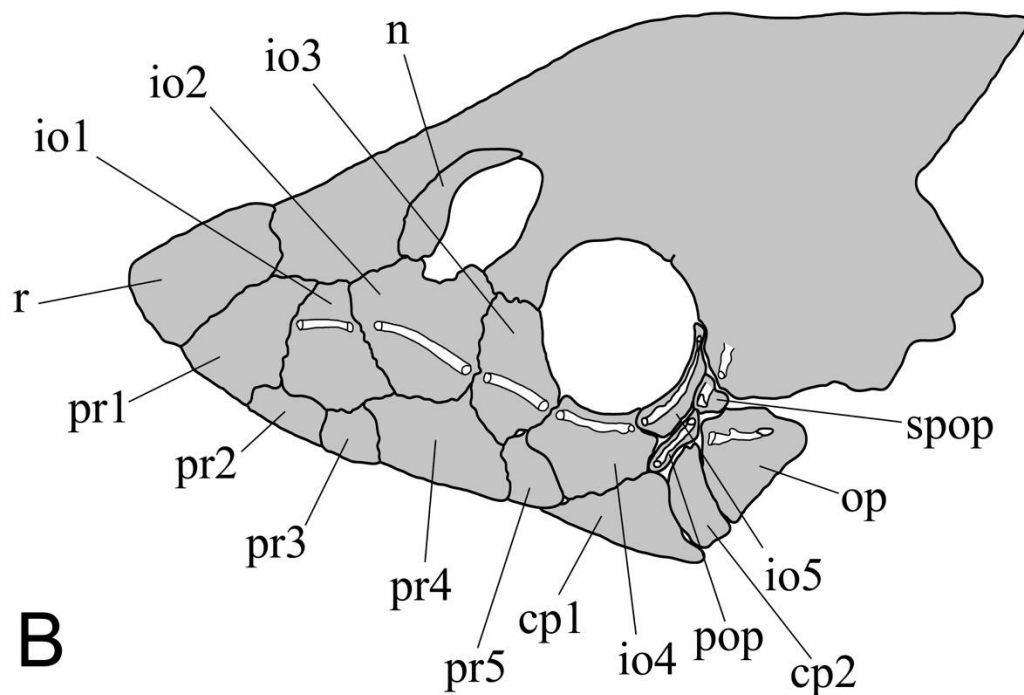
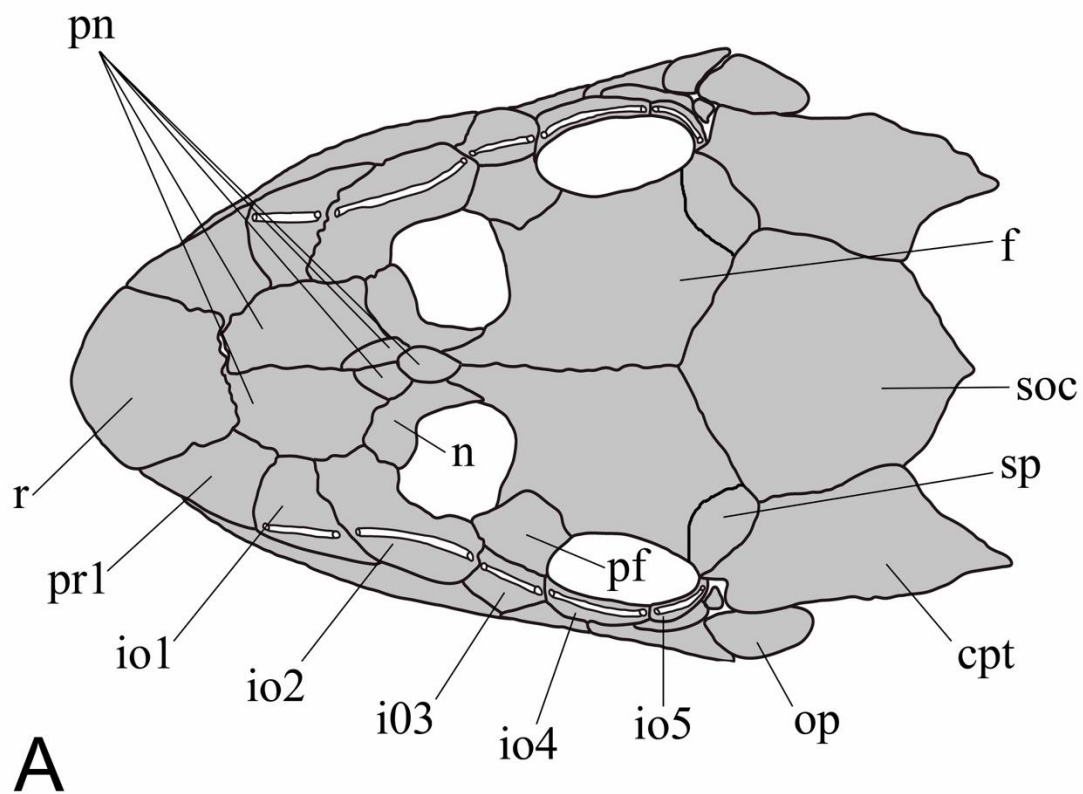


Figure 3. Neurocranium of *Curculionichthys karipuna*, LBP 19017, 29.0 mm SL. **A)** dorsal and **B)** lateral views. Abbreviations are: cpt = compound pterotic; f = frontal; io1-5 = infraorbitals; n = nasal; op = opercle; pr1-5 postrostral plates; pf = prefrontal; pn = prenasal; pop = preopercle; r = rostral plate; sp = sphenotic; cp1-2 = subocular cheek plate; soc = parieto-supraoccipital; spop = supra-opercular plate.



Figure 4. Northern South America showing the distribution of *Curculionichthys karipuna*. Star = rio Cassiporé, type locality; circle = rio Jari, paratypes locality.



Figure 5. Habitats of *Curculionichthys karipuna*. **A)** Type locality, stream at road BR-156 at Campo Alegre farm, affluent of rio Cassiporé. **B)** Paratype locality, an affluent of rio Jari of the Amazon basin.

Capítulo 4

***Avaichthys canoeiro*, description of a new genus and species
of Otothyrinae (Siluriformes: Loricariidae) from rio
Tocantins basin, Central Brazil**

Target jornal: Journal of Fish Biology

***Avaichthys canoeiro*, description of a new genus and species of Otothyrinae (Siluriformes: Loricariidae) from rio Tocantins basin, Central Brazil**

Fábio F. Roxo[†], Gabriel S. C. Silva[†], Luz Eneida Ochoa[†], Cláudio H. Zawadzki[§]

[†]Universidade Estadual Paulista, Departamento de Morfologia, Laboratório de Biologia e Genética de Peixes, municipality of Botucatu, São Paulo State, Brazil

[§]Universidade Estadual de Maringá, Departamento de Biologia, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia), municipality of Maringá, Paraná State, Brazil

Corresponding author: Fábio F. Roxo (roxoff@hotmail.com.br)

Running title: Description of a new catfish genus

Abstract

Avaichthys canoeiro n. gen. and sp. of Otothyrinae is described from small tributaries of the upper rio Tocantins basin. It is distinguished from all other genera of Otothyrinae by having three exclusive characters: the pectoral girdle not exposed in ventral view, the pectoral fin with seven to eight branched rays, and by having the pelvic-fin unbranched ray larger than pectoral-fin unbranched ray in males. Furthermore, the new genus differs from all Otothyrinae genera by having the following combination of characters: absence of dermal plates on snout tip and by the presence of an adipose fin. Maximum likelihood analysis was used to estimate a molecular phylogeny from previously published data of one nuclear (F-Reticulon 4) and three mitochondrial (16S RNA, COI and CytB) genes. The phylogenetic results revealed the new genus and species as a sister taxon of *Gymnotocinclus anosteos* within the Otothyrinae. We also included samples of *Nannoplecostomus eleonora* that appeared sister group of all other Neoplecostominae species, and *Plesioptopoma curvidens* that appeared within the Neoplecostominae forming a sister clade with all species of *Neoplecostomus*, except *N. ribeirensis* and the species of *Pareiorhina*, except *P. rudolphi*.

Keywords. Cascudinhos; catfish; freshwater fishes; molecular phylogeny; taxonomy.

INTRODUCTION

The Loricariidae is the largest Neotropical endemic fish family, which actually includes 916 species (Eschmeyer & Fong, 2016) distributed in seven subfamilies: Delturinae, Hypoptopomatinae, Hypostominae, Lithogeninae, Loricariinae, Neoplecostominae, and Otothyriinae (Armbruster, 2004; Reis *et al.*, 2006; Chiachio *et al.*, 2008; Roxo *et al.*, 2014). Within Loricariidae the Otothyriinae is a monophyletic group including more than 100 species classified in 13 genera: *Corumbataia*, *Curculionichthys*, *Epactionotus*, *Eurycheilichthys*, *Gymnotocinclus*, *Hisonotus*, *Microlepidogaster*, *Otothyris*, *Otothyropsis*, *Parotocinclus*, *Pseudotothyris*, *Rhinolekos* and *Schizolecis* (Eschmeyer, 2016). These fishes are all small, widely distributed throughout South America hydrographic systems from the Guianas to Argentina (Schaefer, 2003).

Reis & Schaefer (1998) suggested that the diversity of Otothyriinae and Hypoptopomatinae should be much greater than they had recognized at that time. Furthermore, for the last 10 years about 50 species of Otothyriinae and 10 species of Hypoptopomatinae has been described (Eschmeyer, 2016), as well as four new genera. Reis *et al.* (2012) described the monotypic genus *Plesioptopoma* from upper rio São Francisco basin as a possible member of the Otothyriinae (recognized as Hypoptopomatinae in that study), despite its shares several characters with members of Neoplecostominae; Carvalho *et al.* (2008) described *Gymnotocinclus* a monotypic genus from rio Tocantins basin; Martins & Langeani (2011) erected *Rhinolekos* to accommodate three new species from rio Paranaíba basin; and Roxo *et al.* (2015) described *Curculionichthys* to reallocate several species previously assigned to *Hisonotus* which were not close related to the type species *H. notatus*.

Recently, during a collecting expedition in tributaries of the upper portion of rio Tocantins basin we recognized an undescribed genus of Loricariidae that after a phylogenetic analysis was assigned to the subfamily Otothyriinae. The present study deals with the description of this new genus and species.

MATERIAL AND METHODS

Morphological analysis

Measurements and counts were taken from the left side of the samples and are shown in Table 1. Body plate nomenclature and counts follow Schaefer (1997), and measurements follow Armbruster (2003), except for mouth width that was not measured. All measurements were taken point to point with digital calipers and were rounded to the nearest 0.1 mm. Osteological examination was made on cleared and double-stained specimens (c&s) according to the procedures of Taylor & Van Dyke (1985). Vertebrae counts included five from the Weberian Apparatus and the compound caudal centrum (PU1 + U1) was counted as one element.

Institutional acronyms follow Fricke & Eschmeyer (2016). Vouchers of all samples were deposited in the collection of the (ANSP) Academy of Natural Sciences of Drexel University, municipality of Philadelphia, USA; (AUM) Auburn University Natural History Museum, municipality of Auburn, USA; (LBP) Laboratório de Biologia e Genética de Peixes, municipality of Botucatu, Brazil; (MZUSP) Museu de Zoologia da Universidade de São Paulo, municipality of São Paulo, Brazil; and (NUP) Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, municipality of Maringá, Brazil. Zoological nomenclature follows the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 1999).

Principal component analysis (PCA)

The PCA - Principal Component Analysis was achieved using all measurements to check overall variation among males and females according to Jolliffe (2002). PCA was performed using covariances of base 10 logarithmically transformed, to reduce the influence of size, and were obtained using Past version 1 28 (Hammer *et al.*, 2001). The PCA Loadings are presented in Table 2.

Phylogenetic analysis - Taxon Sampling

Species included in the present work were all from Roxo *et al.* (2014), except for two samples of *Avaichthys canoeiro*, one sample of *Gymnotocinclus anosteos*, two samples of *Nannoplecostomus eleonora*, two samples of *Plesioptopoma curvidens*, one sample of *Pareiorhina pelicicei*, one sample of *Pareiorhina cepta*, one of *Pareiorhina brachyrhyncha*, one of *Pareiorhina* sp. 1, one of *Microlepidogaster arachas*, one of *Microlepidogaster longicolla*, one of *Microlepidogaster* sp. 1, and one of *Microlepidogaster* sp. 2. It is worthy to note that our molecular character

matrix represents 116 loricariid species (see Table S1 for all taxa). *Diplomystes mesembrinus* (Diplomystidae) was used as outgroup to root our phylogeny (Arratia, 1987; de Pinna, 1993, 1998; Grande, 1987; Grande & de Pinna, 1998; Mo, 1991; Sullivan *et al.*, 2006). Additionally, samples of *Corydoras imitator*, *Corydoras oiapoquensis*, *Hoplosternum littorale*, *Callichthys callichthys* (Callichthyidae), *Astroblepus* sp. 1 and *Astroblepus* sp. 2 (Astroblepidae), *Hemipsilichthys gobio*, *H. papillatus*, *Delturus parahybae* (Loricariidae, Delturinae), *Rineloricaria lanceolata*, *Spatuloricaria* sp. 1 (Loricariidae, Loricariinae), *Hypostomus ancistroides*, *H. nigromaculatus* and *H. microstomus* (Loricariidae, Hypostominae) were included in the analysis as additional outgroups as in the work of Roxo *et al.* (2014).

DNA Extraction and Sequencing

All protocols for DNA extraction and sequencing were previously reported in Roxo *et al.* (2014), however, for convenience of the readers the methods are reintroduced in the present work. The total DNA was extracted from ethanol-preserved muscle, fin and liver samples, following the protocol described by Aljanabi & Martinez (1997), or with the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA). Partial sequences of each gene were amplified by Polymerase Chain Reaction (PCR) with the following primers: Cytochrome c oxidase subunit I (COI - Ward *et al.*, 2005), FishF1 - 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3', FishR1 - 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3'; Cytochrome B (CytB - Oliveira *et al.*, 2011), L14841 - 5'-CCA TCC AAC ATC TCA GCA TGA TGA AA 3', H15915b - 5'-AAC CTC CGA TCT TCG GAT TAC AAG AC 3'; 16S rRNA (Kocher *et al.*, 1989), 16Sar - 5'-ACG CCT GTT TAT CAA AAA CAT-3', 16Sbr - 5'-CCG GTC TGA ACT CAG ATC ACG T-3'; F-Reticulon 4 (Chiachio *et al.*, 2008), Freticul4-D - 5'-AGG CTA ACT CGC TYT SGG CTT TG-3', Freticul4-R - 5'-GGC AVA GRG CRA ART CCA TCT C-3', Freticul4 D2 - 5'-CTT TGG TTC GGA ATG GAA AC-3', Freticul4 R2 - 5'-AAR TCC ATC TCA CGC AGG A-3', Freticul4 iR - 5'-AGG CTC TGC AGT TTC TCT AG-3'. Amplifications were performed in a total volume of 12.5 µl with 1.25 µl of 10 X buffer (10 mM Tris-HCl+15 mM MgCl₂), 0.5 µl dNTPs (200 nM of each), 0.5 µl each 5 mM primer, 0.05 µl Platinum Taq Polymerase (Invitrogen), 1 µl template DNA (12 ng), and 8.7 µl ddH₂O. The PCR reactions consisted of 30–40 cycles, 30 s at 95°C, 15–30 s at 48–58°C, and 45–90 s at 72°C. Nested-PCRs were used to amplify the nuclear marker; the

first amplification was performed using the primers Freticul4-D and Freticul4-R with a total volume of 12.5 μ l for 30–40 cycles (30 s at 95°C, 30 s at 48°C, and 135 s at 72°C); the second amplification was performed using the primers Freticul4 D2 and Freticul4 R2 with a total volume of 12.5 μ l for 30–40 cycles (30 s at 95°C, 30 s at 53–54°C, and 135 s at 72°C). All PCR products were first visually identified on a 1% agarose gel and then purified using ExoSap-IT (USB Corporation) following instructions of the manufacturer. The purified PCR products were sequenced using the “Big Dye™ Terminator v 3.1 Cycle Sequencing Ready Reaction Kit” (Applied Biosystems), purified again by ethanol precipitation and loaded on an automatic sequencer 3130-Genetic Analyzer (Applied Biosystems) in the Instituto de Biociências, Universidade Estadual Paulista (UNESP), municipality of Botucatu, São Paulo State, Brazil.

Phylogenetic analysis

All protocols sequence and phylogenetic analysis also are quite similar to the procedures of Roxo *et al.* (2014), with small modifications reported below. Individual sequences of each species were initially analyzed with BioEdit 5.0.9 software (Hall, 1999), and a consensus sequence was obtained for each DNA segment. Next, all sequences were aligned using MUSCLE (Edgar, 2004) under default parameters and the alignments were visually inspected for any obvious misalignments. To evaluate the occurrence of substitution saturation for each gene separately, we estimated the index of substitution saturation (Iss) as described by Xia *et al.* (2003) and Xia & Lemey (2009) and the rate of transitions/transversions, both evaluated in the software DAMBE 5.2.31 (Xia & Xie, 2001). The Iss estimation was performed without taking into account gaps because unresolved sites reduce the ability of the method to test for phylogenetic signal. The best-fit nucleotide evolution model for the entire matrix was evaluated in the software PartitionFinder (Lanfear *et al.*, 2012) under the information-theoretic measure of Akaike Information Criterion (AICc).

Maximum likelihood analyses (RAxML) were performed on the CIPRES Science Gateway computing cluster (Miller *et al.*, 2010). Bootstrap (BS) resampling (Felsenstein, 1985) was applied to assess support for individual nodes using 1,000 replicates. Random starting trees were used for each independent ML tree search and all other parameters were set on default values. The ML analyses were conducted under GTR+G model as determined by the software PartitionFinder.

RESULTS

AVAICHTHYS, NEW GENUS

Figs. 1, 4

Type species

Avaichthys canoeiro, new species

Diagnosis

Avaichthys differs from all other Otothyrinae genera by having three exclusive characters: (1) pectoral girdle not exposed in ventral view (v. pectoral girdle completely or partially exposed), Fig. 1; (2) pectoral fin with seven (i,7 in 49 specimens) to eight (i,8 in one specimen) branched rays, Fig. 2 (v. six [i,6] branched rays); and (3) by having the pelvic-fin unbranched ray larger than pectoral-fin unbranched ray in males, Fig. 1 (v. the pelvic-fin unbranched ray shorter than pectoral-fin unbranched ray in males). Furthermore, the new genus differs from all Otothyrinae genera, except *Gymnotocinclus* by (4) the absence of dermal plates on snout tip, Fig. 3 (v. presence of plates on snout tip); and from all Otothyrinae genera, except *Parotocinclus* by (5) the presence of an adipose fin, Fig. 1 (v. absence of adipose fin in *Corumbataia*, *Curculionichthys*, *Epactionotus*, *Eurycheilichthys*, *Gymnotocinclus*, *Hisonotus*, *Microlepidogaster*, *Otothyropsis*, *Otothyris*, *Pseudotothyris* and *Rhinolekos*).

Etymology

The generic name “*Avaichthys*” is a combination of the word “Avá” from the Tupi Guarani Language, meaning “man, people”. It is in reference to indigenous people Avá-Canoeiro who used to inhabit vast areas of Central Brazil, including marginal areas of rio Maranhão and Tocantins, actually in Goiás State, Brazil. This name is masculine.

AVAICHTHYS CANOEIRO, NEW SPECIES

Figs. 1, 4

Holotype

MZUSP uncat., male, 51.5 mm **LS**, Brazil, Goiás State, municipality of Cavalcante, unknown river, tributary of rio das Almas, tributary of rio Paranã, rio Tocantins basin, 13° 43'12.6" S; 47° 27'19.8" W; L. H. Roxo, F. F. Roxo, G. S. C. Silva & L. E. Ochoa; 09 November 2014.

Paratypes

All from Brazil, Goiás State, municipality of Cavalcante, unknown river, tributary of rio das Almas, tributary of rio Paranã, rio Tocantins basin; 13° 43'12.6" S; 47° 27'19.8" W (66 specimens in total). ANSP uncat. (3, 43.8–60.7 mm **LS**), collected with holotype. AUM uncat. (3, 49.2–58.3 mm SL), collected with holotype. LBP 19469 (46, 28.0–54.3 mm **LS**, 5 c&s), collected with holotype. LBP 19303 (3, 43.4–56.8 mm **LS**), C. Oliveira, M. Taylor, B. Melo & G. S. C. Silva, 16 August 2014. MZUSP uncat. (3, 47.3–51.9 mm **LS**), collected with holotype. NUP 17787 (3, 45.0–50.6 mm **LS**), collected with holotype.

Diagnosis

Same as for the genus.

Description

Morphometric and counts are presented in Table 1. Large to median size Otothyriinae specimens, ranging from 38.9–62.3 mm **LS**. In lateral view, body elongated, dorsal profile slightly convex to straight from tip of snout to area between orbits; straight from this point to dorsal-fin origin, then descending straightly from dorsal-fin origin to unbranched caudal-fin insertion. Ventral profile slightly convex from snout tip to caudal-fin origin. Largest body depth at dorsal-fin origin. Largest body width at cleithrum region (22.8–39.0 mm of **LS**), progressively narrowing to snout tip and to caudal peduncle. Cross-section of trunk between pectoral and pelvic fins dorsally rounded and ventrally flattened; ellipsoid in caudal peduncle.

Head flat to slightly convex between orbits; snout elongated (54.3–65.0 mm of **LH**) and round in dorsal view. Head without crests even in juveniles. Dorsal surface of head at frontal (f), parieto supraoccipital (soc), and sphenotic (sp) region without odontodes. Eye small (9.0–14.4 mm of **LH**), situated dorsolaterally in midpoint of head. Perforations of compound pterotic distributed on whole bone, greater and more concentrated on its distal margin. Nostril small. Iris operculum present, poor

developed. Mouth wide; oral disk roundish with papillae uniformly distributed on base of dentary and premaxilla; papillae decreasing in size distally. Internally to mouth skin with deep transversal slit at each mandibular articulation. Lower lip almost reaching pectoral girdle and larger than upper; its border fringed. Maxillary barbel very short. Teeth slender, bicuspid, curved inward; crown with long lanceolate mesial cuspid and minute lateral cuspid. Premaxillary teeth 29–61 [47]. Dentary teeth 27–53 [40].

Dorsal fin ii,7; its origin slightly posterior to pelvic-fin origin. Dorsal-fin posterior margin straight to slightly rounded, reaching end of pelvic-fin rays when adpressed. Dorsal-fin spinelet short and ovoid shaped; locking mechanism not functional. Pectoral fin i,7-i,8 (i,7); unbranched ray depressed and slightly curved; posterior margin reaching pelvic-fin insertion when adpressed; unbranched ray covered with backward-oriented odontodes, more developed on ventral portion. Pectoral-fin axillary slit absent. Pelvic-fin i,5–i,6 (i,5); posterior margin nearly straight, reaching anal-fin insertion when adpressed in males and almost reaching anal-fin insertion in females. Pelvic-fin unbranched ray and first branched ray with dermal flap along its dorsal surface in males; unbranched ray ventrally covered with backward-oriented odontodes. Anal-fin i,5; posterior margin almost straight; unbranched ray ventrally covered with back-oriented odontodes. Adipose fin well developed and always present, not preceded by azygous plate. Caudal fin i,7,7,i; emarginate; lower unbranched ray similar in size than upper unbranched ray. Total vertebrae 31 (1 c&s).

Body entire covered by dermal plates, except in ventral portion of head, around pectoral and pelvic-fin origins and dorsal-fin base. Abdomen weakly covered by small platelets surrounded by naked areas (Fig. 1). Cleithrum and coracoid not exposed. Arrector fossae almost totally opened, with a large area at midline. Body lateral entirely covered by plates (Fig. 1); mid-dorsal plates developed and reaching middle of adipose-fin base; median plates series continuous in median portion of body; mid-ventral plates reaching adipose-fin origin.

Head bone plates present in Table 1. Snout tip with a large naked area without plates or odontodes. Nasal (n) almost triangular forming anterior medial nostril margin, contacting posteriorly with frontals (f) and anteriorly with pre-nasals (pn). Pre-nasals (pn) formed by several small triangular, rectangular and oval shaped plates. Dorsal surface of head composed by parieto supraoccipital (soc), compound pterotic

(cpt) and frontal (f), largest bones, and prefrontal (pf) and sphenotic (sp). Compound pterotic (cpt) with random distributed fenestration. Two posterior small and triangular-shaped rostral plates (pr1-pr2); Infraorbital plate series complete (io1-io5) forming inferior orbital margin of eyes, and present just above posterior rostrum series. Infraorbital plate series covered by latero-sensory canal system. Preopercle (pop) elongated and rectangular, covered by latero-sensory canal; preopercle present under io4, and upper cp1 and op. One subocular cheek plate (cp1) and operculum (op) form posterior lateral margin of head. Second subocular cheek plate (cp2) absent.

Color in life

Background color of dorsal surfaces of body brown with several black blocks randomly distributed. Ventral surface light brown with few dark spots mainly in caudal peduncle and lower caudal-fin unbranched ray. Dorsal surface of head dark brown to almost dark. Four dark brown saddles on dorsal surface of trunk, first inconspicuous and below dorsal-fin base; second below end of dorsal fin; third at adipose-fin origin; and fourth at end of caudal peduncle. Lateral portion of body with inconspicuous dark stripe along lateral line from head to caudal fin. All fins with inconspicuous irregular and poorly defined bands (Fig. 4).

Color in alcohol

Same pattern described for living specimens, but with darker brown background color (Fig. 1).

Sexual dimorphism

Mature males have three characters that are absent in females: (1) a conspicuous papilla just posterior urogenital opening; (2) a well-developed membrane along the dorsal portion of the unbranched pelvic-fin ray and slightly developed membranes in dorsal portion of the first four branched pelvic-fin rays; (3) the pelvic-fin unbranched ray larger than pectoral-fin unbranched ray. Additionally, males seem to reach larger size.

Etymology

The specific name “canoeiro” is from the Portuguese language, meaning those person or people who handle and/or build canoes. The name is a reference to the indigenous

people Avá-Canoeiro. In other times numerous and a powerful indigenous force inhabiting the the upper rio Tocantins valley, the Avá-Canoeiro are actually restricted to some small villages due to a series of gradual and abrupt murders, diseases and the lack of legal hunting territories (Pequeno, 2005). Recently, the Avá-Canoeiro were known as the “invisible people” due to the fact that some of the few survivors used to live for more than a decade in caves to avoid contact to civilization, just leaving the caves by night to collect and chase food. The Avá-Canoeiros are menaced to extinction.

Distribution

Avaichthys canoeiro is known from a single locality of a small unknown-named river, a tributary of rio das Almas, which flows to rio Paranã, rio Tocantins basin, Brazil (Fig. 5).

Ecological Notes

The new species *Avaichthys canoeiro* is found in a clear water river, varying from small to medium sized, with rocky outcrops forming small waterfalls and substrates of rocks and sand, Fig. 6. In large portions of the unknown-named river (tributary of rio das Almas), where the new species was found, the marginal vegetation was well preserved, and due to the large trees there is poor light penetration. The species was found at the bottom of the river associated with substrates of rocks and wood.

Phylogenetic analysis

The sequences of 136 specimens are present in Table S1. This list is the same presented in Roxo *et al.* (2014), except for the inclusion of the vouchers and GenBank accession numbers for the *Avaichthys canoeiro*, *Gymnotocinclus anosteos*, *Nannoplecostomus eleonora*, *Plesioptopoma curvidens*, *Pareiorhina pelicicei*, *Pareiorhina cepta*, *Pareiorhina brachyrhyncha*, *Pareiorhina* sp. 1, *Microlepidogaster arachas*, *Microlepidogaster longicolla*, *Microlepidogaster* sp. 1 and *Microlepidogaster* sp. 2. The concatenated dataset resulted in a matrix of 4,406 base pairs (bps), of which 1,462 bps were conserved and 2,561 bps were variable. This matrix was used in the phylogenetic analysis. The data of the concatenated matrix were not saturated, considering that the Iss.c value is higher than the Iss, and the R² value is higher than 0.7 for transitions and transversions.

Our results are very similar to those of Roxo *et al.* (2014), especially considering to the monophyly of the subfamilies Hypoptopomatinae, Neoplecostominae and Ootothyriinae (Fig. 7) with high statistical support (i.e. > 50%). In our analysis the Neoplecostominae was also close related to the Ootothyriinae, with the Hypoptopomatinae forming the sister group to them. *Avaichthys canoeiro* was nested in the Ootothyriinae (Fig. 7), forming a sister group to the monotypic genus *Gymnotocinclus*. We also included samples of *Nannoplecostomus eleonora* that appeared as the sister group to all other species of Neoplecostominae, and *Plesioptopoma curvidens* that appeared within Neoplecostominae forming a sister clade to all species of *Neoplecostomus*, except *N. ribeirensis* and all species of *Pareiorhina*, except *P. rudolphi*.

DISCUSSION

Phylogenetic analysis

Avaichthys

The new genus and species *Avaichthys canoeiro* appeared in this study within the subfamily Ootothyriinae forming a sister group to the monotypic genus *Gymnotocinclus*. Carvalho *et al.* (2008) suggested that *G. anosteos* could be close related to species of the genus *Corumbataia* by the presence of an adnate maxillary barbell. Our present study partially corroborates this study considering that the species of *Corumbataia* are sister group of *Avaichthys canoeiro* plus *Gymnotocinclus anosteos*, and that *Avaichthys canoeiro* also present the adnate maxillary barbell. Furthermore, all this clade appeared in our phylogenetic analysis forming the sister group to the recently described genus *Curculionichthys* (Roxo *et al.*, 2015) (Fig. 8). Carvalho *et al.* (2008) also suggested that *G. anosteos* could represent a basal clade among the hypoptopomatines (considered in that study as the tribes Ootothyriini and Hypoptopomatini), because the genus lacks the pectoral-fin slit, and that according to Reis & Schaefer (1998) and Gauger & Buckup (2005) the absence of this character suggest a basal position within hypoptopomatine. Our results also partially corroborate this hypothesis considering that the clade composed by *Gymnotocinclus anosteos*, *Avaichthys canoeiro*, species of *Corumbataia* and *Curculionichthys* are in a basal position within the Ootothyriinae. However, the pectoral-fin axillary slit is present in all species of both *Corumbataia* and *Curculionichthys*, but it is absent in *Gymnotocinclus anosteos* and *Avaichthys canoeiro*.

In the original description of *Nannoplecostomus eleonora* the phylogenetic analysis using morphological data performed by Ribeiro *et al.* (2012) suggested that *Nannoplecostomus* consists of a distinctive lineage within Loricariidae, not assigned to any of the previously recognized subfamilies (*incertae sedis* within Loricariidae), but forming a sister group to species of Hypostominae (*sensu* Armbruster, 2008). Despite in our present study *Nannoplecostomus eleonora* appears forming a sister clade to all species of Neoplecostominae it is still possible that it makes part of a non described subfamily.

Reis *et al.* (2012) said that *Plesioptopoma curvidens* presented several character shared with species of Otothyriinae (recognized as Hypoptopomatinae in that study) and Neoplecostominae. However, the authors preferred to include this taxon in Otothyriinae based on the presence of: (1) open nasal capsule; (2) enlarged fenestrae on the compound pterotic; (3) metapterygoid channel present; (4) arrector fossae completely opened (a plesiomorphic state of Schaefer, 1998); (5) ventral surface of the coracoid partially exposed. Furthermore, Reis *et al.* (2012) recognized another character shared with species of *Kronichthys* (Neoplecostominae) and species of the genera *Eurycheilichthys*, *Epactionotus*, and some *Parotocinclus* species where the odontodes on the ventral surface of the pelvic-fin unbranched ray are distinctly bent and turned mesially. The authors also suggested that *P. curvidens* has similarities to *Parotocinclus prata*, *Gymnotocinclus* and *Pseudotocinclus* (Otothyriinae). However, our phylogenetic analysis does not corroborate this hypothesis considering that *Plesioptopoma curvidens* appeared forming sister group with all species of *Neoplecostomus*, except *N. ribeirensis* plus all species of *Pareiorhina*, except *P. rudolphi*.

The four species of *Microlepidogaster* (*M. arachas*, *M. longicolla*, *Microlepidogaster* sp. 1 and *Microlepidogaster* sp. 2) included in the present study do not appeared close related to each other suggesting that this genus could be paraphyletic. *Microlepidogaster arachas* described by Martins *et al.* (2013) and the two new species *Microlepidogaster* sp. 1 and *Microlepidogaster* sp. 2 from rio São Francisco basin appeared close related to *Hisonotus bocaiuva* which is also from the rio São Francisco basin. However, a phylogenetic study including a large number of representative species of *Hisonotus*, including its type species *Microlepidogaster perforatus*, would be necessary to corroborate this hypothesis.

In our phylogenetic study we included all the described species of *Pareiorhina*. This genus appeared paraphyletic, with the type species *P. rudolphi* being close related to *Pseudotocinclus tietensis* and *P. juquiae*, than to the remaining species of *Pareiorhina* (i.e. *P. pelicicei*, *P. cepta*, *P. brachyrhyncha*, *P. hyptiorhachis*, *P. carrancas*). Additionally, the possible new species *Pareiorhina* sp. 1 from São Francisco basin appeared in a phylogenetic clade close related to all species of *Neoplecostomus*, except *N. ribeirensis*. This result suggests that the species of *Pareiorhina* which are not close related with the type species *P. rudolphi* may be part of a new genus. Our research group are working in the morphological revision of the genus *Pareiorhina* and the formal description of this new genus are beyond the scope of this present study.

Taxonomical analysis

Avaichthys canoeiro was distinguished from the species of Otothyrinae by three exclusive characters: (1) the pectoral girdle not exposed in ventral view, (2) the pectoral fin with seven to eight branched rays, and by having (3) the pelvic-fin unbranched ray larger than pectoral-fin unbranched ray in males. The first character is widespread in most loricariid subfamilies, except in Otothyrinae and Hypoptopomatinae. Within Otothyrinae only some species of *Parotocinclus* (i.e. *Parotocinclus jegui*, *P. prata* and *P. robustus*) and *Hisonotus pachysarkos* present the pectoral girdle almost completely covered by skin, with a very small area exposed, and not completely covered by skin as in the *Avaichthys canoeiro*. In some species of *Microlepidogaster* (e.g. *M. arachas*) and *Rhinolekos* (e.g. *R. britskii*) the pectoral girdle also have a large area covered by skin with a small area exposed.

The second character (i.e. the pectoral fin with seven to eight branched rays) is common in species of *Lamontichthys* (Paixão & Toledo-Piza, 2009), which all species have seven rays in the pectoral fin, as well as it is present in *Harttia leiopleura* (Oyakawa, 1993) and *Rineloricaria daraha* (Rapp Py-Daniel & Fichberg, 2008). All other loricariid species present six rays on pectoral fin. The third character used to diagnose *Avaichthys canoeiro* is the pelvic-fin unbranched ray larger than the pectoral-fin unbranched ray in males, and it is widespread in some neoplecostomine as *Hirtella carinata*, *Plesioptopoma curvidens* and in species of *Pareiorhaphis* and *Neoplecostomus*, however this character was up to now absent in species of Otothyrinae.

ADDITIONAL SPECIMENS EXAMINED

- Chauliocheilos saxatilis*: paratypes, MZUSP 114758 (2, 38.9–40.2 mm **LS**), municipality of Penha de França, rio Itamarandiba basin.
- Corumbataia cuestae*: LBP 3688 (3, 28.5–29.9 mm **LS**) municipality of Botucatu, upper rio Paraná basin.
- Curculionichthys insperatus*: LBP 4945 (5, 27.3–28.5 mm **LS**, 2 c&s, 28.2–29.9 mm **LS**) municipality of Botucatu, rio Tietê basin.
- Gymnotocinclus anosteos*: LBP 17125 (3, 18.8–33.0 mm **LS**) municipality of Alto Paraíso de Goiás, rio Tocantins basin.
- Hisonotus acuen*: holotype, MZUSP 115350 (25.9 mm **LS**) municipality of Querência, rio Xingu basin; paratypes, LBP 15755 (16, 19.5–26.0 mm **LS**) municipality of Ribeirão Cascalheira, rio Xingu basin.
- Hisonotus bocaiúva*: holotype, MZUSP 112204 (24.2 mm **LS**) municipality of Bocaiúva, rio São Francisco basin; paratypes, LBP 9817 (9, 3 c&s, 18.3–23.2 mm **LS**) municipality of Bocaiúva, rio São Francisco basin.
- Hisonotus notatus*: LBP 18472 (7, 30.1–38.3 mm **LS**) municipality of Silva Jardim, Southeastern Coastal Drainage.
- Hisonotus vespucii*: holotype, MZUSP 115274 (32.6 mm **LS**) municipality of Pirapora, rio São Francisco basin; LBP 10421 (18, 23.6–30.3 mm **LS**) municipality of Pirapora, rio São Francisco basin.
- Isbrueckerichthys alipionis*: LBP 7373 (17, 31.7–81.6 mm **LS**) municipality of Iporanga, rio Ribeira de Iguape basin.
- Kronichthys subteres*: LBP 515 (31, 28.4–61.9 mm **LS**), municipality of Iporanga, rio Ribeira de Iguape basin.
- Lampiella gibbosa*: LBP 7430 (5, 25.6–26.1 mm **LS**), municipality of Jacupiranga, rio Ribeira de Iguape basin.
- Microlepidogaster arachas*: LBP 10882 (3, 22.8–35.3 mm **LS**), municipality of Araxás, rio Paraná basin.
- Nannoplecostomus eleonora*: LBP 19016 (51, 19.9–25.4 mm **LS**) municipality of Guarani de Goiás, rio Tocantins basin.
- Neoplecostomus microps*: LBP 8036 (38, 41.3–65.0 mm **LS**) municipality of Piquete, rio Paraíba do Sul basin.

- Otocinclus vittatus*: LBP 8474 (27, 18.2–21.7 mm **LS**) municipality of Cáceres, rio Paraguay basin.
- Otothyropsis marapoama*: LBP 4698 (6, 23.9–36.3 mm **LS**) municipality of Marapoama, rio Tietê basin.
- Pareiorhaphis steindachneri*: LBP 739 (6, 33.8–49.0 mm **LS**) municipality of Jaraguá do Sul, Southeastern Coastal Drainage.
- Pareiorhina brachyrhyncha*: LBP 12240 (50, 26.4–36.9 mm **LS**) municipality of Pindamonhangaba, rio Paraíba do Sul basin.
- Pareiorhina rudolphi*: LBP 8044 (18, 31.7–48.9 mm **LS**) municipality of Piquete, rio Paraíba do Sul basin.
- Parotocinclus aripuanensis*: LBP 10981 (33, 15.63–18.47 mm **LS**) municipality of Guajará Mirim, rio Lageado basin.
- Parotocinclus maculicauda*: LBP 2869 (15, 20.2–44.7 mm **LS**), municipality of Miracatu, rio Ribeira de Iguape basin.
- Parotocinclus prata*: LBP 1136 (38, 19.8–41.9 mm **LS**) municipality of Presidente Olegário, rio São Francisco basin.
- Pseudotothyris obtusa*: LBP 6822 (70, 22.5–31.7 mm **LS**) municipality of Itanhaém, tributary of Rio Preto.
- Plesioptopoma curvidens*: LBP 17394 (39, 26.1–81.7 mm **LS**) municipality of Cristiano Ottoni, rio São Francisco basin.
- Pseudotocinclus tietensis*: LBP 2931 (3, 38.6–62.3 mm **LS**) municipality of Salesópolis, rio Tietê basin.
- Rhinolekos britskii*: LBP 7253 (15, 21.9–34.7 mm **LS**) municipality of Bela Vista de Goiás, rio Paranaíba basin.
- Schizolecis guntheri*: LBP 14335 (18, 18.3–35.3 mm **LS**) municipality of São Sebastião, Southeastern Coastal Drainage.

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Table 1. Morphometric data for *Avaichthys canoeiro*. SD = Standard Deviation, IO = Interorbital, OD = Orbital Diameter, CP = Caudal Peduncle. Measurements followed Armbruster (2003).

	<i>Avaichthys canoeiro</i> , n=50				Males, n=26			Females, n=24		
	Holotype	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
LS (mm)	51.5	38.9–62.3	48.51	5.07	43.8–62.3	52.0	3.97	40.9–51.0	45.50	2.71
Percents of LS										
Predorsal length	40.9	40.8–47.2	43.20	1.50	40.8–44.8	42.5	1.14	41.1–47.1	43.8	1.46
Head length	30.4	28.1–34.0	31.17	1.14	28.1–32.5	30.7	1.06	29.7–33.5	31.5	1.01
Head-dorsal length	12.0	8.1–18.1	12.48	1.43	10.1–18.1	12.4	1.52	11.1–14.0	12.6	0.90
Cleithral width	26.0	22.8–39.0	26.21	2.09	22.8–27.2	25.3	0.90	25.6–39.0	27.2	2.99
Head-pectoral length	25.1	23.5–29.4	25.72	1.07	23.5–27.1	25.4	0.99	23.9–29.4	26.1	1.13
Thorax length	13.48	12.8–17.3	14.98	0.84	13.4–17.3	15.0	0.87	12.8–16.1	14.7	0.86
Pectoral-spine length	17.39	16.9–21.5	18.95	0.93	17.3–21.5	19.0	0.91	16.9–20.6	18.9	0.99
Abdominal length	27.92	14.5–37.9	25.55	2.57	14.5–27.9	25.2	2.42	24.1–37.9	26.1	3.06
Pelvic-spine length	25.23	20.0–27.0	24.07	1.57	23.0–27.0	25.1	1.06	21.2–25.3	23.1	1.11
Post-anal length	38.76	35.7–41.6	37.97	1.13	35.7–41.6	38.1	1.30	35.8–39.1	37.8	0.90
Anal-fin spine length	16.66	16.1–20.0	17.75	0.93	16.1–20.0	17.8	1.07	16.1–18.8	17.8	0.70
Dorsal-pectoral distance	25.01	23.4–28.0	25.68	1.01	23.4–27.2	25.3	0.95	24.0–28.0	26.0	0.91
Dorsal spine length	21.43	20.0–23.7	21.90	0.80	20.0–23.7	21.9	0.84	21.0–23.4	22.0	0.78
Dorsal-pelvic distance	19.88	14.7–21.1	18.22	1.43	14.7–21.1	18.6	1.40	16.1–20.3	18.0	1.17
Dorsal-fin base length	15.90	12.3–17.7	14.14	0.88	13.2–17.7	14.5	0.95	12.8–15.0	13.8	0.53

Dorsal-adipose distance		22.59	20.2–27.1	23.22	1.52	20.6–27.1	23.8	1.50	21.0–25.2	22.9	1.14
Adipose-spine length		6.13	5.8–10.6	8.51	1.06	6.1–10.6	8.8	1.13	5.8–9.6	8.0	0.96
Dorsal adipose-caudal distance		22.88	11.8–25.0	21.62	2.04	18.2–24.5	21.8	1.64	11.8–25.0	21.1	2.76
Caudal peduncle depth		9.77	8.6–10.1	9.52	0.30	8.6–10.1	9.5	0.33	9.1–9.9	9.5	0.29
Ventral adipose-caudal distance		23.42	20.1–26.0	24.03	1.32	20.1–26.0	24.0	1.43	21.3–26.0	23.7	1.21
Adipose anal distance		21.23	18.1–23.0	20.31	1.19	18.1–23.0	20.8	1.31	18.8–21.3	19.8	0.66
Dorsal anal distance		16.93	14.9–17.7	16.36	0.60	14.9–17.3	16.3	0.67	15.5–17.7	16.4	0.52
Pelvic dorsal distance		23.73	18.5–25.7	22.53	1.77	18.5–25.7	22.6	2.05	20.2–25.0	22.4	1.48
Percents of LH											
Head-eye length		36.50	31.0–39.0	35.00	1.97	31.7–38.8	34.7	1.84	31.0–38.4	35.2	2.15
Orbital diameter		12.99	9.0–14.4	11.89	1.06	9.0–14.4	11.4	1.11	10.9–13.2	12.3	0.71
Snout length		59.17	54.3–65.0	60.79	2.48	57.7–64.3	61.3	1.69	56.1–65.0	60.9	2.67
Internares width		15.35	12.4–17.7	14.91	1.12	12.4–17.3	14.8	1.01	12.9–17.2	14.9	1.08
Interorbital width		32.17	30.6–36.4	33.52	1.41	30.6–36.4	33.6	1.65	32.1–35.9	33.6	1.12
Head depth		62.10	56.0–68.1	60.92	2.77	56.0–65.1	60.5	2.12	56.0–68.1	61.7	3.52
Mouth length		50.19	47.3–56.9	53.00	2.58	48.7–56.9	53.1	2.57	47.3–56.9	52.9	2.87
Barbel length		8.22	3.2–9.0	6.27	1.31	3.2–8.4	5.9	1.49	4.6–9.0	6.3	1.02
Dentary tooth cup length		21.34	16.9–25.3	20.51	1.80	18.0–22.2	20.2	1.27	16.9–25.2	20.4	2.06
Premaxillary tooth cup length		22.42	17.9–23.6	21.06	1.26	17.9–23.6	20.9	1.34	18.5–23.09	21.3	1.11
Counts		Holotype	Range	Mode	SD	Range	Mode	SD	Range	Mode	SD

Dorsal plates		28	25–28	26	–	26–28	26	–	25–27	26	–
Mid-dorsal plates		24	20–26	23	–	20–26	22	–	20–25	22	–
Median plates		28	25–28	27	–	27–28	27	–	25–28	27	–
Mid-ventral plates		24	20–25	22	–	20–24	22	–	20–25	22	–
Ventral plates		21	19–22	21	–	19–22	21	–	20–22	21	–
Dorsal-fin branched rays		ii,7	ii,7–ii,7	ii,7	–	ii,7–ii,7	ii,7	–	ii,7–ii,7	ii,7	–
Pectoral-fin branched rays		i,7	i,7–i,8	i,7	–	i,6–i,8	i,7	–	i,6–i,7	7	–
Pelvic-fin branched rays		i,5	i,5–i,6	i,5	–	i,5–i,5	i,5	–	i,5–i,6	5	–
Anal-fin branched rays		i,5	i,5–i,5	i,5	–	i,5–i,5	i,5	–	i,5–i,5	5	–
Caudal-fin branched rays		i,14	i,14–i,14	i,14	–	i,14–i,14	i,14	–	i,14–i,14	14	–
Premaxillary teeth		44	29–61	47	–	29–61	44	–	35–61	57	–
Dentary teeth		35	27–53	40	–	27–52	38	–	32–53	42	–

Table 2. Variable loadings in the Axis 1 and Axis 2 of size-free Principal Component Analysis of samples of *Avaichthys canoeiro*.

	Axis 1	Axis 2
Standard length	0.1725	0.01251
Predorsal length	0.1384	-0.0007908
Head length	0.1335	0.0204
Head dorsal length	0.188	-0.2402
Cleithral width	0.1166	-0.01365
Head pectoral length	0.1495	0.0004579
Torax length	0.1796	0.01392
Pectoral spine length	0.1859	-0.0235
Abdominal length	0.1758	0.2061
Pelvic-spine length	0.2336	0.004468
Post-anal length	0.1763	0.009115
Anal-fin spine length	0.1826	0.03538
Dorsal-pectoral depth	0.1598	-0.0297
Dorsal spine length	0.1756	-0.03496
Dorsal pelvic depth	0.2412	-0.05525
Dorsal-fin base length	0.2091	0.05129
Dorsal-adipose distance	0.2302	0.003059
Adipose-spine length	0.2446	-0.01122
Dorsal-adipose caudal distance	0.1884	-0.09621
Caudal peduncle depth	0.1763	0.01204
Ventral adipose-caudal distance	0.1636	-0.04995
Adipose anal distance	0.2195	0.09699
Dorsal anal distance	0.1709	0.04413
Pelvic dorsal distance	0.1739	-0.03045
Head-eye length	0.1274	-0.03378
Orbital diameter	0.07964	0.1676
Snout length	0.1725	0.007984
Internares width	0.1518	-0.00704
Minimal interorbital Distance	0.1515	0.03001
Mouth Length	0.155	0.01172
Barbel Length	-0.0127	0.9097

Dentary tooth cup length	0.1267	0.08233
Premaxillary tooth cup length	0.1162	-0.009248
Head depth	0.145	0.007288



FIG. 1. *Avaichthys canoeiro*, MZUSP uncat., holotype, male, 51.5 mm *LS*, from Brazil, Goiás State, municipality of Cavalcante, unknown river, tributary of rio Paraná, rio Tocantins basin.



FIG. 2. *Avaichthys canoeiro*, LBP 19469, paratype, 56.8 mm *LS*, in ventral view showing the pectoral fin with one unbranched ray and seven branched rays.

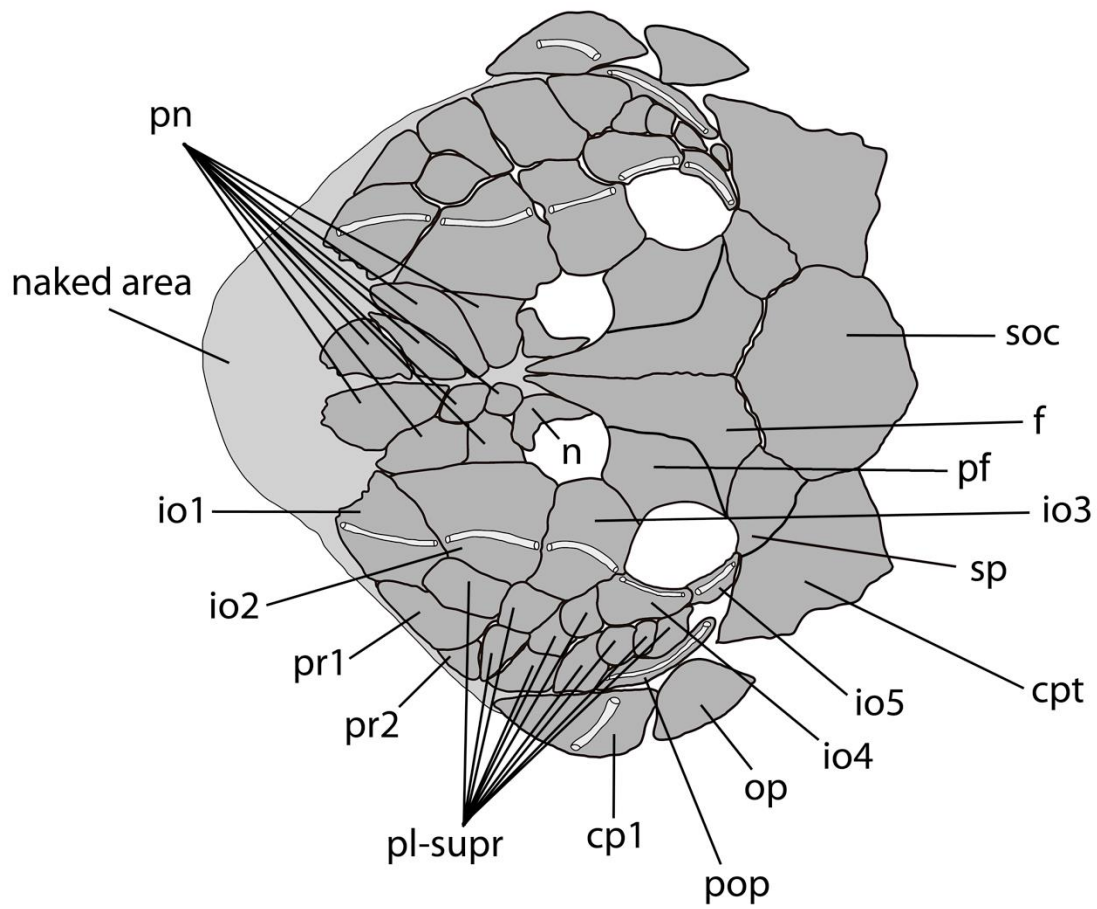


FIG. 3. *Avaichthys canoeiro*, LBP 19469, paratype, 44.7 mm *LS*, showing the head bone plates in dorsal view.



FIG. 4. Live specimen of *Avaichthys canoeiro*. Upper picture, paratype, LBP 19303, 49.3 mm *LS*. Photo: M. Taylor. Lower picture, holotype, MZUSP uncat., 51.5 mm *LS*.

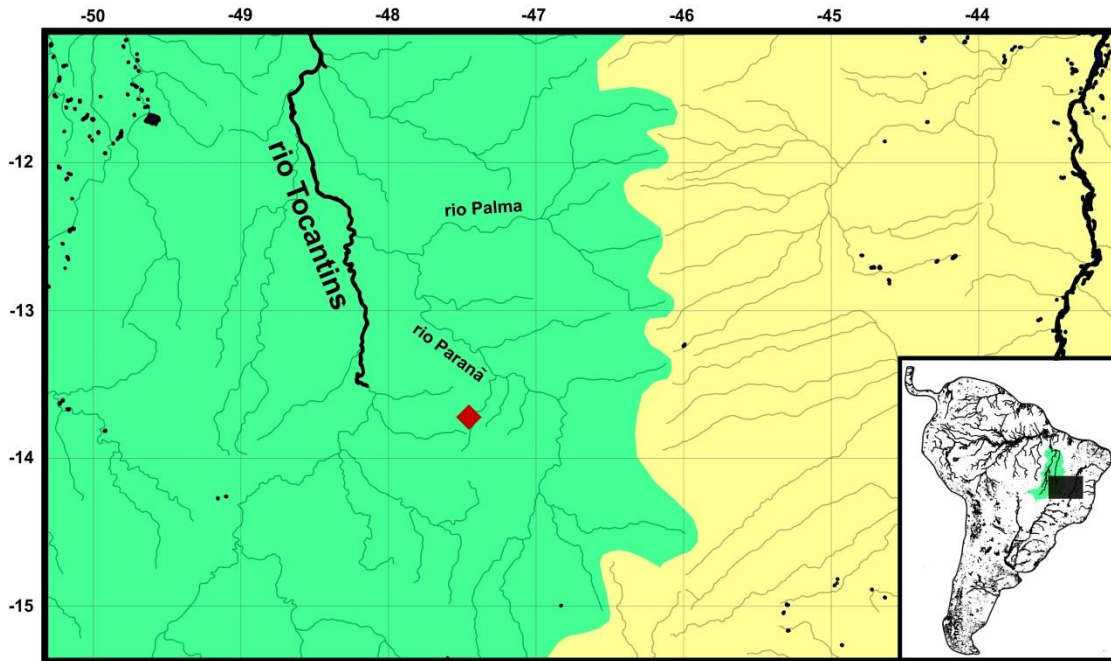


FIG. 5. Type locality of *Avaichthys canoeiro*, unknown-named river, tributary of rio Parana, tributary of rio das Almas, rio Tocantins basin (green color), 13°43'12.6"S, 47°27'19.8"W. The yellow color in the map represent the drainages of the rio São Francisco basin.



FIG. 6. Type locality and habitat of *Avaichthys canoeiro*. Specimens were collected associated with loose stones on the stream bed.

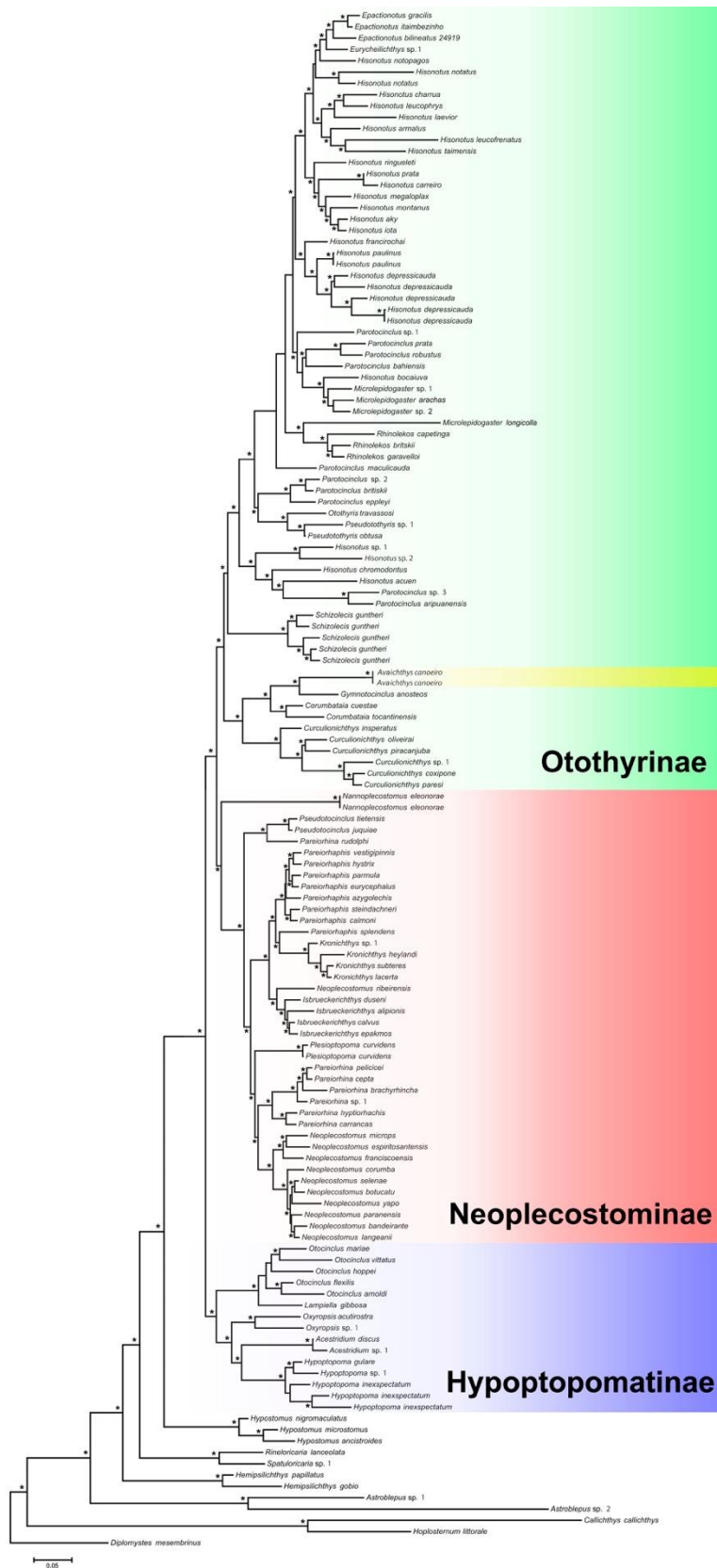


FIG. 7. Maximum likelihood tree showing the relationship among species of the subfamilies Hypoptopomatinae (blue color), Neoplecostominae (red color) and Ootothyriinae (green color). The yellow color highlights the new genus *Avaichthys canoeiro*, described in the present study. Asterisk on branches of the phylogeny denote the bootstrap values higher that 50%.

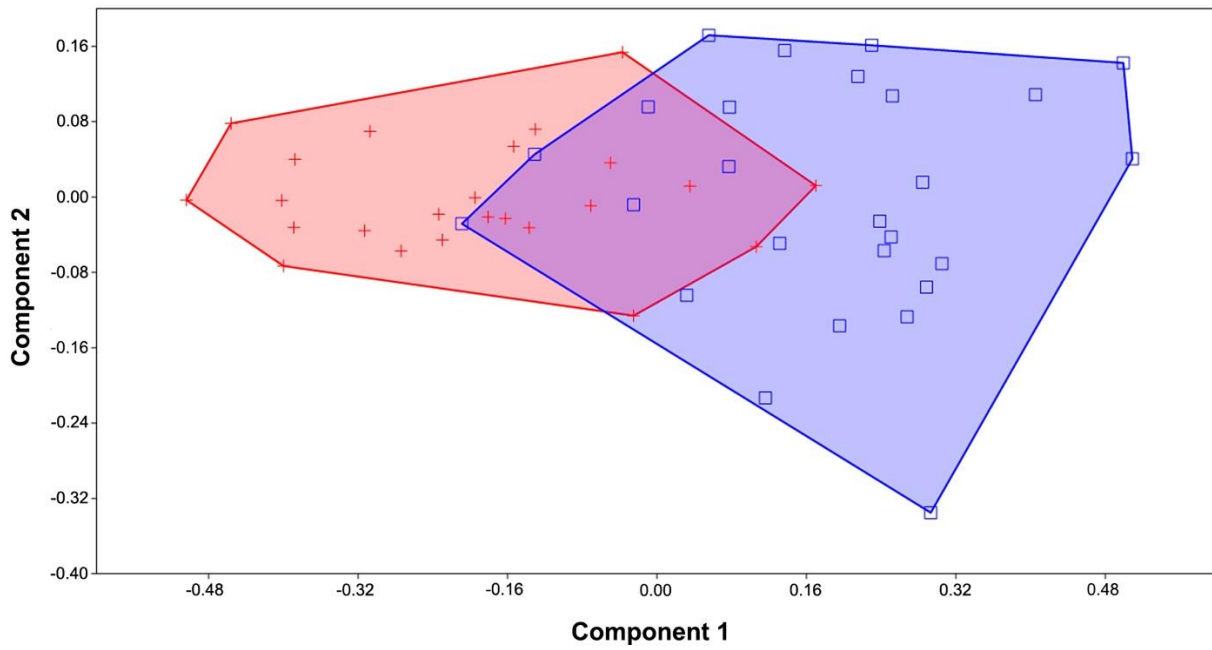


FIG. 8. Scatter plot of Principal Component Analysis (PCA) of *Avaichthys canoeiro*. Pink filled squares = females (n = 24); Blue empty squares = males (n = 26). Specimens size included in this analysis varies from 38.9–62.3 mm **LS**.

Supplementary table 1. Species included in Roxo et al. (2014) and in the present study (*). ANSP = Academy of Natural Sciences of Drexel University, Philadelphia; AUM = Auburn University Natural History Museum; LBP = Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista; MCP = Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul; MNRJ = Museu Nacional da Universidade Federal do Rio de Janeiro; NUP = Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá; MHNG = Museum of Natural History of the City of Geneva.

Number	Collection No	Fish No	GenBank (Reticulon/16S/COI/CytB)	Species
1	MCP 23606		-/-/EU371005/-	<i>Epactionotus gracilis</i>
2	MCP 23683		-/-/EU371004/-	<i>Epactionotus itaimbezinho</i>
3	LBP 4871	24919	KM205132/KM104344/EU817532/KM104528	<i>Epactionotus bilineatus</i>
4	LBP 4723	24951	KM205131/KM104343/EU817529/KM104527	<i>Eurycheilichthys</i> sp. 1
5	MCP 40762		-/KM104342/KM104436/KM104526	<i>Hisonotus notopagos</i>
6	LBP 3472	20258	KM205120/KM104329/JN998579/KM104517	<i>Hisonotus notatus</i>
7	MNRJ 37474		KM205121/KM104330/JN998532/KM104518	<i>Hisonotus notatus</i>
8	MCP 21644		KM205125/KM104336/JN998529/KM104522	<i>Hisonotus charrua</i>
9	MCP 41351		KM205124/KM104335/JN998546/KM104521	<i>Hisonotus leucophrys</i>
10	LBP 13187	51070	KM205127/KM104338/JQ015384/KM104524	<i>Hisonotus laevior</i>
11	LBP 6037	29054	KM205118/KM104327/JN998540/KM104515	<i>Hisonotus armatus</i>
12	MCP 31819		KM205123/KM104334/JN998552/KM104520	<i>Hisonotus leucofrenatus</i>
13	LBP 13147	51066	-/KM104332/KM104434/KM104519	<i>Hisonotus taimensis</i>
14	LBP 4863	24930	KM205136/KM104349/KM104438/KM104532	<i>Hisonotus ringueleti</i>
15	MCP 40942		-/KM104351/KM104440/KM104533	<i>Hisonotus prata</i>
16	MCP 40943		-/KM104350/KM104439/-	<i>Hisonotus carreiro</i>
17	MCP 41352		KM205137/KM104352/JN998543/KM104534	<i>Hisonotus megaloplax</i>
18	LBP 13055	51035	-/KM104353/KM104441/-	<i>Hisonotus montanus</i>
19	MCP 41474		-/KM104354/JN998533/KM104535	<i>Hisonotus aky</i>
20	MCP 40029		KM205139/KM104356/JN998544/KM104536	<i>Hisonotus iota</i>
21	LBP 13923	58218	KM205143/KM104364/KM104449/KM104543	<i>Hisonotus francirochai</i>
22	LBP 871	8801	KM205144/KM104365/KM104450/KM104544	<i>Hisonotus paulinus</i>

23	LBP 3932	22661	KM205145/KM104366/KM104451/KM104545	<i>Hisonotus paulinus</i>
24	LBP 17583	69477	-/KM104367/KM104452/KM104546	<i>Hisonotus depressicauda</i>
25	LBP 17583	69479	-/KM104368/KM104453/KM104547	<i>Hisonotus depressicauda</i>
26	LBP 8051	37802	KM205146/KM104369/KM104454/KM104548	<i>Hisonotus depressicauda</i>
27	MCP 22602		-/KM104370/KM104455/KM104549	<i>Hisonotus depressicauda</i>
28	LBP 2910	18756	-/KM104371/KM104456/KM104550	<i>Hisonotus depressicauda</i>
29	LBP 3457	20293	-/KM104363/KM104448/KM104542	<i>Parotocinclus</i> sp. 1
30	LBP 8249	38464	-/KM104357/KM104442/KM104537	<i>Parotocinclus prata</i>
31	LBP 8258	38298	-/-/KM104443/KM104538	<i>Parotocinclus robustus</i>
32	LBP 7182	34694	KM205140/KM104359/KM104444/KM104539	<i>Parotocinclus bahiensis</i>
33	LBP 17402	67143	-/KM104361/KM104446/KM104540	<i>Hisonotus bocaiuva</i>
34	LBP 11757	60286		* <i>Microlepidogaster</i> sp. 1
35	LBP 7244	33302		* <i>Microlepidogaster arachas</i>
36	LBP 5867	28345		* <i>Microlepidogaster</i> sp. 2
37	LBP 17060	68278		* <i>Microlepidogaster longicolla</i>
38	LBP 5848	28365	KM205149/KM104375/KM104460/-	<i>Rhinolekos capetinga</i>
39	LBP 7253	33309	KM205147/KM104373/KM104458/-	<i>Rhinolekos britskii</i>
40	LBP 7246	34410	KM205150/KM104376/KM104461/-	<i>Rhinolekos garavelloi</i>
41	LBP 2869	18571	EU817527/KM104378/KM104463/KM104552	<i>Parotocinclus maculicauda</i>
42	LBP 6950	35328	KM205153/KM104382/KM104467/-	<i>Parotocinclus</i> sp. 2
43	ANSP 179131		-/-/GQ225426/-	<i>Parotocinclus britskii</i>
44	LBP 4787	25579	EU817528/KM104383/KM104468/KM104554	<i>Parotocinclus eppleyi</i>
45	LBP 1971	13685	EU817526/KM104381/KM104466/KM104553	<i>Otothyris travassosi</i>
46	LBP 4722	24946	EU817525/KM104379/KM104464/-	<i>Pseudotothyris</i> sp. 1
47	LBP 2094	13851	KM205152/KM104380/KM104465/-	<i>Pseudotothyris obtusa</i>
48	LBP 334	4276	KM205156/KM104386/KM104471/KM104555	<i>Hisonotus</i> sp. 1
49	LBP 8276	38487	KM205157/KM104387/KM104472/KM104556	<i>Hisonotus</i> sp. 2
50	LBP 7948	35749	KM205160/KM104390/KM104475/-	<i>Hisonotus chromodontus</i>
51	LBP 16277	61771	KM205158/KM104388/KM104473/KM104557	<i>Hisonotus acuen</i>
52	LBP 2414	16276	KM205161/KM104393/KM104478/KM104560	<i>Parotocinclus</i> sp. 3

53	LBP 10981	50459	-/KM104391/KM104476/KM104558	<i>Parotocinclus aripuanensis</i>
54	LBP 2514	13846	EU817539/KM104394/KM104479/-	<i>Schizolecis guntheri</i>
55	LBP 3238	19471	EU817536/KM104395/KM104480/-	<i>Schizolecis guntheri</i>
56	LBP 2513	15240	EU817535/KM104396/KM104481/-	<i>Schizolecis guntheri</i>
57	LBP 2401	15272	EU817538/KM104397/KM104482/-	<i>Schizolecis guntheri</i>
58	LBP 2988	19646	EU817537/KM104398/KM104483/KM104561	<i>Schizolecis guntheri</i>
59	LBP 19303	77929		* <i>Avaichthys canoeiro</i>
60	LBP 19303	77930		* <i>Avaichthys canoeiro</i>
61	LBP17125	68274		* <i>Gymnotocinclus anosteos</i>
62	LBP 2001	12191	EU817521/KM104400/FJ965499/FJ965509	<i>Corumbataia cuestae</i>
63	LBP 1653	11477	EU817520/KM104399/KM104484/-	<i>Corumbataia tocantinensis</i>
64	LBP 5529	25381	EU817518/KM104401/KM104485/KM104562	<i>Curculionichthys insperatus</i>
65	LBP 1325	11238	KM205162/KM104402/KM104486/KM104563	<i>Curculionichthys oliveirai</i>
66	LBP 17256	66651	KM205163/KM104403/KM104487/KM104564	<i>Curculionichthys piracanjuba</i>
67	MNRJ 29502	490	-/KM104405/KM104489/KM104566	<i>Curculionichthys</i> sp. 1
68	LBP 5062	26057	KM205165/KM104407/KM104491/-	<i>Curculionichthys coxipone</i>
69	LBP 17532	68706	-/KM104406/KM104490/KM104567	<i>Curculionichthys paresi</i>
70	LBP 19016			* <i>Nannoplecostomus eleonorae</i>
71	LBP 19016			* <i>Nannoplecostomus eleonorae</i>
72	LBP 2931	18994	JN689285/JN089823/JN089799/JN089774	<i>Pseudotocinclus tietensis</i>
73	LBP 616	7564	JN689286/FJ625810/FJ625819/FJ965511	<i>Pseudotocinclus juquiae</i>
74	LBP 4391	24189	JN689280/ FJ434517/FJ434540/FJ965510	<i>Pareiorhina rudolphi</i>
75	LBP 1161	8935	JN689320/FJ434512/FJ434535/FJ965530	<i>Pareiorhaphis vestigipinnis</i>
76	LBP 701	7363	JN689321/FJ434513/FJ434536/FJ965531	<i>Pareiorhaphis hystrix</i>
77	MCP 41747		EU359443/-/-/-	<i>Pareiorhaphis parmula</i>
78	MCP 41458		EU359439/-/-/-	<i>Pareiorhaphis eurycephalus</i>
79	MCP 41909		-/-/EU359437/-	<i>Pareiorhaphis azygolechis</i>
80	LBP 902	7989	JN689319/FJ434514/FJ434537/FJ965532	<i>Pareiorhaphis steindachneri</i>
81	MCP 41275		-/-/EU359438/-	<i>Pareiorhaphis cameroni</i>
82	LBP 748	8257	JN689318/FJ625811/FJ625820/FJ965524	<i>Pareiorhaphis splendens</i>

83	LBP 1766	12886	JN689317/FJ965493/FJ965501/FJ965521	<i>Kronichthys</i> sp. 1
84	LBP 2122	15096	JN689314/FJ434502/FJ434525/FJ965520	<i>Kronichthys heylandi</i>
85	LBP 515	6334	JN689316/FJ965492/FJ965500/FJ965523	<i>Kronichthys subteres</i>
86	LBP 795	8304	JN689315/FJ434503/FJ434526/FJ965522	<i>Kronichthys lacerta</i>
87	LBP 7384	34837	JN689323/JN089845/JN089820/JN089796	<i>Neoplecostomus ribeirensis</i>
88	LBP 2650	17402	JN689325/FJ625812/FJ625821/FJ965528	<i>Isbrueckerichthys duseni</i>
89	LBP 7373	34853	JN689297/FJ965490/FJ965506/FJ965525	<i>Isbrueckerichthys alipionis</i>
90	LBP 6389	29765	JN689326/FJ965489/FJ965503/FJ965529	<i>Isbrueckerichthys calvus</i>
91	LBP7385	34852	JN689324/FJ965491/FJ965502/FJ965526	<i>Isbrueckerichthys epakmos</i>
92	LBP 17394	67137		* <i>Plesioptopoma curvidens</i>
93	LBP 17394	67138		* <i>Plesioptopoma curvidens</i>
94	LBP 18899	75263		* <i>Pareiorhina pelicicei</i>
95	LBP 10287	47213		* <i>Pareiorhina cepta</i>
96	LBP 12240	52133		* <i>Pareiorhina brachyrhyncha</i>
97	LBP 17393	67147		* <i>Pareiorhina</i> sp. 1
98	LBP 8380	37559	JN689288/JN089826/JN689277/JN089777	<i>Pareiorhina hyptiorhachis</i>
99	LBP 8368	37559	JN689291/JN089829/JN089804/JN089780	<i>Pareiorhina carrancas</i>
100	LBP 645	7593	JN689311/FJ434508/FJ434531/FJ965518	<i>Neoplecostomus microps</i>
101	LBP 2551	15243	JN689312/FJ434507/FJ434530/ FJ965512	<i>Neoplecostomus espiritosantensis</i>
102	LBP 6537	31681	JN689313/FJ965494/FJ965504/FJ965519	<i>Neoplecostomus franciscoensis</i>
103	NUP 2528	9423	JN689295/FJ434520/FJ434543/FJ965513	<i>Neoplecostomus corumba</i>
104	LBP 7383	34843	JN689310/ FJ965496/FJ965507/FJ965517	<i>Neoplecostomus selenae</i>
105	LBP 7525	34832	-/JN089843/JN089818/JN089794	<i>Neoplecostomus botucatu</i>
106	NUP 3560	9701	JN689293/ FJ434506/FJ434529/FJ965516	<i>Neoplecostomus yapo</i>
107	LBP 2732	17444	JN689303/FJ965495/FJ965505/FJ965515	<i>Neoplecostomus paranensis</i>
108	LBP 2861	18616	JN689298/JN089833/JN089808/JN089784	<i>Neoplecostomus bandeirante</i>
109	LBP 5901	27990	JN689304/JN089837/JN089812/JN089788	<i>Neoplecostomus langeanii</i>
110	MHNG 2601.060	BR98-040	EU817557/KM104423/KM104507/KM104574	<i>Otocinclus mariae</i>
111	LBP 5132	26233	EU817544/KM104421/KM104505/KM104573	<i>Otocinclus vittatus</i>
112	LBP 5310	26831	KM205171/KM104419/KM104503/-	<i>Otocinclus hoppei</i>

113	LBP 877	8564	EU817546/KM104418/KM104502/KM104572	<i>Otocinclus flexilis</i>
114	MCP 25234		-/-/EU370983/-	<i>Otocinclus arnoldi</i>
115	LBP 2652	17407	EU817545/KM104417/KM104501/-	<i>Lampiella gibbosa</i>
116	LBP 4300	23945	EU817542/KM104416/KM104500/KM104571	<i>Oxyropsis acutirostra</i>
117	LBP 6973	35324	KM205170/KM104415/KM104499/KM104570	<i>Oxyropsis</i> sp. 1
118	LBP 3165	19315	EU817543/KM104413/KM104497/KM104569	<i>Acestridium discus</i>
119	LBP 7204	35332	KM205169/KM104414/KM104498/-	<i>Acestridium</i> sp. 1
120	LBP 3081	19713	EU817541/KM104411/KM104495/-	<i>Hypoptopoma gulare</i>
121	MHNG 2709.024	MUS 388	EU817554/KM104412/KM104496/-	<i>Hypoptopoma</i> sp. 1
122	LBP 4042	22905	KM205167/KM104409/KM104493/-	<i>Hypoptopoma inexpectatum</i>
123	LBP 693	7084	KM205168/KM104410/KM104494/KM104568	<i>Hypoptopoma inexpectatum</i>
124	MHNG 2678.015	PR 12	JN689282/FJ965486/FJ965498/FJ965508	<i>Hypoptopoma inexpectatum</i>
125	LBP 3510	21309	JN689283/FJ625809/FJ625818/FJ965533	<i>Hypostomus nigromaculatus</i>
126	NUP 1725	16652	KM205172/KM104424/KM104508/-	<i>Hypostomus microstomus</i>
127	LBP 2544	10887	KM205173/KM104425/KM104509/-	<i>Hypostomus ancistroides</i>
128	LBP 1557	11505	KM205174/KM104426/KM104510/-	<i>Rineloricaria lanceolata</i>
129	LBP 5049	11506	KM205175/KM104427/KM104511/-	<i>Spatuloricaria</i> sp. 1
130	LBP 4956	10241	JN689279/FJ625808/FJ625817/FJ965534	<i>Hemipsilichthys papillatus</i>
131	LBP 2368	15363	JN689278/FJ434499/FJ434524/FJ965535	<i>Hemipsilichthys gobio</i>
132	LBP 1352	11454	-/KM104428/KM104512/-	<i>Astroblepus</i> sp. 1
133	LBP 3284	20010	KM205176/KM104429/KM104513/-	<i>Astroblepus</i> sp. 2
134	LBP 485	6040	-/KM104430/KM104514/-	<i>Callichthys callichthys</i>
135	LBP 210	4134	-/GU210868/-/-	<i>Hoplosternum littorale</i>
136	LBP 449	5815	-/KM104431/EU179801/-	<i>Diplomystes mesembrinus</i>

Transcontinental dispersal, ecological opportunity and origins of an adaptive radiation in the Neotropical catfish genus *Hypostomus* (Siluriformes: Loricariidae)

GABRIEL S. C. SILVA,^{*1} FÁBIO F. ROXO,^{*1} NATHAN K. LUJAN,^{†‡} VICTOR A. TAGLIACOLLO,[§] CLAUDIO H. ZAWADZKI[¶] and CLAUDIO OLIVEIRA^{*}

^{*}Departamento de Morfologia, Laboratório de Biologia e Genética de Peixes, IB–UNESP, Campus de Botucatu, Botucatu, 18618–970 São Paulo, Brazil, [†]Center for Systematic Biology and Evolution, Academy of Natural Sciences of Drexel University, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103, USA, [‡]Department of Biology, University of Toronto Scarborough, Toronto, ON M1C1A4, Canada, [§]Programa de Pós Graduação Ciências do Ambiente (CIAMB), Universidade Federal do Tocantins, Palmas, Tocantins 77001–090, Brazil, [¶]Departamento de Biologia, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia), Universidade Estadual de Maringá, Maringá, 87020–900, Paraná, Brazil

Abstract

Ecological opportunity is often proposed as a driver of accelerated diversification, but evidence has been largely derived from either contemporary island radiations or the fossil record. Here, we investigate the potential influence of ecological opportunity on a transcontinental radiation of South American freshwater fishes. We generate a species-dense, time-calibrated molecular phylogeny for the suckermouth armored catfish subfamily Hypostominae, with a focus on the species-rich and geographically widespread genus *Hypostomus*. We use the resulting chronogram to estimate ancestral geographical ranges, infer historical rates of cladogenesis and diversification in habitat and body size and shape, and test the hypothesis that invasions of previously unoccupied river drainages accelerated evolution and contributed to adaptive radiation. Both the subfamily Hypostominae and the included genus *Hypostomus* originated in the Amazon/Orinoco ecoregion. *Hypostomus* subsequently dispersed throughout tropical South America east of the Andes Mountains. Consequent to invasion of the peripheral, low-diversity Paraná River basin in southeastern Brazil approximately 12.5 Mya, Paraná lineages of *Hypostomus*, experienced increased rates of cladogenesis and ecological and morphological diversification. Contemporary lineages of Paraná *Hypostomus* are less species rich but more phenotypically diverse than their congeners elsewhere. Accelerated speciation and morphological diversification rates within Paraná basin *Hypostomus* are consistent with adaptive radiation. The geographical remoteness of the Paraná River basin, its recent history of marine incursion, and its continuing exclusion of many species that are widespread in other tropical South American rivers suggest that ecological opportunity played an important role in facilitating the observed accelerations in diversification.

Keywords: freshwater fish, geodispersal, macroevolution, molecular evolution, systematics

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Introduction

A central aim of evolutionary biology is to understand why species richness and phenotypic diversity are unevenly distributed across the tree of life (Stanley

Correspondence: Fábio F. Roxo, Fax: + 14 38116264 120;
E-mail: roxoff@hotmail.com.br

¹These authors contributed equally to this work.

1998; Mace *et al.* 2003; Venditti *et al.* 2010; Rabosky *et al.* 2012). Life is replete with examples of both ‘explosive radiations’ – characterized by rapid diversification into staggering numbers of species and phenotypes (e.g. African cichlid fishes, Caribbean *Anolis* lizards, Hawaiian silversword plants) – and ‘living fossil’ lineages – characterized by low species richness and morphological stasis for much of their evolutionary history (e.g. lungfishes, tuatara, *Equisetum*; Rabosky *et al.* 2013). Causes for such disparity can be broadly divided into mechanisms that accelerate speciation and/or phenotypic diversification rates (e.g. adaptive radiation, mimicry rings) and those that require no differences in underlying rates (e.g. differences in clade age or extinction rate). Only recently, with the advent of low-cost, fast and comprehensive molecular data (e.g. Boubli *et al.* 2012; Rabosky *et al.* 2012; Sorenson *et al.* 2014) and a growing plethora of comparative statistical techniques (e.g. Ricklefs 2007; Alfaro *et al.* 2009; Paradis 2011; Revell 2012; Rabosky 2014) have both robust phylogenies and techniques for identifying potential mechanisms been available to biologists. A major current goal to link results of these evolutionary models to climatic and geophysical processes to understand how large-scale Earth processes influence biological diversification.

Broadly distributed radiations of freshwater fishes are excellent systems in which to investigate linkages between biological diversification and geomorphological processes that shape the Earth’s surface. Freshwater fishes are highly diverse, and most are entirely restricted to freshwater habitats. Moreover, physical connections and slopes between and within these habitats shift in response to geomorphic processes. Phylogenies of freshwater fishes often reflect these events (Smith 1981; Hocutt & Wiley 1986; Mayden 1988; Lundberg *et al.* 1998; Cardoso *et al.* 2012; Lujan *et al.* 2015a; Tagliacollo *et al.* 2015). Indeed, geomorphological processes involving both the division of previously contiguous populations and the passive geodispersal of species between drainages have been linked to freshwater fish speciation and diversification both theoretically and empirically (Grant *et al.* 2007; Muneeppeerakul *et al.* 2008; Winemiller *et al.* 2008; Bertuzzo *et al.* 2009; Albert & Crampton 2010).

When an evolutionary lineage reaches a new environment, such as an island or drainage basin that lacks similar species, it may gain access to ecological opportunities that promote diversification by relaxing selection on ecologically important traits (Ehrlich & Raven 1964; Yoder *et al.* 2010). In some noteworthy instances, this leads to adaptive radiation in which a clade displays accelerated diversification rates, correlation between ecological and phenotypic traits, and newly derived traits that permit increased performance

(Schluter 2000). Although causal connections between ecological opportunity and biological diversification are often difficult to establish and differentiate from other drivers, macroevolutionary evidence for ecological opportunity’s importance is strong. First, long-term decreases in rates of lineage and morphological diversification are common in many lineages throughout the fossil and extant biological record (Soltis *et al.* 2002; Mahler *et al.* 2010). Such decreases are primarily attributed to the saturation of niche space – that is the decline of ecological opportunity due to increasing exploitation – that is an expected consequence of species accumulation (Simpson 1949, 1953). Second, fossil and molecular data for some groups (e.g. trilobites, Hallam & Wignall 1997; mammals, Meredith *et al.* 2011) suggest that speciation rates have increased following mass extinctions – that is periods of increased ecological opportunity due to niche vacancies (Sepkoski 1981, 1998, 2002; Erwin *et al.* 1987; Erwin 2001).

In addition to these macroevolutionary patterns, theory predicts a number of shorter-term, population-level, ecological and physiological responses to increased ecological opportunity. For example, after a lineage reaches a new environment, its population may increase, it may occupy a broader range of microhabitats, and it may display a broader range of morphological or behavioural characteristics as a result of decreased competitive pressure (Crowell 1962; Losos & de Queiroz 1997). Easier access to new or alternative resources created by ecological opportunity should effectively flatten the adaptive landscape, making a wider range of phenotypes viable (Roughgarden 1972; Travis 1989; Lathi *et al.* 2009). This is supported by empirical studies of populations that show a broader range of phenotypes following release from predation, competition or other sources of stabilizing selection (Roughgarden 1972; Houle *et al.* 1994). Access to the extrinsic opportunities and mechanisms that are linked to invasion of a new environment are often dependent upon intrinsic aspects of a species’ life history (e.g. vagility, fecundity, body size, ecological tolerance). Vagile species are more likely to invade a new habitat than philopatric species, and eurytopic species, which exhibit broad physiological and habitat tolerances, are more likely to succeed in a new habitat (Vrba 1980; Ribbink 1994).

Hypostominae is a species-rich (*c.* 447 valid species and 38 genera; Eschmeyer & Fong 2015) and monophyletic (Lujan *et al.* 2015b) group of primarily herbivorous and detritivorous catfishes restricted to the freshwaters of tropical Central and South America. Despite being broadly constrained to the primary consumer niche, Hypostominae lineages are known to display a wide range of jaw morphologies consistent with their fine-scale partitioning of basal food resources

including algae, detritus and wood (Lujan *et al.* 2011, 2012; Lujan & Armbruster 2012). Containing approximately 141, or about 30%, of all valid Hypostominae species, *Hypostomus* is the most species-rich genus in the subfamily and is among the most common and ubiquitous fish genera throughout the continental freshwaters of tropical South America. Our goal in this study was to examine the hypothesis that geographical dispersal via landscape-scale geomorphological events has accelerated rates of diversification of lineages (cladogenesis), maximum body size (MBS), and body shape within Hypostominae, particularly in the genus *Hypostomus*. We do this by (i) generating a species-dense, time-calibrated phylogeny for Hypostominae with a focus on *Hypostomus*, (ii) using the obtained chronogram to estimate ancestral geographical ranges and (iii) infer historical diversification rates in occupied habitat, body size and body shape.

Material and methods

Taxon sampling

Our objective in taxonomic sampling was to comprehensively sample genera within Hypostominae (*sensu* Lujan *et al.* 2015b) and to maximize the breadth of genera representing other loricariid subfamilies and tribes. Our in-group (Hypostominae) included 124 specimens spanning 24 genera and four to five tribes (*sensu* Lujan *et al.* 2015b). To test for monophyly of Hypostominae, we compiled a broad range of outgroups representing all other subfamilies of Loricariidae plus Astroblepidae and Callichthyidae. Specimens sequenced for this study are listed in Tables S1 and S2 (Supporting information). Sequence data for many taxa outside the Hypostominae, including members of the subfamilies Loricariinae, Hypoptopomatinae and Rhinelepininae, were obtained from previously published studies (Montoya-Burgos *et al.* 1998; Chiachio *et al.* 2008; Covain & Fisch-Muller 2012; Roxo *et al.* 2012, 2014) via the GenBank database.

Members of two other Loricarioidei families (Astroblepidae: *Astroblepus* sp. and Callichthyidae: *Hoplosternum littorale*, *Corydoras imitator* and *C. oiapoquensis*) were selected as outgroups based on relationships established in previous larger-scale phylogenetic analyses of both morphological (de Pinna 1993, 1998) and molecular (Sullivan *et al.* 2006) data. Vouchers of all sequenced samples were deposited in the Auburn University Natural History Museum, Auburn, Alabama, USA (AUM); Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brasil (LBP); the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá,

Paraná, Brasil (NUP); Museum d'Histoire Naturelle, Département d'Herpétologie et Ichthyologie, Ville de Genève, Genève, Switzerland (MHNG); or at the Smithsonian Tropical Research Institute, Balboa, Ancón, Panama (STRI). Specimens were identified directly by the authors GSCS and CHZ, except in the case of GenBank identifications, which were not changed.

DNA extraction and sequencing

Whole-genomic DNA was extracted from muscle, fin or liver samples preserved in 95% ethanol using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Partial sequences of the 16S rRNA, cytochrome *b* (*cyt b*) and F-reticulon 4 (RTN4) genes were amplified by polymerase chain reaction (PCR; primers listed in Table S3, Supporting information). Amplifications were performed in a total volume of 12.5 μ L with 1.25 μ L of 10 \times buffer (10 mM Tris-HCl+15 mM MgCl₂), 0.5 μ L dNTPs (200 nM of each), 0.5 μ L of each 5 mM primer, 0.05 μ L Platinum[®] *Taq* Polymerase (LTI: Life Technologies Inc., Carlsbad, CA, USA), 1 μ L template DNA (approximately 12 ng) and 8.7 μ L ddH₂O. PCRs consisted of 30–40 cycles of 30 s at 95 °C, 15–30 s at 48–58 °C and 45–90 s at 72 °C (annealing temperature and duration depended on primers and species, Table S3, Supporting information). Nested PCRs were used to amplify the nuclear marker RTN4; the first amplification was performed using the primers Freticul4-D and Freticul4-R with a total volume of 12.5 μ L for 30–40 cycles (30 s at 95 °C, 30 s at 48 °C and 135 s at 72 °C); and the second amplification was performed using the primers Freticul4 D2 and Freticul4 R2 with a total volume of 12.5 μ L for 30–40 cycles (30 s at 95 °C, 30 s at 53–54 °C and 135 s at 72 °C). All PCR products were first visually identified on a 1% agarose gel and then purified using ExoSap-IT[®] (USB Corporation, Cleveland, OH, USA) following manufacturers' instructions. Purified PCR products were sequenced using the Big Dye[®] Terminator v3.1 Cycle Sequencing Ready Reaction Kit (ABI: Applied Biosystems Inc., Foster, CA, USA), purified again by ethanol precipitation and sequenced on a 3130 Genetic Analyzer (ABI) in the Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo State.

Sequence alignment and phylogenetic inference

Two intronic regions of the RTN4 gene, totalling 63% of total locus length, were amplified and sequenced by our primers. All loci, including the entire RTN4 sequence, were first aligned using the MUSCLE algorithm (default parameters; Edgar 2004) followed by visual inspection and correction of the alignment requiring

almost no changes in base position. The total matrix consisted of 157 individuals \times 3433 base pairs, or a total of approximately 548 800 characters, of which 40% were missing data. To detect potential sequencing errors due to contamination or paralogy, alignments for each gene were separately analysed by maximum likelihood (ML; Stamatakis *et al.* 2008) using the web server RAXML BLACKBOX (Stamatakis 2006). Sequences that resulted in obviously misplaced taxa in resulting gene trees were resequenced or eliminated from subsequent analyses. Because terminal redundancy can affect results of biogeographical analyses using dispersal–extinction–cladogenesis (DEC) models, we trimmed our final data set to include only one individual of each species.

We estimated the index of substitution saturation (Iss) and rates of transitions/transversions for each gene separately in DAMBE 5.2.31 (Xia & Xie 2001), as described by Xia *et al.* (2003) and Xia & Lemey (2009). Iss was estimated without considering gaps because unresolved sites reduce the ability of the method to test for phylogenetic signal. The best-fit partitioning scheme and nucleotide substitution model for each partition were determined using the greedy algorithm in the software PARTITIONFINDER (Lanfear *et al.* 2012), which was programmed to use PHYML to estimate likelihoods and the Akaike information criterion with correction for finite sample sizes (AICc) to choose the best model.

Maximum-likelihood (ML) analyses (Stamatakis *et al.* 2008) were performed using RAXML on the CIPRES Science Gateway computing cluster (Miller *et al.* 2010). RAXML implements a fast algorithm of heuristic searches using bootstrap (BS) resampling (Felsenstein 1985). Support for individual nodes was assessed using random start trees, the GTR+G model for all partitions of the matrix as determined by the software PARTITIONFINDER (Lanfear *et al.* 2012; Table S4, Supporting information), and independent ML tree searches consisting of 1000 pseudoreplicates each with all other parameters set to default values.

Bayesian inference (BI; Huelsenbeck & Ronquist 2001) was used to determine statistical support for alternative tree topologies through the estimation of posterior probabilities using the software MRBAYES v3.2.2 (Ronquist *et al.* 2012). This was made under different models for each partition of the matrix as determined by the software PARTITIONFINDER (Table S4, Supporting information), using a random tree to start the Markov chain Monte Carlo searches, and a duplex of four chains (nchains = 4) set to run simultaneously for 10 million generations, sampling tree space every 500th generation. This analysis was performed twice to ensure similarity of converged trees. Log-likelihood scores were calculated using the program TRACER 1.5 (Rambaut &

Drummond 2007a) to determine whether independent searches of tree space had achieved stationarity based on their convergence within a stable – Ln range of values and effective sample sizes (ESS) exceeding 200. All sampled topologies beneath the asymptote (40%) were discarded as burn-in, and remaining trees were used to construct a 50% majority-rule consensus tree in TREEANNOTATOR v1.7.5 (Rambaut & Drummond 2007b).

Tree topology tests

Alternative tree topologies hypothesized by Lujan *et al.* (2015a,b) were evaluated in the program TREEFINDER (Jobb *et al.* 2004) using the Shimodaira and Hasegawa (SH) test (Shimodaira & Hasegawa 1999), the Approximately Unbiased (AU) test (Shimodaira 2002) and the Expected Likelihood Weights (ELW) method (Strimmer & Rambaut 2002). All tests were conducted under ML with the same 12-partition model as implemented in the RAXML analysis (Table S4, Supporting information).

Time calibration

The uncorrelated relaxed molecular clock (log-normal) was estimated using BEAST v1.7.5. (Drummond & Rambaut 2007). We used a clade-based approach that included each Hypostominae lineage in our analysis. The GTR+G model was used for all partitions (Table S4, Supporting information). The calibration prior was implemented using a log-normal distribution offset to 55 Mya with a mean and standard deviation of 1 for the origin of the genus *Corydoras* lineage (node including all Callichthyidae: *Corydoras imitator*, *C. oiapoquensis* and *Hoplosternum littorale*). The oldest known loricarioid fossil, *Corydoras revelatus* Cockerell (1925), was dated by Marshall *et al.* (1997) as Paleocene, which we used to assign a 55 My minimum age for the family Callichthyidae. We used a birth–death model for speciation likelihood and a starting tree obtained from ML. The analysis was run for 10 million generations and sampled every 1000th generation. Stationary and sufficient mixing of parameters (ESS > 200) was checked using TRACER v1.5 (Rambaut & Drummond 2007a). A consensus tree was built using TREEANNOTATOR v1.7.5 (Rambaut & Drummond 2007b).

Ancestral area estimation

Data on the geographical distributions of species in the Hypostominae were taken from the original species descriptions and information available from the online Catalog of Fishes (Eschmeyer & Fong 2015). To maintain consistency with previous biogeographical analyses of the subfamily Hypoptomatinae (*sensu* Lujan *et al.*

2015b) by Chiachio *et al.* (2008) and Roxo *et al.* (2014), we assigned taxa to geographical areas using the ecoregion classification of Vari (1998), which was derived from patterns of endemism in the Neotropical endemic characiform family Curimatidae. Our samples were drawn from the following six of Vari's (1998) ecoregions: (i) Atlantic Coastal drainages, (ii) upper Paraná basin, (iii) Paraguay, lower Paraná and Uruguay basins, (iv) Amazon and Orinoco basins, (v) São Francisco basin and Northeastern Brazilian drainages and (vi) Pacific Coastal drainages and Magdalena basin.

Maximum-likelihood inference of geographical range evolution was performed using the dispersal–extinction–cladogenesis (DEC) model as implemented in LAGRANGE v2.0 (Ree *et al.* 2005; Ree & Smith 2008). Four DEC models were tested to estimate distribution ranges inherited by descending lineages at each node of the tree. Each model differed in the rates of dispersal between adjacent vs. nonadjacent areas (see Table S5, Supporting information for likelihood values and dispersal rates for each model). Model 3 (M3), which constrained dispersal rates between adjacent areas at 0.5 and areas separated by one or more intervening areas at 0.0001, obtained the highest ML values.

Speciation rates

We used BMM version 2.1.0 (Rabosky 2014) to estimate speciation and extinction rates across the Hypostominae phylogeny. We assessed the effects of missing taxa on our tree by counting missing species for each lineage of our phylogeny (see Table S2, Supporting information for summary of missing genera and species). The analysis was conducted with two chains running simultaneously for a total of 5 million generations and tree space being sampled every 1000th generation. We used a burn-in value of 0.5 and checked for MCMC convergence using the BAMMTOOLS package (Rabosky *et al.* 2014) in the R statistical environment (R Core Team 2014) to plot log-likelihood values. To account for effects of phylogenetic uncertainty on our analyses, we conducted BAMM analyses of species diversification across 2500 trees sampled from the posterior distribution of topologies and branch lengths. To visualize the evolutionary rate dynamics from BAMM output results, we also used BAMMTOOLS.

Morphometric data

We measured 33 morphometric characters (doi: 10.5061/dryad.c8q11) on the bodies of two adult specimens from each of 124 specimens and 24 genera of Hypostominae, using 31 homologous landmarks originally proposed by Armbruster (2003a): 14 on the head, five on the mouth and the remainder on the trunk or fins. Although the suite

of measurements we used was originally intended to maximize homology and taxonomic discrimination (Armbruster 2003a), many of the measurements are known to be functional, ecological correlates (e.g. mouth width, tooth row length, eye size, eye position, body depth, fin size; Gatz 1979; Winemiller 1991; Lujan & Armbruster 2012). All analyses described later are based on means of log-transformed trait values computed for each species. To remove the influence of body size on morphometric data, we used the program PAST v1.28 (Hammer *et al.* 2004) to normalize two coordinate dimensions, to divide all coordinate values by the centroid size for each specimen and to conduct a Procrustes fitting of the left half to a mirrored version of the right half (Dryden & Mardia 1998). We conducted a principal component analysis (PCA) using the covariance matrix of phylogenetically corrected residuals from the PAST output. The first principal component (PC1) in the matrix of residuals accounted for 27.5% of variance in body shape for Hypostominae (values used for BMM and phylomorphospace analyses) and 22.4% for *Hypostomus* (values used for an independent phylomorphospace analysis). PCA loadings are presented in Table S6 (Supporting information).

Diversification rates

We used BMM version 2.1.0 (Rabosky 2014) to estimate rates of continuous trait evolution in body shape and maximum body size (MBS) values across the Hypostominae phylogeny. These analyses were conducted with two chains running simultaneously for a total of 50 million generations, with tree space sampled every 5000th generation. We used a burn-in value of 0.5 and checked for MCMC convergence using the BAMMTOOLS package in R (Rabosky *et al.* 2014). We conducted BAMM analyses of phenotypic evolution across 5000 trees sampled from the posterior distribution and visualized the evolutionary rate dynamics from BAMM output using BAMMTOOLS.

Habitat data and ancestral state estimation

Each Hypostominae species was classified according to one or more of the following five habitat categories (from Albert *et al.* 2011) based on original species descriptions, the online Catalog of Fishes (Eschmeyer & Fong 2015) and the authors' personal field experience: channels, rapids, streams, and low or high altitudes (see Table S2, Supporting information for habitat classifications and values for each species). Habitats for each species were coded using five binary (presence/absence) values, and the resulting matrix was examined in 1000 stochastic character map simulations using Simmap (Bollback 2006) in R (R Core Team 2014).

Phylomorphospace analysis

We generated two phylomorphospace biplots (Sidlauskas 2008) using the 'phylomorphospace' function in the PHYTOOLS v0.3-10 (Revell 2012) package in R (R Core Team 2014): one for all Hypostominae species and the second for only the genus *Hypostomus*. For each plot, the first principal component of size-corrected body shape for each taxonomic grouping was plotted against log maximum body size (MBS). Species colours in both plots correspond to ecoregion colour in the ancestral area estimation. In the Hypostominae plot, a convex hull was drawn around all *Hypostomus* species, and in the *Hypostomus* plot, a convex hull was drawn around all species from the Paraná River basin.

Results

DNA sequence alignment and substitution rates

The concatenated alignment of sequence data for 16S rRNA, *cyt b* and RTN4 genes resulted in a matrix consisting 3433 base pairs (bp); 971 bp was conserved and 2283 was variable. We found no evidence for saturation: the Iss.c value was greater than the Iss value (Xia *et al.* 2003; Xia & Lemey 2009), and the determination coefficients of transitions and transversions vs. genetic distance were higher than 0.7 (transitions $R^2 = 0.94$; transversions $R^2 = 0.98$). The observed transition/transversion ratio was 1.9, with nucleotide frequencies of 25.7% for adenine, 29.2% for thymine/uracil, 26.9% for cytosine and 18.1% for guanine.

Phylogenetic relationships

Within Hypostominae, our results (Fig. S1–S4, Supporting information) supported monophyly of six of the nine tribe-level clades identified by Lujan *et al.* (2015b): the *Chaetostoma* Clade (BS = 99, PP = 1), the '*Pseudancistrus*' Clade (BS = 91, PP = 1), Ancistrini (BS = 50), the *Pseudancistrus* Clade (BS = 97, PP = 1), the *Peckoltia* clade (BS = 92, PP = 1) and Hypostomini (BS = 99, PP = 1; see Figs S1–S4, Supporting information). One of Lujan *et al.*'s (2015b) tribe level clades was not examined (the *Lithoxus* Clade, consisting of nine small species restricted to the Guiana Shield), and two tribe level clades from that study were not supported as monophyletic: Monophyly of the *Hemiancistrus* Clade, containing the genus *Panaque* (BS = 100, PP = 1) and a clade of four other genera (*Baryancistrus*, *Hemiancistrus*, *Parancistrus* and *Spectracanthicus*; BS = 65), was not supported. Also, monophyly of the *Acanthicus* Clade, containing our *Leporacanthicus* clade (BS = 99, PP = 1) and *Megalancistrus* clade (BS = 100,

PP = 1), was not supported. However, a topology test did not reject the hypothesis of monophyly of Lujan *et al.*'s (2015b) *Acanthicus* Clade (i.e. *Megalancistrus* being sister to *Leporacanthicus* + *Pseudacanthicus*). See Table S7 (Supporting information) for results of our topology tests.

Relaxed clock and ancestral area estimation

The mean substitution rate across all loci estimated using BEAST was 0.29% per My. The subfamily Hypostominae was estimated by BEAST to have originated during the Eocene approximately 43.1 Mya (26.3–67.2 Mya 95% HPD; see Fig. 1 for molecular clock tree including outgroups) and inferred by Lagrange to have originated in the Amazon/Orinoco ecoregion (Region D). Modern Hypostominae species are distributed across all six ecoregions in Fig. 1.

The ancestor of all tribe Hypostomini genera in our analysis was also hypothesized to have originated in the Amazon/Orinoco ecoregion approximately 15.1 Mya (8.5–23.6 Mya 95% HPD), as was the genus *Hypostomus*. *Hypostomus* was hypothesized to have reached the upper Paraná basin (Region B) approximately 12.5 Mya (7.2–19.2 Mya 95% HPD). All together, ancestral *Hypostomus* lineages were hypothesized to have dispersed into new drainages several times from 12.5 Mya to the present (see Fig. 1 for all dispersal events), resulting in their modern distribution across all but two of the ecoregions in Fig. 1 (i.e. samples missing only from Region E: São Francisco basin plus North-eastern Brazilian drainages, and Region F: Pacific Coastal drainages plus Magdalena basin).

Speciation rates

The mean phylorate plot for speciation suggests that the tribe Hypostomini experienced consistently higher cladogenesis rates than any other lineage within Hypostominae (Fig. 2). Within Hypostomini, speciation rates were highest in the genus *Hypostomus*. Moreover, the consensus phylorate plot and marginal probabilities of rate shifts plot support shifts in speciation rate at the node and along the branch (respectively) giving rise to *Hypostomus*. Our speciation rate-through-time plot suggests that speciation rates throughout Hypostominae began a steady increase approximately 15 Mya, coincident with the origin of the genus *Hypostomus* (Fig. 2).

Morphometric diversification rates

The mean phylorate plot for morphometric diversification (Fig. 3) suggests that the tribe Hypostomini

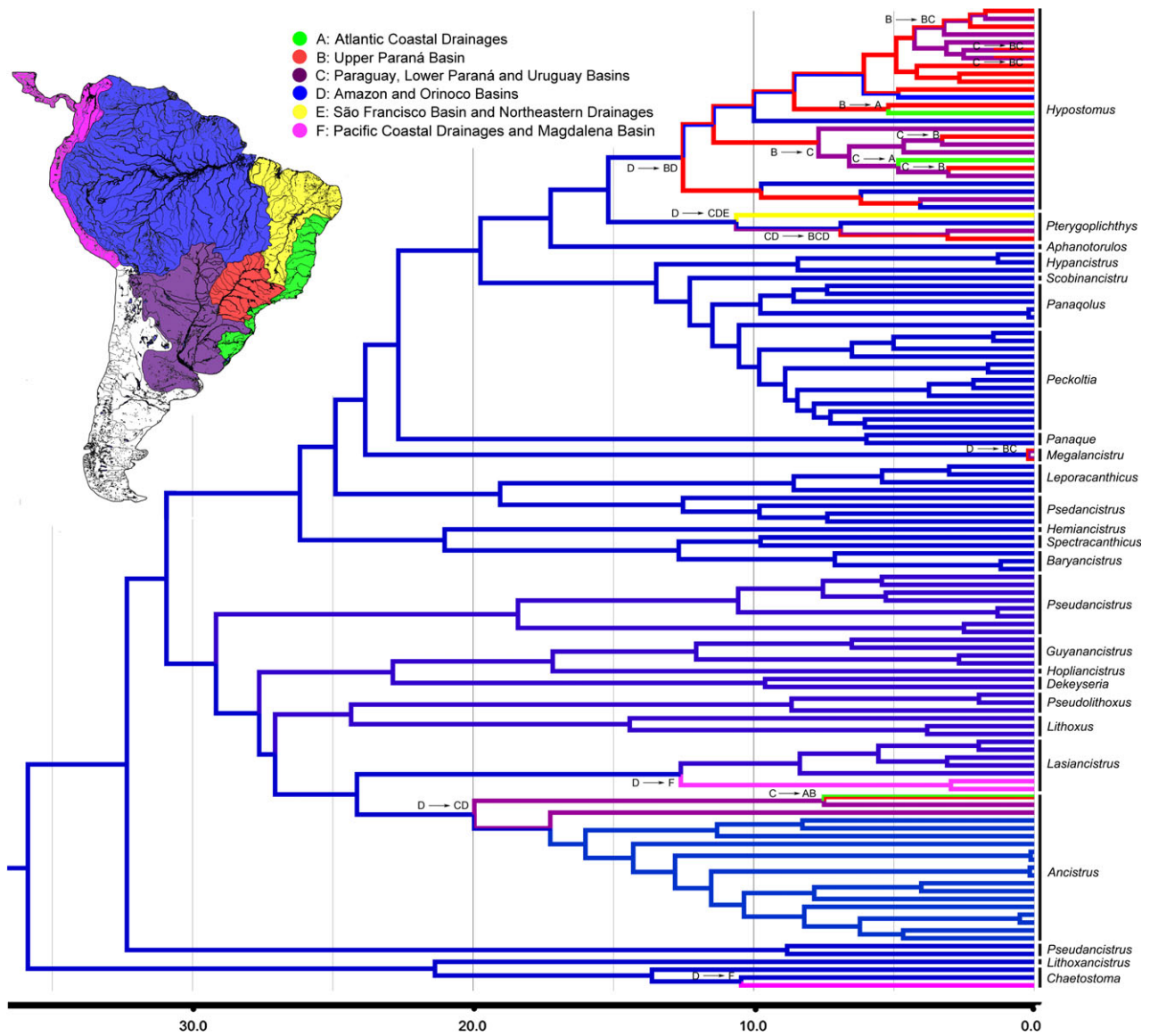


Fig. 1 Time-calibrated phylogeny for the loriciid catfish subfamily Hypostominae, illustrating ancestral range estimation and species range expansion through time. The chronogram was calibrated with a log-normal distribution offset to 55 Mya with a mean and standard deviation of 1 for the origin of the node including all Callichthyidae (*Corydoras imitator*, *C. oiapoquensis* and *Hoplosternum littorale*). The maximum-likelihood inference of geographical range evolution was performed using a dispersal–extinction–cladogenesis (DEC) model. The order of taxa in the phylogeny follows the order in Table S1–S2 and Figs S1–S4 (Supporting information).

experienced elevated rates of body shape diversification throughout its evolution, with highest rates observed along branches leading to members of the genus *Hypostomus*. Moreover, marginal probabilities of shifts in body shape diversification show high probabilities of shifts within the tribe Hypostomini, with shifts concentrated within *Hypostomus* (Fig. 3). The trait rate-through-time plot indicates that overall rates of Hypostominae body shape diversification increased slightly and steadily until approximately 5 Mya.

Body size diversification rates

Evolutionary rates of change in log maximum body size (MBS) were highest in a clade that included a diverse assemblage of Hypostominae genera (*Aphanotorulus*, *Hypancistrus*, *Hypostomus*, *Leporacanthicus*, *Megalancistrus*, *Panaqolus*, *Panaque*, *Peckoltia*, *Pterygoplichthys* and *Scobinancistrus*). Plots for both the consensus phylorate and marginal shift probabilities support a shift in body size diversification rate at the node giving rise to these genera (Fig. 4).

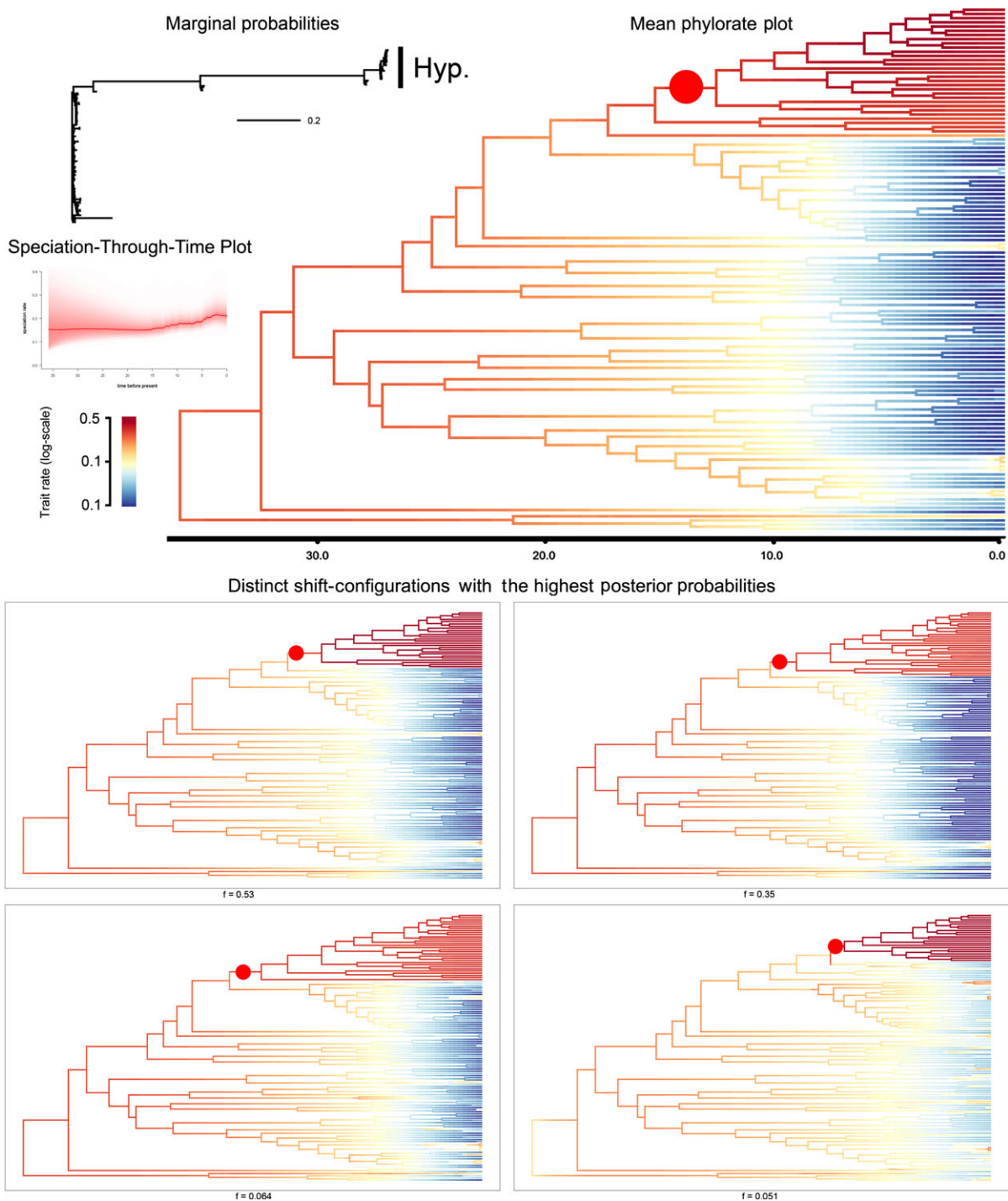


Fig. 2 Phylorate plots showing rates of cladogenesis in the subfamily Hypostominae. Colours along branches denote instantaneous rates (cool colours = slow, warm = fast). The large tree at top depicts the Mean Phylorate Plot, with colours indicating the mean evolutionary rate across all shift configurations sampled during simulation. The circle in this plot indicates the most frequent shift along all sampled trees of the Bayesian analysis. Smaller phylogenies at bottom show the Distinct Shift-Configurations with the Highest Posterior Probabilities. For each distinct shift configuration, the locations of rate shifts are shown as red circles (rate increases). Text labels denote the posterior probability of each shift configuration. The small Marginal Probabilities plot at top left shows rate shifts along all branches, with branch length scaled by the probability that it contains a shift event. The small Speciation-Through-Time Plot at left displays the cumulative speciation rate from the root of the tree to the present computed from the joint posterior density in BAMM. The order of taxa in the phylogeny follows the order in Table S1–S2 and Figs S1–S4 (Supporting information).

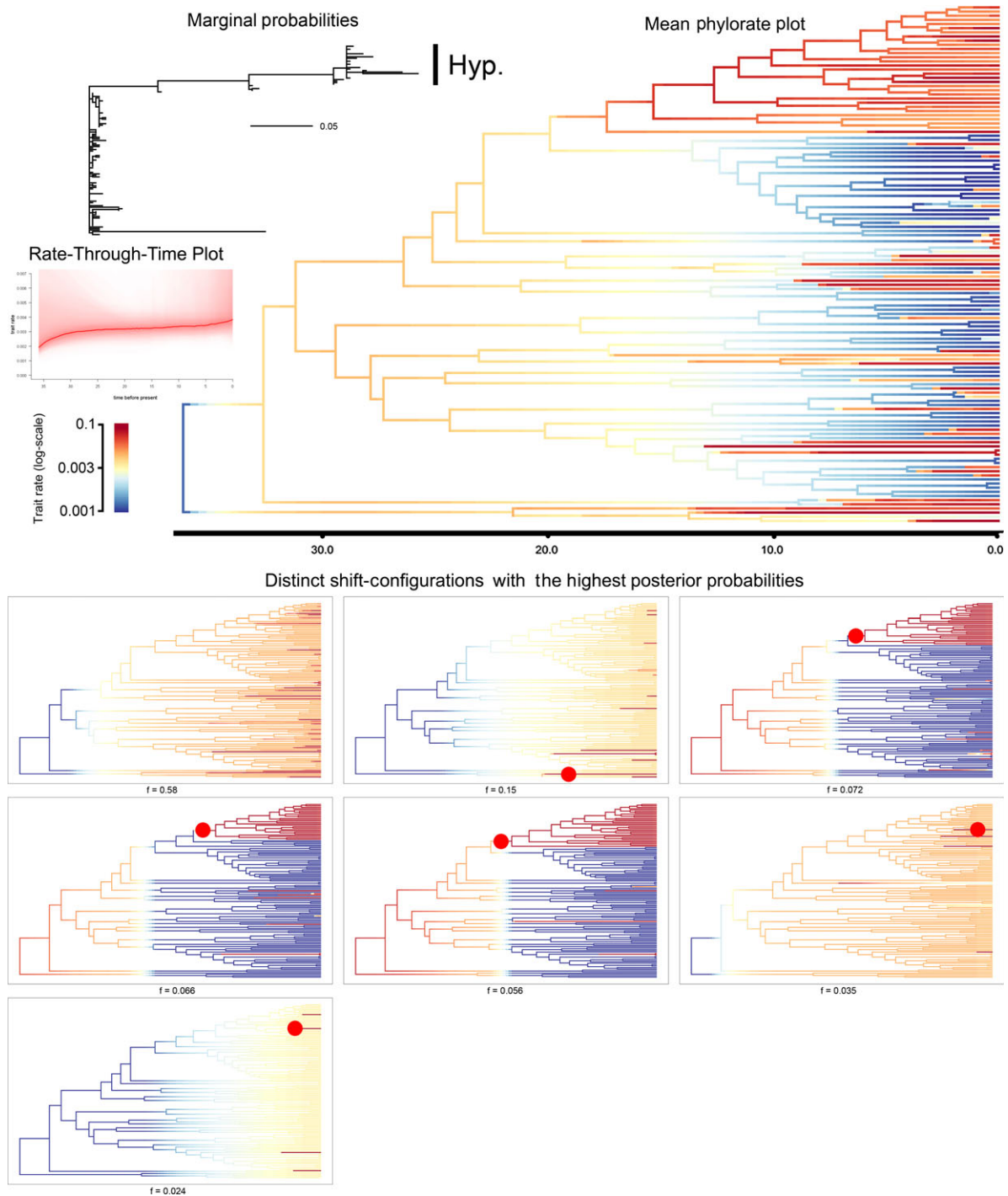


Fig. 3 Phylorate plots showing rates of body shape diversification in the subfamily Hypostominae. Colours along branches denote instantaneous rates (cool colours = slow, warm = fast). The large tree at top depicts the Mean Phylorate Plot, with colours indicating the mean evolutionary rate across all shift configurations sampled during simulation. The circle in this plot indicates the most frequent shift along all sampled trees of the Bayesian analysis. The smaller phylogenies at bottom show the Distinct Shift-Configurations with the Highest Posterior Probabilities. For each distinct shift configuration, the locations of rate shifts are shown as red circles (rate increases). Text labels denote the posterior probability of each shift configuration. The small Marginal Probabilities plot at top left shows rate shifts along all branches, with branch length scaled by the probability that it contains a shift event. The small Rate-Through-Time Plot at left displays the cumulative body shape diversification rate from the root of the tree to the present computed from the joint posterior density in BAMM. The order of taxa in the phylogeny follows the order in Table S1–S2 and Figs S1–S4 (Supporting information).

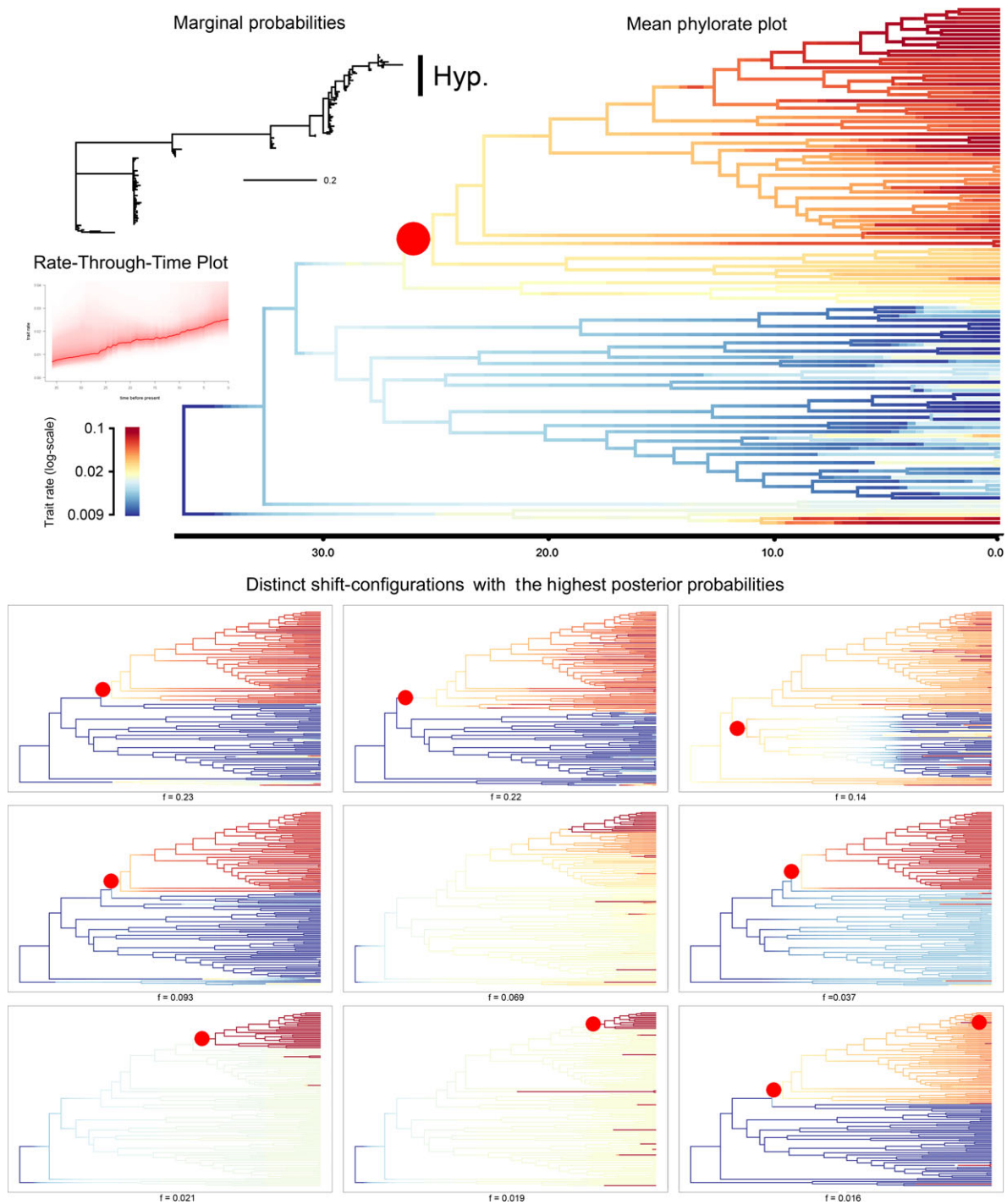


Fig. 4 Phylorate plots showing rates of log maximum body size (MBS) diversification for the subfamily Hypostominae. Colours along branches denote instantaneous rates (cool colours = slow, warm = fast). The large tree at top depicts the Mean Phylorate Plot, with colours indicating the mean evolutionary rate across all shift configurations sampled during simulation. The circle in this phylogeny indicates the most frequent shift along all sampled trees of the Bayesian analysis. The smaller phylogenies at bottom show the Distinct Shift-Configurations with the Highest Posterior Probabilities. For each distinct shift configuration, the locations of rate shifts are shown as red circles (rate increases). Text labels denote the posterior probability of each shift configuration. The small Marginal Probabilities phylogeny at top left shows rate shifts along all branches, with branch length scaled by the probability that it contains a shift event. The small Rate-Through-Time Plot at left displays the cumulative rate of body size diversification from the root of the tree to the present computed from the joint posterior density in BAMM. The order of taxa in the phylogeny follows the order in Table S1–S2 and Figs S1–S4 (Supporting information).

Ancestral habitat estimation

The stochastic character map analysis indicated that ancestors of the genus *Hypostomus* occupied a greater diversity of habits (i.e. channels, riffles, streams, and low and high elevations) than any other Hypostominae lineage (Fig. 5), whereas ancestors of the genus *Ancistrus* occupied the lowest diversity of habitats.

Morphological disparity

The first principal component (PC1) axis explained 27.5% of the variation for Hypostominae species (Fig. 6A) and 22.4% for *Hypostomus* species (Fig. 6B). Characters that loaded strongly on the first principal component for both Hypostominae and the genus *Hypostomus* were anal-fin spine length, mouth width, barbel length, dentary tooth cup length and premaxillary tooth cup length (Table S6). Characters that varied more in *Hypostomus* species than in Hypostominae as a whole were mostly related to the head (e.g. head–eye length, snout length and interorbital width) and fins (e.g. pectoral–spine length, pelvic–spine length, dorsal–pectoral distance, dorsal–spine length, adipose–spine length, dorsal–anal distance; Table S6). Within Hypostominae morphospace, most of the body size/PC1 range occupied by the genus *Hypostomus* overlapped the distributions of other genera (Fig. 6A), whereas within *Hypostomus*, the body size/PC1 area occupied by species from the Paraná River spanned most of the range for the entire genus (Fig. 6B).

Discussion

Macroevolutionary patterns in the suckermouth armored catfish subfamily Hypostominae indicate that, beginning approximately 12.5 Mya (95% HPD: 7.2–19.2 Mya; Fig. 1), the genus *Hypostomus* underwent transcontinental dispersal synchronized with accelerated rates of speciation (Fig. 2) and diversification in body shape (Figs 3, 6 and 7), body size (MBS, Fig. 4) and habitat diversity (Fig. 5). These and other lines of evidence (Cardoso *et al.* 2012) suggest that *Hypostomus*' accelerated diversification was at least partly a response to its repeated invasion of new drainages that likely had reduced predation and competition for food and habitat resources – that is ecological opportunity. Although specific linkages between organismal phenotype and either environment or ecological function remain tenuous at this time, our evidence for monophyly and accelerated diversification in *Hypostomus* suggest that such ecological opportunities promoted an adaptive radiation within a lineage that invaded the upper Paraná River basin in southeastern Brazil approximately 12.5 Mya (95% HPD: 7.2–19.2 Mya; Fig. 1).

The Paraná River is the third largest river in South America by discharge, after the Amazon and Orinoco, yet it is geographically isolated from the hydrologically contiguous Amazon/Orinoco basins to the northwest. It drains southward from highlands of the Brazilian Shield that separate it from the São Francisco drainage to the northeast and the Tocantins drainage to the northwest. Moreover, much of the Paraná basin was dominated by marine conditions as recently as the late Miocene (approx. 10 Mya; Lundberg *et al.* 1998). Due to its geographical remoteness and recent marine incursions, the Paraná River has likely long had a much more depauperate fish fauna than the Amazon. To this day, many of the largest and most distinctive Amazonian piscivores and herbivores are missing from the Paraná, including all osteoglossiform fishes (e.g. *Arapaima* and *Osteoglossum* spp.), the electric eel (*Electrophorus electricus*), the pacu (*Piaractus brachyomus*), as well as the manatee (*Trichechus inunguis*) and freshwater dolphins (*Inia* spp.). The peacock bass genus *Cichla*, which comprises some of the most ubiquitous and iconic piscivorous fishes throughout the Amazon/Orinoco ecoregion, has only recently been introduced to the Paraná by man (Kullander & Ferreira 2006).

The synchronized cladogenesis and geographical, ecological and morphological diversifications of *Hypostomus* across tropical South America, combined with this genus' accelerated diversification upon entering the species-poor Paraná River basin, are highly suggestive of ecological opportunity's influence on this clade's evolution. Historical contributions of ecological opportunity to evolutionary diversifications are often difficult to conclusively demonstrate, especially in large, continental radiations like the Hypostominae (Hughes & Eastwood 2006; Antonelli *et al.* 2009). However, both intrinsic (i.e. organismal) and extrinsic (i.e. environmental) drivers of ecological opportunity can be identified in *Hypostomus*, contributing to a mechanistic understanding of how this genus may have uniquely exploited ecological opportunities historically present in the Paraná River basin.

Intrinsic drivers of accelerated diversification: Evidence for key innovations

Loricariid catfishes are distinguished from all other fishes by having a ventral oral disc, diets consisting largely of algae and detritus, and bodies covered in bony plates and external teeth (i.e. odontodes). *Hypostomus* has no known anatomical traits that might give it a competitive advantage over other loricariids; however, *Hypostomus* species are known to have broad physiological tolerances for diverse diets and habitats. These tolerances may have facilitated their dispersal and been a

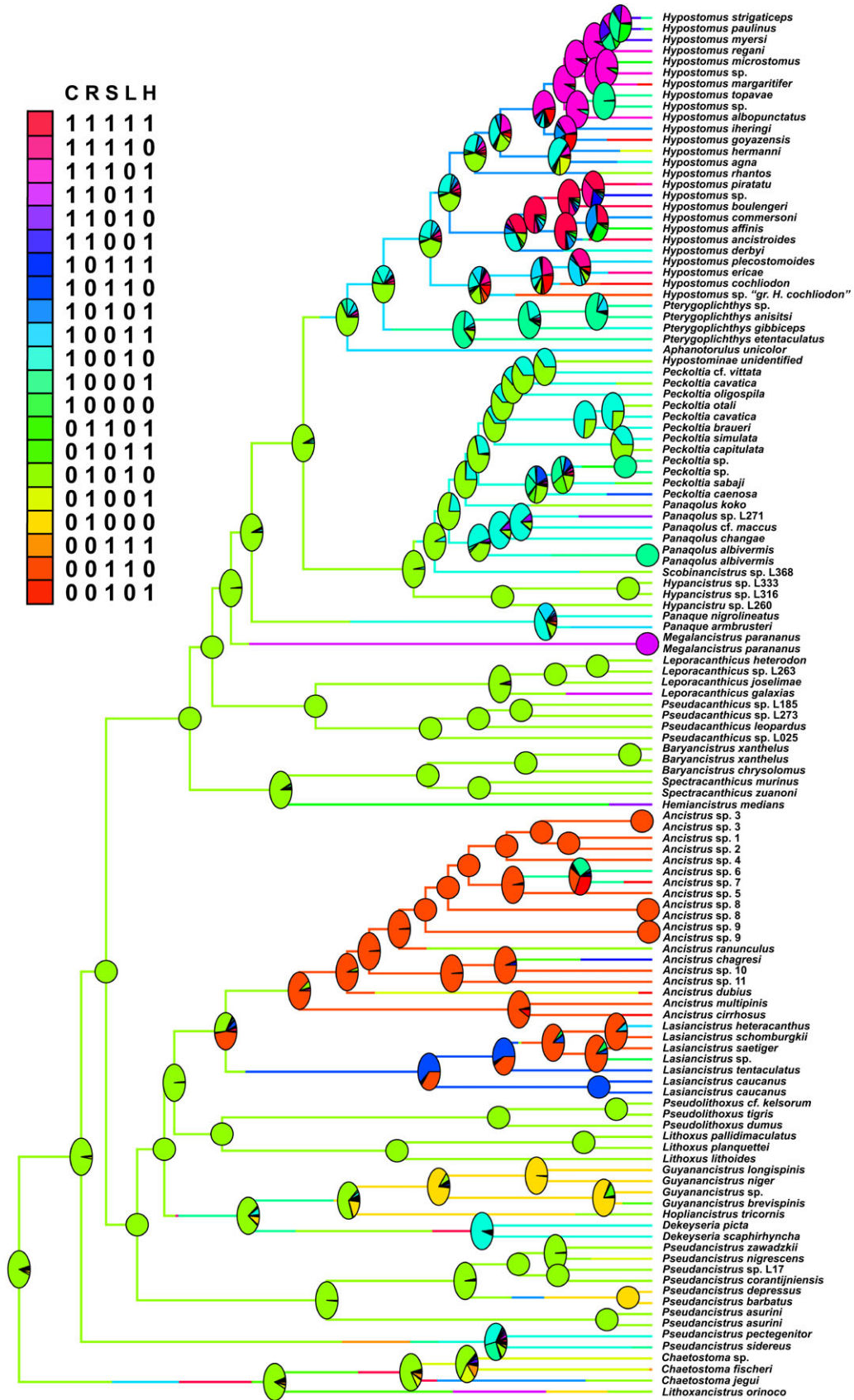


Fig. 5 Ancestral habitat estimates for the subfamily Hypostominae generated using SIMMAP (Bollback 2006). Habitat abbreviations are as follows: C = channel, R = rapids, S = stream, L = low altitude, H = high altitude (habitat characterizations originally proposed by Albert *et al.* 2011). Numbers indicate presence (1) or absence (0) of species in each habitat. Each colour indicates a unique combination of habitats occupied. The genus *Hypostomus*, for example, displays a wider range of unique combinations of habitats than any other genus.

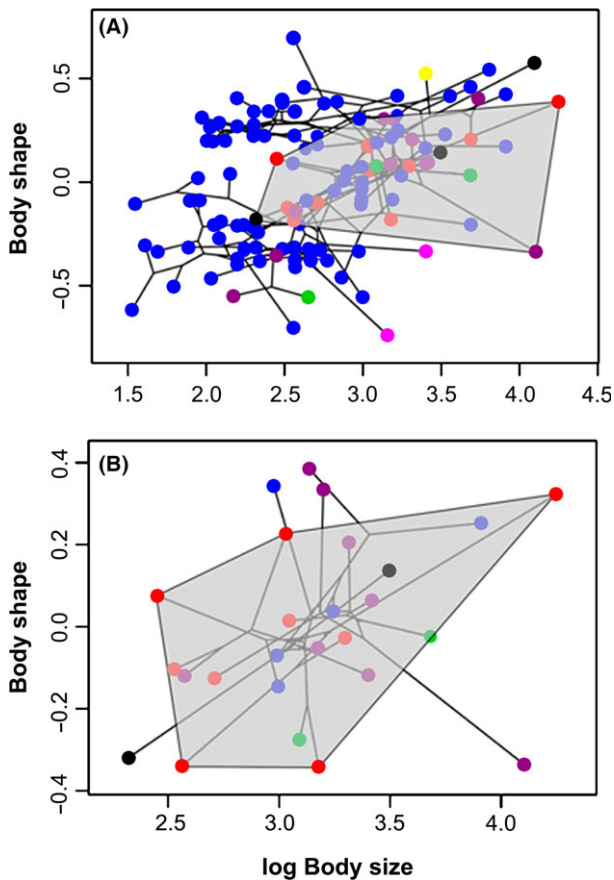


Fig. 6 Phylomorphospace plot for subfamily Hypostominae (A) and genus *Hypostomus* (B). Colours correspond to geographical area classifications used in the ancestral area estimation (Fig. 1). The *y*-axis is the first principal component (PC) from a size-corrected PC analysis of log-normalized linear distances between 31 homologous landmarks distributed across the head, body and fins (following landmarks originally proposed by Armbruster 2003a,b) and the *x*-axis is log maximum body size (MBS). Shaded convex hulls enclose all examined species of *Hypostomus* (A) and all examined species of *Hypostomus* from the Paraná River basin (B).

key to their exceptional invasion and diversification within the Paraná River basin.

In an evolutionary analysis of trophic diversity across 19 sympatric assemblages of loricariid catfishes, Lujan *et al.* (2012) found that *Hypostomus* species tended to specialize on the most nutrient-poor (i.e. protein depauperate or ^{15}N -depleted; Kelly & del Rio 2010) subsets of food resources being ingested and assimilated.

Likewise, in a detailed study of resource partitioning among sympatric wood-eating loricariids in northern Peru, Lujan *et al.* (2011) found that a wood-eating member of the genus *Hypostomus* ingested and assimilated the lowest protein (i.e. most ^{15}N -depleted) fraction of submerged woody detritus compared to two unrelated but also wood-eating loricariid genera (i.e. *Panaque* and *Panaqolus*).

The preference or tolerance of *Hypostomus* species for presumably nutrient-poor, low-protein subsets of the food spectrum available to loricariids is complemented by their occupation of a wide range of habitats. Not only are *Hypostomus* ubiquitous and often locally abundant in a wide range of habitats throughout their native range in tropical South America (Fig. 5; Saint-Paul *et al.* 2000), they are also among the most adept of all loricariid genera at invading novel habitats outside their native range. Invasive and often ecologically destructive populations of *Hypostomus* are now established in many drainages around the world, including rivers in Texas (Pound *et al.* 2011), Sri Lanka (Bambaradeniya 2002) and China (Xu *et al.* 2012). *Hypostomus* is one of only two loricariid genera to have broadly established invasive populations outside their native range, the other being the closely related genus *Pterygoplichthys* (Capps & Flecker 2013).

Together, the broad dietary and habitat tolerances of *Hypostomus* species indicate that, although this genus appears to lack key morphological innovations, it likely has key physiological adaptations that facilitate its invasion and survival in environments that are inhospitable to most other loricariids. However, more robust comparative analyses of physiological tolerances and dietary and habitat preferences across the Loricariidae are needed to identify specific traits that allow *Hypostomus* to be so successful.

Extrinsic drivers of accelerated diversification: Evidence for open ecological niches

One of the most distinctive and prominent events in the transcontinental dispersal of *Hypostomus* is the repeated invasion of the Paraná River basin in southeastern Brazil. Results of this and previous (Cardoso *et al.* 2012) studies support at least six independent invasions by ancestral lineages of *Hypostomus* in the upper Paraná (coloured red in Fig. 1; with one large clade occupying

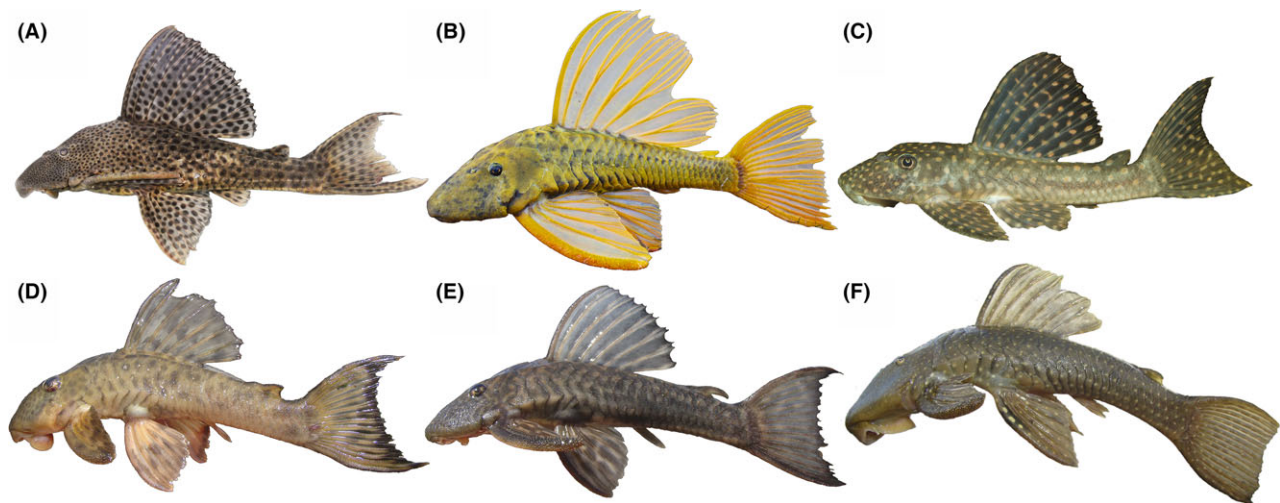


Fig. 7 Variation in colour patterns across the genus *Hypostomus*, with (A) *Hypostomus niceforoi* representing a typical Amazon basin species, and (B) *Hypostomus luteus* (Uruguay basin), (C) *Hypostomus* aff. *roseopunctatus* (Uruguay basin), (D) *Hypostomus hermanni* (rio Tietê basin), (E) *Hypostomus strigaticeps* (rio Tietê basin), and (F) *Hypostomus albopunctatus* (rio Paraná-Paraguay basin) representing diversity throughout the Paraná River basin. Photograph A by NKL, D, E and F by CHZ, B and C by O. Lucanus.

the upper Paraná basin almost exclusively); four invasions into the Paraguay, lower Paraná and Uruguay basins (coloured purple in Fig. 1); and two invasions into Atlantic Coastal drainages (coloured green in Fig. 1). To this day, *Hypostomus* is one of only four Hypostominae genera to have successfully invaded the Paraná River basin, resulting in 52 species, or approximately 37% of all *Hypostomus* species, being present there today. The only other Hypostominae taxa now in the Paraná are 13 species of *Ancistrus*, three species of *Pterygoplichthys* and one species of *Megalancistrus* – each of which occupies a much narrower range of habitats than *Hypostomus* (Fig. 5). The first two of these genera share with *Hypostomus* a preference for or tolerance of ^{15}N -depleted food resources (Lujan *et al.* 2012). *Ancistrus*, though, is phylogenetically distantly related to *Hypostomus*, as is *Megalancistrus* (Lujan *et al.* 2015b).

The only other loricariid genera present in the Paraná basin include two genera of the morphologically distinct whiptail catfish subfamily Loricariinae (*Rineloricaria* with 21 species in Paraná basin and *Harttia* with two species in Paraná basin), and a total of 32 much smaller-bodied species (<10 cm SL) in the subfamily Hypoptopomatinae (*sensu* Lujan *et al.* 2015b; e.g. *Hisonotus*, 17 spp.; *Neoplecostomus*, eight spp.; *Otothyropsis*, four spp.; *Pareiorhina*, two spp.; *Pseudotocinclus*, one sp.; Roxo *et al.* 2012, 2014). There is no fossil or other evidence to suggest that any other loricariid genera occupied this watershed prior to *Hypostomus*.

In contrast to the Paraná Basin, the Amazon and Orinoco basins host diverse assemblages of over 30 Hypostominae genera (Lujan *et al.* 2015b), most of which

overlap *Hypostomus* in morphospace (Fig. 6). Given the highly distinctive ecological niche occupied by loricariids as a whole (i.e. benthic, surface-scraping, algivore–detritivores), the very few other similar loricariid species present in the Paraná, and this drainage's distinctive history of biogeographical isolation and marine incursion, it becomes increasingly plausible that when *Hypostomus* first invaded the Paraná River basin, it experienced at least an initial reduction in competitive pressure compared with other parts of its range. Of course, in response to *Hypostomus*' adaptive diversification and consequent saturation of available niches, much of the ecological opportunity historically present in the Paraná is predicted to have been reduced over time (Simpson 1949, 1953).

Linking phenotype to environment and ecological function: Evidence for adaptive radiation in Hypostomus of the Paraná River basin

A hallmark of any adaptive radiation (e.g. African cichlids, Caribbean anoles, Hawaiian silverswords) is tremendous phenotypic diversity as compared with closely related lineages (Rabosky *et al.* 2013; Brawand *et al.* 2014). This disparity is echoed in species of *Hypostomus* from the Paraná River basin, which exhibit as great a range of body shapes and sizes (Fig. 6) and a greater range of colour patterns (Fig. 7) and jaw and tooth morphologies (authors' pers. obs.) than congeners throughout the remainder of the genus' range. Moreover, many morphometric traits that are highly variable across Hypostominae, such as mouth width and premaxillary

and dentary tooth cup lengths (Table S6, Supporting information), are known to be functionally relevant to the distinctive feeding biomechanics of loricariids (Lujan & Armbruster 2012).

Most Amazonian *Hypostomus* exhibit minor variations on a colour pattern consisting of black or dark brown spots on a light brown base colour (Fig. 7A) and exhibit one of only two distinctive ecomorphotypes representative of reciprocally monophyletic subgenera (Lujan *et al.* 2015b). The wood-eater morphotype (subgenus *Cochliodon* or the *Hypostomus cochliodon* group) has a tall head and supraoccipital crest and acute rows of few, large and spoon-shaped teeth. The algivorous morphotype (subgenus *Hypostomus* or the *Hypostomus plecostomus* group) has a depressed head and oblique rows of many, small, villiform teeth. In contrast to bimodal Amazonian diversity, *Hypostomus* in the Paraná basin exhibit a broader and more continuous range of body, jaw and tooth shapes. In addition to both the Amazonian ecomorphotypes, the Paraná basin hosts many distinctive ecomorphotypes not seen elsewhere. These include dorsoventrally depressed species with white polka dots on a dark base colour (e.g. *Hypostomus albopunctatus*, *H. roseopunctatus*; Fig. 7C and F), longitudinally truncate species with angled jaws and few but small, villiform teeth (e.g. *H. peckoltoides*, *H. margaritifera* and *H. microstomus*; not illustrated), and a large-finned species with a stunning yellow-gold base colour (e.g. *H. luteus*; Fig. 7B). Because some of the most distinctive yet rare Paraná basin *Hypostomus* species (e.g. *H. peckoltoides*) were omitted from this study, our results showing an acceleration in body shape diversification (Fig. 3) and broad morphometric diversity (Fig. 6) in Paraná River *Hypostomus* likely underestimate the true diversity of this radiation.

Schluter (2000) proposed four prerequisites to adaptive radiation: (i) common ancestry, (ii) accelerated diversification, (iii) phenotype–environment correlation and (iv) trait utility. Given the monophyly and accelerated diversification in species of *Hypostomus* of the upper Paraná River basin, and a consistent pattern in other loricariid studies of correlation between phenotypic characters examined herein and both environment and ecological function (Casatti & Castro 2006; Lujan & Armbruster 2012; Lujan *et al.* 2012), we find the support for adaptive radiation in *Hypostomus* from the upper Paraná River basin to be compelling. However, specific environmental and functional data from upper Paraná River basin *Hypostomus* are still needed and are the subject of ongoing research by ourselves and collaborators.

Conclusion

There are few clear examples of evolutionary diversification, let alone adaptive radiation, in response to

ecological opportunity either in river systems or across continents. The most convincing examples remain those on islands (e.g. birds and lizards, Losos & Ricklefs 2009), in lakes (e.g. African cichlid fishes, Kocher 2004) or at high mountain elevations (e.g. Andean *Lupinus* plants, Hughes & Eastwood 2006), where relatively recent dates of origin for entire ecosystems can be established and rates of speciation and diversification are often striking in an absolute sense. In contrast, the geologic and hydrologic history of the upper Paraná River basin is poorly resolved, and accelerated speciation and diversification rates of *Hypostomus* are striking in a more relative sense. The diversification of *Hypostomus* in the upper Paraná River basin has occurred over a time frame on the order of 10–15 Myr vs. only 100 000 years for African cichlids of Lake Victoria (reference), 1–2 Myr for Andean *Lupinus* (Hughes & Eastwood 2006) and 5 Myr for cichlids of Lake Malawi (reference) and Hawaiian silverswords (Baldwin & Sanderson 1998). Evidence nonetheless remains strong for a single *Hypostomus* lineage having invaded the upper Paraná River basin and experienced accelerated cladogenesis and morphological diversification in response. Moreover, the current and likely historical absence in the Paraná of many competitors and predators that are diverse and abundant in the Amazonian drainages where *Hypostomus* first originated suggests that ecological opportunity played a role in facilitating this accelerated evolution.

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G.S.C.S., F.F.R., N.K.L. and C.O. conceived the ideas. F.F.R. and G.J.C.S. collected the data. F.F.R. and V.A.T. analysed the data. G.S.C.S., F.F.R. and N.K.L. led the writing, and G.S.C.S. and C.H.Z. identified all samples.

Data accessibility

DNA sequences: GenBank Accession nos at Table S1 (Supporting information).
 Habitat and Ecological information: available at Table S2 (Supporting information).
 Morphological characters and time-calibrated tree available at Dryad (doi: 10.5061/dryad.c8q11).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. Taxa list, collection vouchers and accessing numbers for sequence data analyzed in the present study ($n = 157$).

Table S2. Species included in the present study with habitat and Maximum Body Size (MBS) classifications for the internal group.

Table S3. Primers used in the present study to amplify partial sequences of F-ribulose 1,6-bisphosphate carboxylase, 16S rRNA, cytochrome oxidase subunit I (COI) and cytochrome B (CytB).

Table S4. Nucleotide substitution models for each partition evaluated in the software PartitionFinder (Lanfear *et al.* 2012) and used in the phylogenetic analyses.

Table S5. DEC models tested to estimate distribution ranges inherited by the descending lineages at each node of the tree.

Table S6. Variable loadings in the first Principal Component Analysis (PCA 1) for body shape of combined samples of Hypostominae and for the genus *Hypostomus*.

Table S7. Likelihood-based tests for alternative topologies.

Fig. S1. Tree showing relationships between the Hypostominae, and other loricariid subfamilies (Delturinae, Loricariinae, Otothyriinae, Neoplecostominae, Hypoptopomatinae), Clade A and B) obtained by maximum likelihood and Bayesian analyses.

Fig. S2. Tree showing the relationships among species of the *Ancistrus*, *Lithoxus*, *Dekeyseria* and *Pseudancistrus* clades obtained by maximum likelihood and Bayesian analyses.

Fig. S3. Tree showing relationships among species of the *Hemiancistrus*, *Leporacanthicus*, *Megalancistrus*, *Panaque* and *Peckoltia* clades obtained by maximum likelihood and Bayesian analyses.

Fig. S4. Tree showing relationships among species of the *Hypostomus* clade obtained by maximum likelihood and Bayesian analyses.

Description of a new catfish genus (Siluriformes, Loricariidae) from the Tocantins River basin in central Brazil, with comments on the historical zoogeography of the new taxon

Gabriel S. C. Silva¹, Fábio F. Roxo¹, Luz E. Ochoa¹, Claudio Oliveira¹

¹ *Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, IBB–UNESP, Campus de Botucatu, 18618–970, Botucatu, São Paulo state, Brazil*

Corresponding author: *Fábio F. Roxo* (roxoff@hotmail.com.br)

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Abstract

This study presents the description of a new genus of the catfish subfamily Neoplecostominae from the Tocantins River basin. It can be distinguished from other neoplecostomine genera by the presence of (1) three hypertrophied bicuspid odontodes on the lateral portion of the body (character apparently present in mature males); (2) a large area without odontodes around the snout; (3) a post-dorsal ridge on the caudal peduncle; (4) a straight tooth series in the dentary and premaxillary rows; (5) the absence of abdominal plates; (6) a conspicuous series of enlarged papillae just posterior to the dentary teeth; and (7) caudal peduncle ellipsoid in cross section. We used maximum likelihood and Bayesian methods to estimate a time-calibrated tree with the published data on 116 loricariid species using one nuclear and three mitochondrial genes, and we used parametric biogeographic analyses (DEC and DECj models) to estimate ancestral geographic ranges and to infer the colonization routes of the new genus and the other neoplecostomines in the Tocantins River and the hydrographic systems of southeastern Brazil. Our phylogenetic results indicate that the new genus and species is a sister taxon of all the other members of the Neoplecostominae, originating during the Eocene at 47.5 Mya (32.7–64.5 Mya 95% HPD). The present distribution of the new genus and other neoplecostomines may be the result of a historical connection between the drainage basins of the Paraguay and Paraná rivers and the Amazon basin, mainly through headwater captures.

Keywords

Molecular phylogeny, Freshwater fishes, headwater capture, catfish, taxonomy

Introduction

The Loricariidae, an endemic Neotropical family of freshwater fish, is the largest group of catfish, with about 900 valid species (Eschmeyer and Fong 2015). Within the Loricariidae, the subfamily Neoplecostominae has a long complex taxonomic and systematic history, with a number of major morphological and molecular studies being conducted since the nineteenth century (e.g. Eigenmann and Eigenmann 1890; Regan 1904; Gosline 1947; Isbrücker 1980; Howes 1983; Schaefer 1987; Montoya-Burgos et al. 1998; Armbruster 2004; Chiachio et al. 2008; Roxo et al. 2012a, 2014).

The neoplecostomines are small-bodied catfishes which were, until now, restricted to southern and southeastern Brazil, where they are found in small- to medium-sized streams with clear and shallow water, of up to 1 m in depth (Langeani 1990). Previous studies (e.g. Chiachio et al. 2008; Roxo et al. 2012a, 2014) concluded that the considerable diversity of this subfamily can be accounted for primarily by the geomorphological processes (i.e. tectonics and erosion) that have shaped the South American continent over the past 100 Mya, influencing fish distribution and speciation patterns (Ribeiro 2006; Albert and Reis 2011). In this context, one of the principal processes is river capture (also known as stream capture or headwater capture), an important landscape-level mechanism that can isolate lineages and promote diversification (Waters et al. 2006; Winemiller et al. 2008; Albert and Crampton 2010) by changing the connectivity of adjacent river basins (Smith 1981; Hocutt and Wiley 1986; Mayden 1988; Lundberg et al. 1998). The consequences of this process for the local fauna can be profound, changing watershed boundaries and allowing previously isolated species to disperse and colonize new environments (Grant et al. 2007; Muneeppeerakul et al. 2008; Bertuzzo et al. 2009).

Here, we recognize a new genus and species of neoplecostomine catfish based on specimens collected during a recent expedition to the Tocantins River basin in Goiás state, Brazil. The new taxon is described in detail below.

Material and methods**Morphological analysis**

Body plate nomenclature follows Schaefer (1997) and measurements, Armbruster (2003), except for the dorsal-adipose distance, adipose-spine length, dorsal adipose-caudal distance, ventral adipose-caudal distance, adipose-anal distance and mouth width. Measurements and counts were taken on the left side of the specimens and were taken point to point, to the nearest 0.1 mm with digital calipers. Specimens were

cleared and stained (c&s) according to the method of Taylor and Van Dyke (1985). Dorsal fin ray counts include the spinelet as the first unbranched ray. Counts of vertebrae include the five vertebrae that comprise the Weberian apparatus, while the compound caudal centrum (PU1 + U1) was counted as a single element. Zoological nomenclature follows the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999).

Molecular analysis

Taxon sampling

The molecular analysis included 157 specimens representing 116 loricariid species (115 species from the study of Roxo et al. [2014], and one sample of the new genus, see Suppl. material 1 for all taxa). *Diplomystes mesembrinus* (Ringuelet, 1982) was used as the outgroup to root all phylogenies (Arratia 1987; de Pinna 1993, 1998; Grande 1987; Grande and de Pinna 1998; Mo 1991; Sullivan et al. 2006). Samples of *Corydoras imitator* Nijssen & Isbrücker, 1983, *Corydoras oiapoquensis* Nijssen, 1972, *Hoplosternum littorale* (Hancock, 1828), *Callichthys callichthys* (Linnaeus, 1758), *Astroblepus* spp. 1 and 2, *Hemipsilichthys gobio* (Lütken, 1874), *H. papillatus* Pereira, Oliveira & Oyakawa, 2000, *Delturus parahybae* Eigenmann & Eigenmann, 1889b, *Rineloricaria lanceolata* (Günther, 1868), *Spatuloricaria* sp. 1, *Hypostomus ancistroides* (Ihering, 1911), *H. nigromaculatus* (Schubart 1964) and *H. microstomus* Weber, 1987 were also included in the analysis as outgroups.

Vouchers of the samples were those catalogued by Roxo et al. (2014), except for the samples of the new genus, which was deposited in the collection of Auburn University Natural History Museum (AUM), Auburn; Laboratório de Biologia e Genética de Peixes (LBP), Botucatu; and Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo.

DNA extraction and sequencing

Total DNA was extracted from muscle samples collected from two specimens of the new genus preserved in ethanol using the protocol described by Aljanabi and Martinez (1997). Partial sequences for two genes, Cytochrome B (CytB), forward 5'-CCA TCC AAC ATC TCA GCA TGA TGA AA 3', reverse 5'-AAC CTC CGA TCT TCG GAT TAC AAG AC 3' (Oliveira et al. 2011), and 16S rRNA, forward 5'-ACG CCT GTT TAT CAA AAA CAT-3', reverse 5'-CCG GTC TGA ACT CAG ATC ACG T-3' (Kocher et al. 1989) were amplified by polymerase chain reaction (PCR). The amplification was conducted in a total volume of 12.5 µl with 1.25 µl of 10 X buffer (10 mM Tris-HCl+15 mM MgCl₂), 0.5 µl of the dNTPs (200 nM of each), 0.5 µl of each 5 mM primer, 0.05 µl of platinum Taq polymerase (Invitrogen), 1 µl of template

DNA (12 ng), and 8.7 μ l of dd H₂O. The PCR reactions consisted of 30–40 cycles, 30 s at 95 °C, 15–30 s at 48–58 °C, and 45–90 s at 72 °C. All the PCR products were first identified visually on a 1% agarose gel and then purified using ExoSap-IT (USB Corporation) following the manufacturer's instructions. The purified PCR products were sequenced using the Big Dye™ Terminator v 3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems), purified by ethanol precipitation and loaded into a 3130-Genetic Analyzer automatic sequencer (Applied Biosystems).

Sequencing and phylogenetic analysis

The individual sequences of each species were initially analyzed in the BioEdit 5.0.9 software (Hall 1999), and a consensus sequence was obtained for each DNA segment. The sequences were then aligned in MUSCLE (Edgar 2004) using the default parameters, and inspected visually. To evaluate the saturation of the matrix by substitution, we calculated the index of substitution saturation (Iss), as described by Xia et al. (2003) and Xia and Lemey (2009), and the transition/transversion rate, in DAMBE 5.2.31 (Xia and Xie 2001). The Iss was calculated without taking gaps into account.

Maximum likelihood analyses were run in RAxML Web-Servers (Stamatakis et al. 2008). Bootstrap (BS) resampling (Felsenstein 1985) was used to evaluate the support for each node, based on 1000 replicates. Random starting trees were used for each independent ML tree search, while all other parameters were set at the default values. The ML analyses were based on the GTR model.

Time calibration and estimates of ancestral ranges

The uncorrelated relaxed (lognormal) molecular clock was calibrated using BEAST v.1.7.5. All clade-age estimates are presented as the mean and 95% highest posterior density (HPD) values. We included two calibration points to constrain the divergence dates for the 157 clades identified in our phylogenetic tree. The first calibration point was implemented as a normally-distributed prior, with an offset of 125 million years ago (Mya), and a standard deviation of 15 million years. Data from the stratigraphic record and the geographic distribution of living taxa indicate that the Siluriformes originated during the Lower Cretaceous (145–100 Mya; Lundberg 1993; Sullivan et al. 2006; Lundberg et al. 2007).

The second calibration point was implemented using a log-normal prior set at 55 Mya, with a mean and standard deviation of 1 for the origin of the family Callichthyidae. The oldest known callichthyid fossil, *Corydoras revelatus* Cockerell (1925) was dated to the Paleocene by Marshall et al. (1997), assuming 55 Mya as a minimum age. We used a macroevolutionary Birth–Death model to estimate diversification likelihood values, with a starting tree obtained from the RAxML analysis. These analyses were conducted under the GTR model. The ML tree obtained in this analysis was used as a starting tree for the MCMC searches. This analysis was run for 100 million generations and sampled every

10,000th generation. Stationarity and the sufficient mixing of parameters ($ESS > 200$) were verified using Tracer v1.5 (Rambaut and Drummond 2007a). A consensus tree was built in TreeAnnotator v1.7.5 (Rambaut and Drummond 2007b).

Data on the geographic distribution of the species in each of the three subfamilies analyzed here (Hypoptopomatinae, Neoplecostominae and Otothyriinae) were obtained from the original species descriptions and the catalog of Eschmeyer (2015), with the species classification following Roxo et al. (2014). Species ranges are located within five biogeographic regions: A, Drainage basins of the Atlantic coast of southeastern Brazil; B, Upper Paraná basin; C, Paraguay, Lower Paraná and Uruguay basins; D, Amazon and Orinoco basins; E, São Francisco basin and the coastal drainage basins of northeastern Brazil.

We estimated the likelihood of ancestral range evolution using the Dispersal-Extinction-Cladogenesis (DEC: Ree and Smith 2008) and jumping (DECj: Matzke 2013a) models of species range evolution. These models are composed of two (DEC) or three (DECj) parameters including: 1) dispersal (D), where ancestral ranges expand by adding new geographic units, 2) extinction (E), where ancestral ranges are reduced by extirpating geographic units, and 3) jumping events (j), where j specifies the weight of the jumping events beyond an ancestral range (Matzke 2014). The two models of range evolution (i.e. DEC and DECj) were implemented in the R package BioGeoBEARS (Matzke 2013b). The global likelihood of the six biogeographic scenarios found using the two models (i.e. DEC and DEC+J models) were compared using the Akaike Information Criterion (AIC) (Akaike 1973) (Suppl. material 2). The model that obtained the lowest AIC values was model 2 with the DEC+J model (M2 – DEC + J), which constrained the dispersal rates between adjacent areas at 1.0 and areas separated by one or more intercalated areas at 0.5.

Results

Microplecostomus gen. n.

<http://zoobank.org/077BD513-6BF2-47D9-AB4C-4A496FE33115>

Figs 1, 6

Type species. *Microplecostomus forestii* sp. n.

Diagnosis. The new genus and species differs from all members of the Loricariidae by having (1) three hypertrophied bicuspid odontodes on the lateral portion of the body (character apparently present only in mature males – observed in the holotype, but not present in the paratypes) (Fig. 2a, b); and differs from all members of the Neoplecostominae by having (2) a large area without odontodes around the snout, observed in all specimens, Fig. 3 (*vs.* margin of snout bearing odontodes); and from all members of the Neoplecostominae, except *Hirtella carinata* Pereira, Zanata, Cetra & Reis, 2014, *Pareiorhina carrancas* Bockmann & Ribeiro, 2003 and *Pareiorhina hypiorhachis* Silva, Roxo & Oliveira, 2013 by (3) the presence of a post-dorsal ridge on



Figure 1. *Microplecostomus forestii* sp. n., MZUSP 118673, holotype, male, 38.3 mm SL, Goiás state, Brazil, Tocantins River basin.

the caudal peduncle, see dorsal view of holotype in Figs 1, 4 (*vs.* the absence of a post-dorsal ridge). *Microplecostomus forestii* sp. n. differs from species of the genera *Isbrueckerichthys*, *Neoplecostomus* and *Pseudotocinclus* by (4) the absence of abdominal plates, Fig. 1 (*vs.* abdomen covered by pentagonal or hexagonal platelets); from *Kronichthys* by having (5) the tooth series in dentary and premaxillary rows straight (*vs.* tooth series strongly curved medially); from *Neoplecostomus* by (6) the absence of a conspicuous

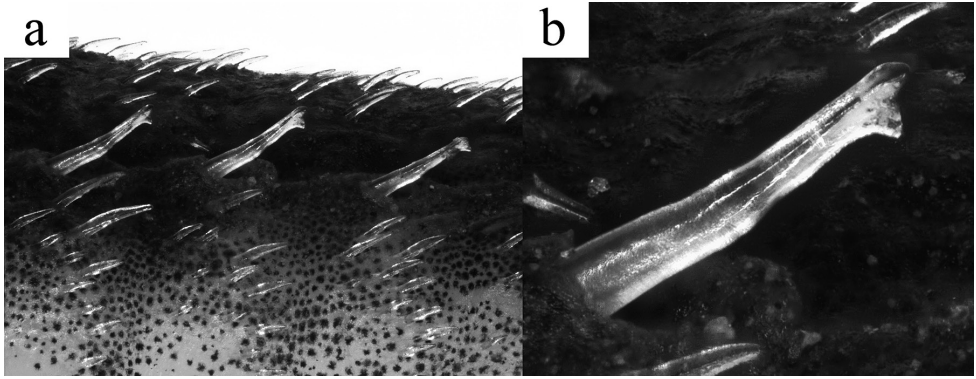


Figure 2. Photographs showing **a** the three hypertrophied bicuspid odontodes on the lateral portion of the body of the holotype of *Microplecostomus forestii* sp. n., MZUSP 118673; **b** Detail of the hypertrophied bicuspid odontodes.

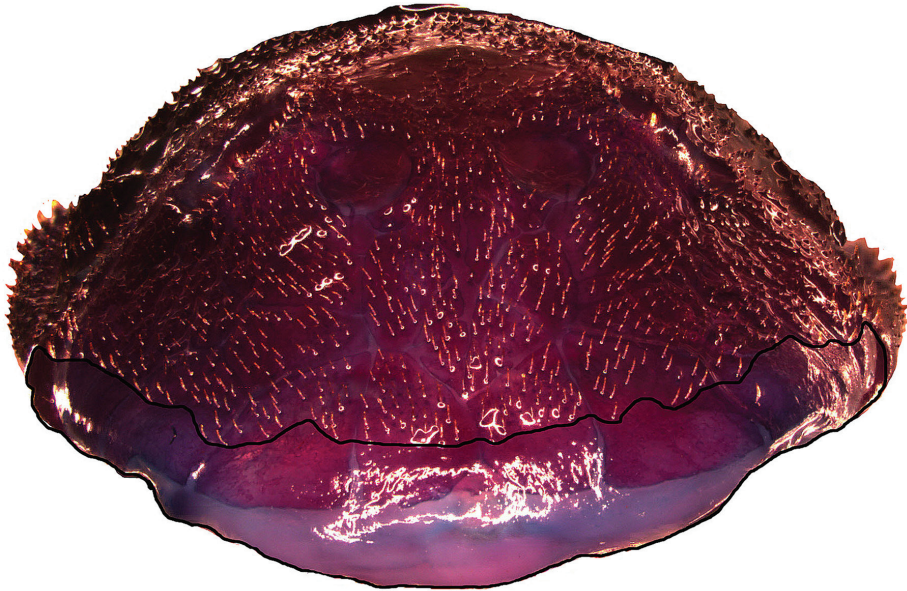


Figure 3. *Microplecostomus forestii* sp. n. showing a large area without odontodes around the snout, LBP 19017, 29.0 mm SL.

series of enlarged papillae just posterior to the dentary teeth (*vs.* presence of enlarged papillae); and from *Pseudotocinclus* by having (7) the caudal peduncle ellipsoid in cross section (*vs.* caudal peduncle square in cross-section).

Etymology. The generic name is a combination of Greek, *micro* (mikrós) = small, related to the small size of the adult type-specimen, and *plecostomus* = a former generic name of species currently included in Loricariidae, also in reference to the small adult size of the type-species. A masculine name.

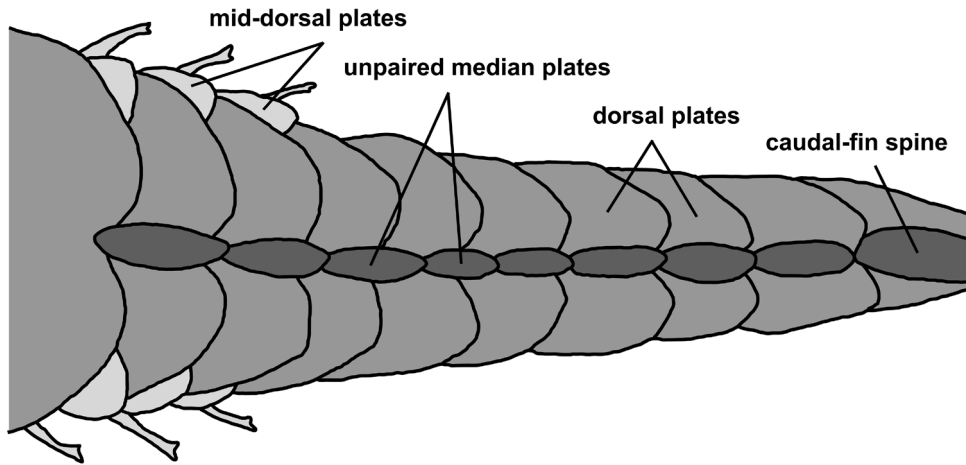


Figure 4. Dorsal view of the caudal peduncle in *Microplecostomus forestii* sp. n., LBP 19017, 29.0 mm SL, showing the presence of a series of unpaired median plates that form a post-dorsal ridge.

***Microplecostomus forestii* sp. n.**

<http://zoobank.org/2A1A0D93-ED90-4C5F-9562-D3209D951630>

Figs 1, 6; Table 1

Holotype. MZUSP 118673 (adult male, 38.3 mm SL), Brazil, Goiás state, municipality of São João D’Aliança, Roncador Stream, a tributary of das Brancas Stream, tributary of the Tocantzinho River, Tocantins River basin, 14°53’47.2”S, 47°34’58.4”W, 9 November 2014, FF Roxo, GSC Silva, LEO Ochoa, LH Roxo.

Paratypes. All from Brazil, Goiás state, Tocantins River basin (15 specimens). AUM 67015, 1, 29.4 mm SL, municipality of Água Fria de Goiás, córrego das Brancas, tributary of rio Tocantzinho, 14°53’47.2”S, 47°34’58.4”W, 9 November 2014, FF Roxo, GSC Silva, LEO Ochoa, LH Roxo. LBP 17318, 2, 24.2–30.3 mm SL, municipality of São João D’Aliança, Roncador Stream, a tributary of das Brancas Stream, 14°43’51.3”S, 47°32’34.0”W, 21 November 2012, BF Melo, GSC Silva, JHM Martinez, R Devidé. LBP 19000, 2, 29.8–32.2 mm SL, collected with the holotype. LBP 19017, 1, 24.8 mm SL, 1 c&s 29.0 mm SL, municipality of Água Fria de Goiás, das Brancas Stream, a tributary of the Tocantzinho River, 14°53’47.2”S, 47°34’58.4”W, 30 June 2014, FF Roxo, GSC Silva, LE Ochoa. LBP 19319, 3, 24.4–28.4 mm SL, municipality of Água Fria de Goiás, das Brancas Stream, tributary of the Tocantzinho River, 14°53’47.2”S, 47°34’58.4”W, 16 August 2014, BF Melo, C Oliveira, GSC Silva, MI Taylor. LBP 19467, 2, 27.6–28.4 mm SL, municipality of Água Fria de Goiás, das Brancas Stream, a tributary of the Tocantzinho River, 14°53’47.2”S, 47°34’58.4”W, 9 November 2014, FF Roxo, GSC Silva, LEO Ochoa, LH Roxo. LBP 19468, 1, 27.7 mm SL, municipality of São João D’Aliança, Roncador Stream, a tributary of das Brancas Stream, 14°43’51.3”S, 47°32’34.0”W, 9 November 2014, FF Roxo, GSC Silva, LE Ochoa, LH Roxo. MZUSP 113919, 2, 21.7–25.0 mm SL, municipality of Água Fria

de Goiás, das Brancas Stream, a tributary of the Tocantinzinho River, 14°53'47.2"S, 47°34'58.4"W, 27 November 2012, AM Zanata, P Camelier, M Melo, OT Oyakawa.

Diagnosis. Same as for the genus.

Description. Morphometric and meristic data in Table 1. In lateral view, dorsal profile of head strongly convex from snout tip to distal margin of supraoccipital; straight from supraoccipital to dorsal-fin origin; concave and slightly decreasing to end of caudal peduncle. Ventral surface of body, slightly concave at head, straight to convex from posterior end of head to pelvic-fin insertion, and straight but angled to posterior caudal peduncle. Snout tip rounded in dorsal view. Nostril small. Trunk and caudal peduncle rectangular in cross-section. Greatest body depth at dorsal-fin origin. Body progressively narrowing posteriorly from cleithrum to caudal peduncle. Head flat to slightly convex between orbits; superior margin of orbits elevated. Head lacking crests. Head and body plates covered with minute, uniformly sized and evenly distributed odontodes. Head with large area without odontodes around snout. Eye small, situated dorsolaterally just posterior of midpoint.

Tip of snout formed by two triangle rostral plates, without odontodes. Nasal plates almost rectangular forming medial nostril margin and contacting pre-nasals anteriorly. Nasal plates posteriorly contacting frontal bones. Lateral margin of head formed by four or five postrostral plates. Complete infraorbital plate series composed of five plates; all infraorbital plates containing latero-sensory canals; first and second infraorbitals largest and third, fourth and fifth smallest. Preopercle elongate, bearing a branch of laterosensory canal. Subocular cheek plates present ventral to preopercle plate. Top of head composed of compound pterotic, supraoccipital, prefrontal, frontal, and sphenotic (Fig. 5); compound pterotic as with fenestrae irregularly distributed and with different sizes and shapes. Anterior margin of mesethmoid pointed and projected anteriorly to condyle.

Lateral ethmoid exposed without odontodes in dorsal view. Lateral ethmoid strut short and broad, nasal capsule partially closed, lateral ethmoid surrounding more than 50% of nasal capsule. Compound pterotic roughly quadrangular, without posterior process, with several fenestrae non-uniform in shape and size. Parieto-supraoccipital not contributing to dorsal portion of swimbladder capsule. Metapterygoid channel present. Hyomandibular square and not sutured to compound pterotic, hyomandibular adductor palatine crest present. Quadrangle triangle. Lips large; oral disk rounded and papillose. Premaxillary teeth 44–65 (mode 46). Dentary teeth 45–69 (mode 48). Teeth bicuspid. Maxillar barbel short. Upper pharyngeal tooth-plate small and triangular. Five ceratobranchials with accessory process present and long. Five teeth in ceratobranchial. Four branchiostegal rays.

Dorsal-fin rays II,7; dorsal-fin originating at vertical through posterior end of pelvic-fin base; distal margin slightly convex; dorsal-fin spinelet short and oval in shape. Pectoral-fin rays I,6; distal margin slightly convex; unbranched pectoral-fin ray reaching pelvic-fin origin; unbranched pectoral-fin ray covered with large and pointed odontodes. Pectoral girdle not exposed ventrally. Arrector fossae, partially enclosed by ventral lamina of coracoids, opening relatively large, extending laterally towards base

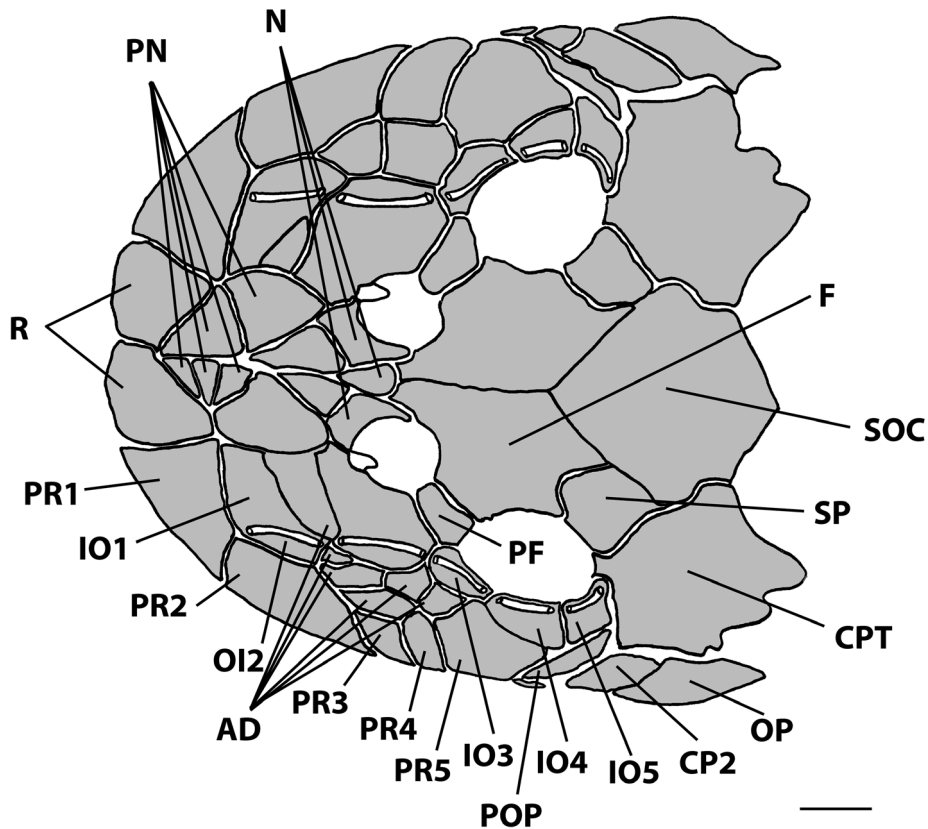


Figure 5. Dorsal view of the head plates in *Microplecostomus forestii* sp. n., LBP 19017, 29.0 mm SL. CPT = compound pterotic; F = frontal; IO1-5 = infraorbitals; N = nasal; OP = opercle; PR1-4 postrostral plates; PF = prefrontal; PN = prenasal; POP = preopercle; R = rostral plate; SP = sphenotic; CP2 = subocular cheek plate; SOC = parieto-supraoccipital; AD = additional plates.

of pectoral fin. Pelvic-fin rays I,5; distal margin of fin slightly convex; tip of adpressed pelvic-fin almost reaching anal-fin origin; unbranched pelvic-fin ray covered with conspicuously pointed, and uniformly distributed odontodes, larger at ventral portion. Pelvic girdle with slender lateropterigium. Basipterygium lacking anterior fenestrae. Anal-fin rays I,5; distal margin slightly convex. Adipose-fin absent. Caudal-fin rays I,7-7,I, truncated with ventral unbranched principal ray longer than dorsal ray.

Compound hypurals 1 and 2 almost completely fused to compound hypurals 3-5, and lower and upper halves fused to last vertebra. Upper and lower lobes of hypural plates of same length. Epural present and separated from hypural plate. Body entirely covered by bony plates, except for ventral surface of head, abdomen and region between compound pterotic and first medial plate. Dorsal series of plates 22-23, mid-dorsal 4-7, median perforated plates 22-23, mid-ventral 11, and ventral 18-20. Trunk with conspicuous, elongated, post-dorsal ridge formed by 14-15 raised, unpaired, median



Figure 6. *Microplecostomus forestii* sp. n., live specimen, LBP 19319, paratype, 28.4 mm SL, Tocantins River, Goiás state, Brazil. Photograph: MI Taylor.

Table 1. Morphometric data for *Microplecostomus forestii* sp. n. SD = standard deviation.

	<i>Microplecostomus forestii</i> sp. n., 15 paratypes and the holotype			
	Holotype	Range	Mean	SD
SL	38.3	21.7–38.3	27.9	–
Percentage of SL				
Predorsal length	45.5	44.5–50.8	47.9	1.8
Head length	34.9	34.5–39.9	37.4	1.5
Head-dorsal length	12.5	10.0–13.5	11.4	1.1
Cleithral width	32.5	31.2–35.2	33.3	1.0
Head-pectoral length	29.7	22.4–32.7	30.1	2.4
Thorax length	19.1	17.1–20.5	19.1	1.0
Pectoral-spine length	19.9	19.2–25.3	21.4	1.7
Abdominal length	21.8	19.4–24.3	21.8	1.2
Pelvic-spine length	20.8	17.0–22.3	20.3	1.6
Post-anal length	34.2	31.8–34.9	33.2	0.9
Anal-fin spine length	12.2	10.6–13.6	12.1	0.8
Dorsal-pectoral distance	25.7	25.3–34.5	28.1	2.2
Dorsal spine length	19.4	18.2–23.0	20.9	1.4
Dorsal-pelvic distance	20.2	16.8–22.3	20.1	1.6
Dorsal-fin base length	18.5	15.1–19.4	17.6	1.2
Caudal peduncle depth	9.5	8.1–10.5	9.6	0.6
Dorsal-anal distance	13.6	13.6–16.8	15.0	1.0
Pelvic-dorsal distance	22.5	20.3–25.7	23.2	1.6
Percentage of HL				
Head-eye length	32.3	30.9–36.7	33.9	1.9
Orbital diameter	15.8	13.2–17.2	15.1	1.2
Snout length	61.8	52.9–61.8	57.8	2.8
Internares width	17.0	14.8–19.2	16.7	1.2
Interorbital width	31.5	28.8–34.3	32.1	1.5
Head depth	58.8	55.8–66.6	61.1	2.6
Mouth length	55.8	45.6–66.9	58.7	5.8
Barbel length	4.8	1.2–5.5	3.2	1.2
Dentary tooth cup length	24.2	20.1–27.0	23.3	1.6
Premaxillary tooth cup length	21.7	18.3–25.2	23.3	1.8

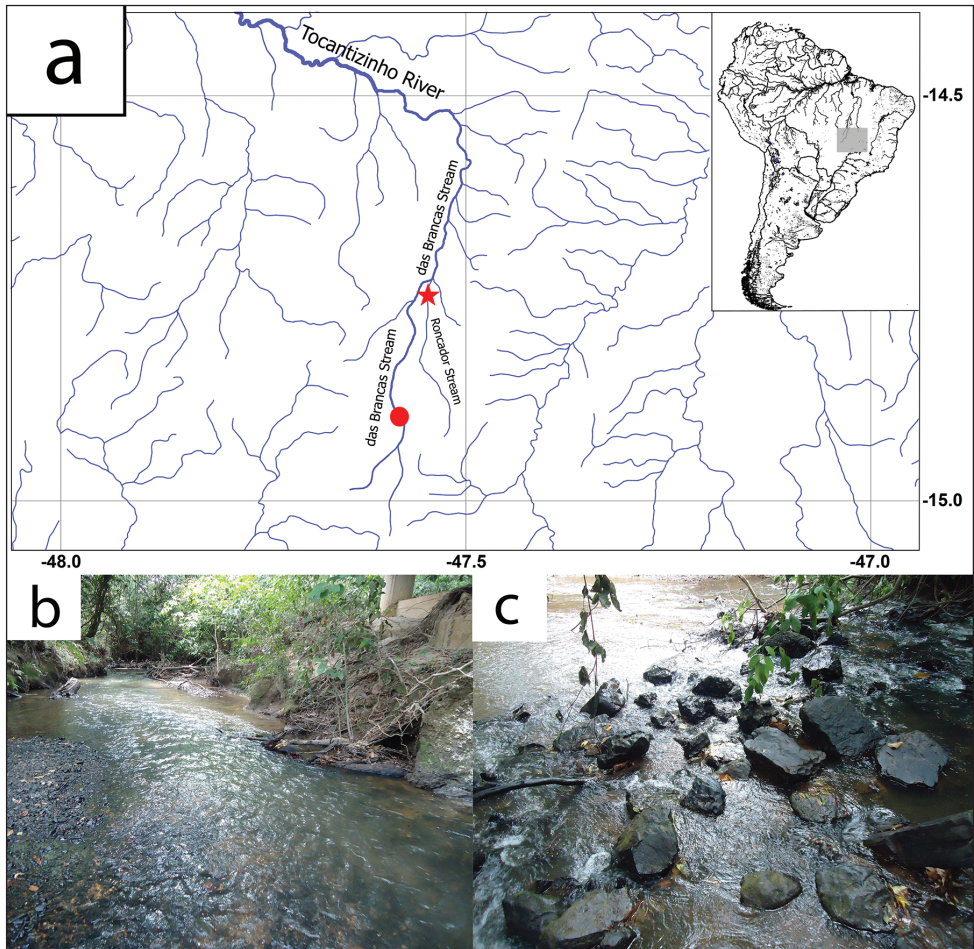


Figure 7. **a** Map showing the distribution of *Microplecostomus forestii* sp. n. Type locality at Roncador Stream, red star – $14^{\circ}43'51.3''\text{S}$, $47^{\circ}32'34.0''\text{W}$. Paratype locality at das Brancas Stream, red circle – $14^{\circ}53'47.2''\text{S}$, $47^{\circ}34'58.4''\text{W}$. Habitat where *Microplecostomus forestii* sp. n. is found at **b** Roncador Stream and **c** das Brancas Stream. These are small size streams with a depth of less than 1 m, clear water, the bottom covered with loose stones and shaded margins. Photographs: LH Roxo.

plates; ridge continuous posteriorly with procurrent caudal-fin rays. Six pairs of ribs associated with vertebrae 7–13. Ribs slender and poorly ossified. Total vertebrae 27.

Color in life. Background color of dorsal and ventral surfaces of body yellowish tan. Dorsal surface of head dark brown. Four dark brown saddles on dorsal surface of trunk, most anterior inconspicuous and below dorsal-fin origin, second below end of dorsal-fin, third typically in adipose-fin region, and fourth at end of caudal peduncle. Lateral portion of body with inconspicuous dark stripe from head to caudal fin. Pectoral, pelvic and dorsal fins with three irregular, poorly defined bands. Caudal fin with variegated blotches (Fig. 6).

Color in alcohol. Similar to pattern described for living individuals, but with darker brown color, and darker saddles and stripes (Fig. 1).

Sexual dimorphism. Specimens lacking main sexual dimorphic characters usually present in loricariid species, particularly in Neoplecostominae members, such as (1) a papilla present posteriorly to urogenital opening; (2) an expanded flap skin on dorsal surface of first pelvic-fin ray; and (3) a larger pelvic-fin and body size (all characters present in males), but absent in females. Three hypertrophied bicuspid odontodes are present on lateral portion of body (a characteristic that may be related to mature males), however it is only present in holotype.

Etymology. The specific name, *forestii*, is given in honor of Fausto Foresti, Professor of the university of São Paulo state “Júlio de Mesquita Filho” (Unesp) in Brazil, for his contributions to fish genetics, with more than 250 papers published in this field.

Distribution. *Microplecostomus forestii* sp. n. is known from two localities, the Roncador Stream and the das Brancas Stream, both tributaries of the Tocantinzinho River, in the Tocantins basin (Fig. 7a).

Habitat. *Microplecostomus forestii* sp. n. was collected in shallow, clear waters of about 0.5 m in depth and fast-flowing currents, with an underlying substrate of rock, in areas of flat terrain. The fishes captured were associated with pebbles (Fig. 7b, c). This species is relatively hard to collect and is not abundant. In seven expeditions to the Roncador and das Brancas streams in different periods of the year, we were able to collect only 16 specimens. *Microplecostomus forestii* sp. n. is sympatric with species such as *Creagrutus* sp., *Rhinolekos capetinga* Roxo, Ochoa, Silva & Oliveira, 2015, *Hypostomus* sp., *Phenacorhamdia* sp., *Ancistrus* sp., and *Ituglanis* sp.

Sequencing and phylogenetic analysis

The sequences of all 157 specimens are shown in Suppl. material 1 (the same list of species presented by Roxo et al. 2014, but with the inclusion of the voucher and GenBank accession numbers for the specimens of the newly described genus). The concatenated dataset resulted in a matrix of 4,102 base pairs (bps), used in all the phylogenetic and biogeographic analyses, of which 1,361 bps were conserved and 2,657 bps were variable. There was no evidence of saturation in these data, considering that the Iss.c value is higher than the Iss, and the R^2 value is higher than 0.8 for transitions and transversions, for the concatenated matrix.

Our results are very similar to those of Roxo et al. (2014), in particular, that the Hypoptopomatinae, Neoplecostominae and Otothyriinae clades are monophyletic (Figs 8–9) with strong statistical support (BS = 99 for Hypoptopomatinae; BS = 80 for Neoplecostominae; BS = 84 for Otothyriinae), that the Neoplecostominae is more closely related to the Otothyriinae than to the Hypoptopomatinae (BS = 95), and that these two clades together form the sister group of the Hypoptopomatinae (BS = 96). The new genus *Microplecostomus forestii* sp. n. was placed in the subfamily Neoplecostominae (Fig. 8), forming a sister group with all its members, with strong statistical support (BS = 80).

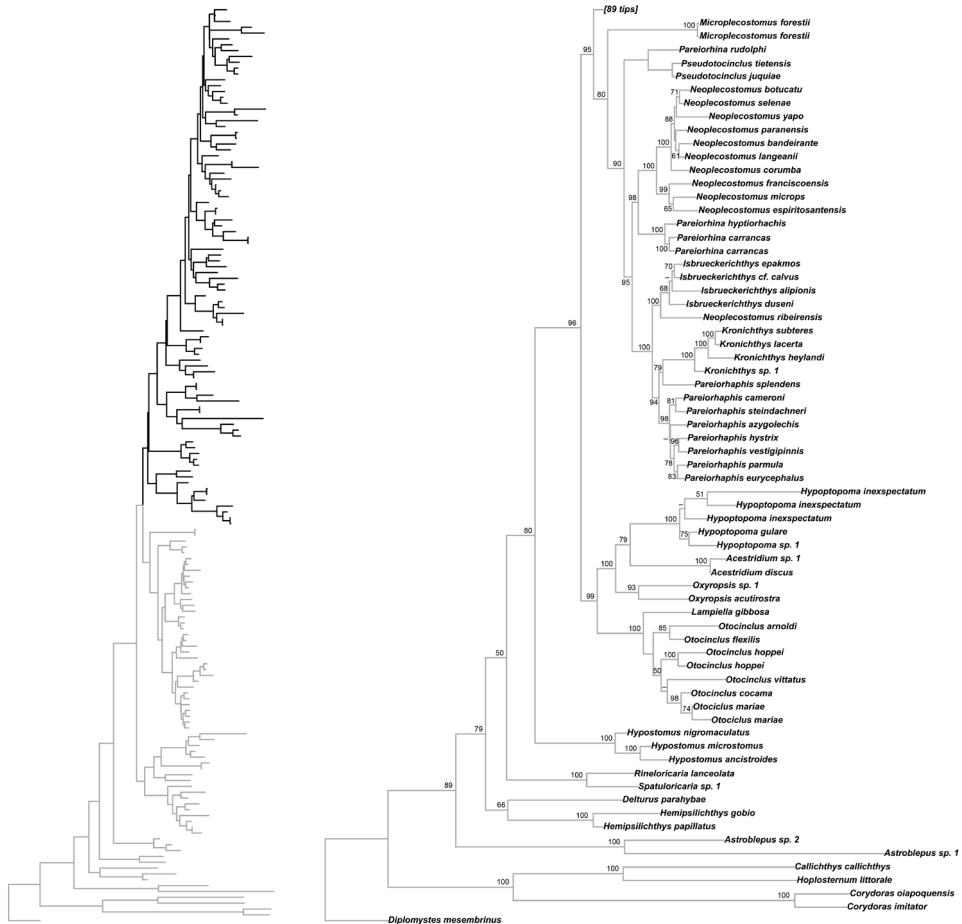


Figure 8. Partial ML tree showing the relationship among the species of the subfamilies Hypoptopomatinae and Neoplecostominae. Numbers above the branches are bootstrap values from 1000 bootstrap pseudoreplicates obtained from the ML analysis. Bootstrap values below 50% (-) are not shown.

Time calibrated tree and historical biogeography

Our time-calibrated tree estimated that the origin of the hypoptopomatine lineage was in the Paleocene, about 63.1 Mya (44.5–83.8 Mya 95% HPD), and is inferred by the DEC+J model to have been located in areas A (Atlantic Coast drainage basins) + D (Amazon and Orinoco basins) (Fig. 10 Region AD). The clade composed of the Neoplecostominae (Fig. 10 Region AD) + Othothyriinae (Fig. 11 Region AD) is estimated by BEAST to have also originated during the Paleocene, about 59.1 Mya (41.4–77.6 Mya 95% HPD), and once again, according to the DEC+J model, in areas A and D. *Microplecostomus forestii* sp. n. is found in the headwaters of the Tocantins River, one of the principal rivers of the Amazon basin (Area D). Our time-calibrated phylogeny estimated that the lineage of the new genus and species arose during the Eocene, 47.5 Mya (32.7–64.5 Mya 95% HPD).



Figure 9. Partial ML tree showing the relationship among the species of the subfamily Otothyriinae. Numbers above the branches are bootstrap values from 1000 bootstrap pseudoreplicates obtained from the ML analysis. Bootstrap values below 50% (-) are not shown.

Discussion

Taxonomy and phylogenetic relationship

The results of our molecular analyses indicated that *Microplecostomus forestii* sp. n. is the sister-group of all the other members of the Neoplecostominae (Fig. 8), with strong statistical support. *Microplecostomus forestii* sp. n. is distinguished from all other

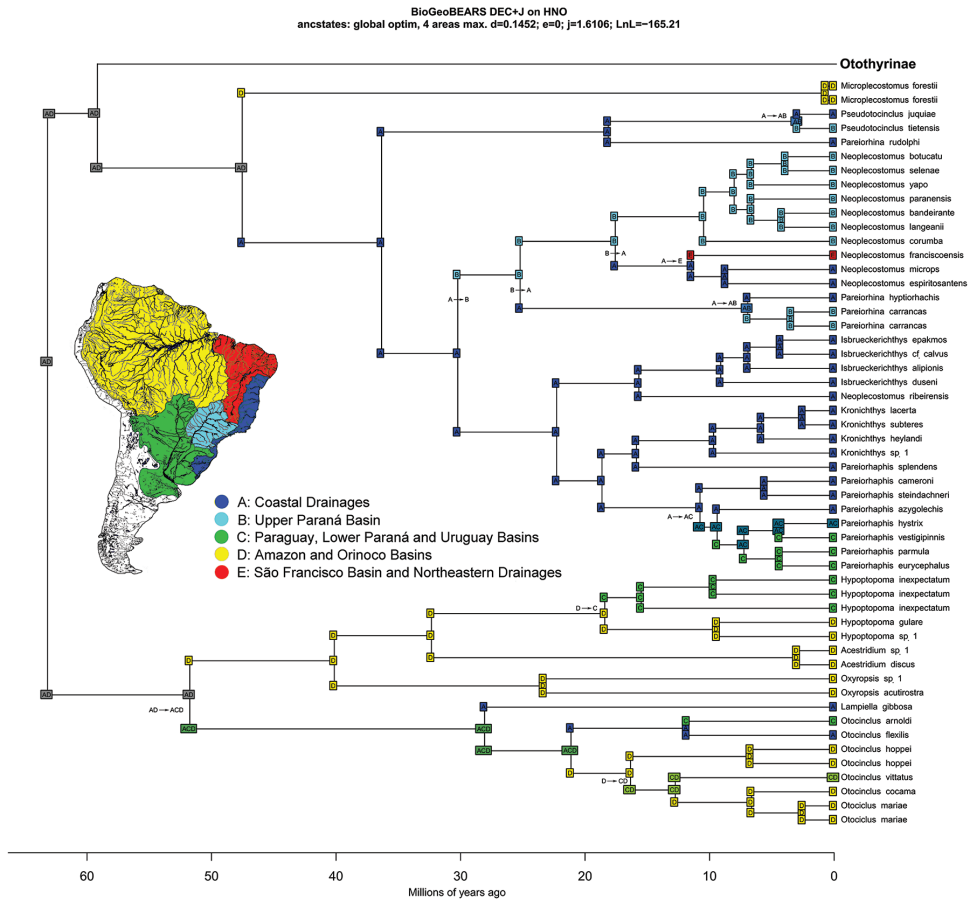


Figure 10. Time-calibrated phylogeny and ancestral range estimates for the Hypoptopomatinae and Neoplecostominae. Divergence ages were calibrated by the origins of the Siluriformes (120 Mya) and the Callichthyidae (55 Mya).

neoplecostomine species by the presence of two autapomorphic characters: (1) three hypertrophied bicuspid odontodes on the lateral portion of the body and (2) a large area without odontodes around the snout, observed in all specimens. Only one (the holotype) of the 16 specimens analyzed presented the three hypertrophied bicuspid odontodes on the lateral portion of the body. We believe that this is a sexually dimorphic character found only in large mature males, although this remains uncertain because the new taxon does not have any other obvious sexual dimorphism, and the trait is only present in the holotype, which cannot be dissected. Sexual dimorphism is very common in other neoplecostomines, in particular species of the genera *Neoplecostomus* (Langeani 1990; Zawadzki et al. 2008; Roxo et al. 2012b) and *Pareiorhaphis* (Pereira and Reis 2002; Pereira 2005). As in the present study, these species have been diagnosed primarily on the basis of the characteristics of the mature males.

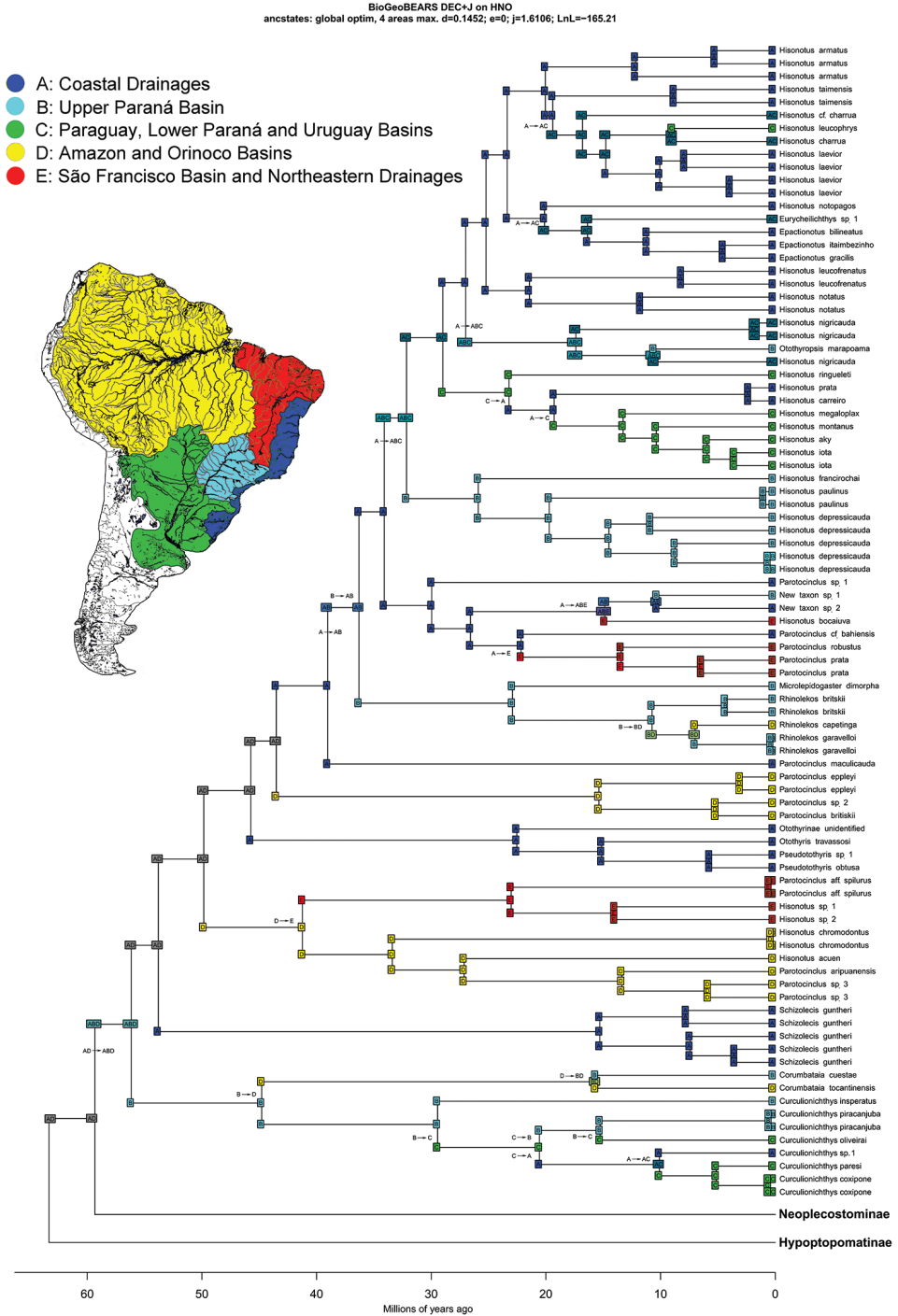


Figure II. Time-calibrated phylogeny and ancestral range estimates for the Otothyridae. Divergence ages were calibrated by the origins of the Siluriformes (120 Mya) and the Callichthyidae (55 Mya).

Another character used to distinguish *Microplecostomus forestii* sp. n. from other neoplecostomines is the presence of a post-dorsal ridge on the caudal peduncle. Bockmann and Ribeiro (2003) were the first authors to report this character in a neoplecostomine species (*Pareiorhina carrancas*), and Silva et al. (2013) also reported the structure in a second new species of the same genus, *P. hyptiorhachis*. This character is also present in species of *Corymbophanes* (Armbruster et al. 2000), a genus assigned to the subfamily Hypostominae, which is found throughout the Potaro River in Guyana, northern South America. Considering that these species are not closely related (Armbruster 2004; Roxo et al. 2014), the presence of a post-dorsal ridge appears to have arisen more than once during the evolution of these species.

The subfamily Neoplecostominae, as defined by Roxo et al. (2014), appears to be monophyletic and forms a sister group to the Otothyriinae, which together form a sister group to the Hypoptopomatinae (this relationship was first reported by Chiachio et al. 2008). The main deviation that we found from the arrangement proposed by Roxo et al. (2014) is in the relationship among the members of the genus *Hisonotus*. In the present study, the species *H. depressicauda* (Miranda Ribeiro, 1918b), *H. francirochai* (Ihering, 1928) and *H. paulinus* (Regan, 1908) appeared as a monophyletic group which includes most of the *Hisonotus* species found in southern Brazil, the type species of *Hisonotus* (i.e. *H. notatus* Eigenmann & Eigenmann, 1889), *Otothyropsis marapoama* Ribeiro, Carvalho & Melo, 2005, *Eurycheilichthys* and *Epactionotus*. In Roxo et al. (2014) *Hisonotus depressicauda*, *H. francirochai* and *H. paulinus* were not closely related to the former species, but formed a clade with *Parotocinclus* and *Hisonotus* species from the São Francisco River basin, albeit with reduced statistical support (see Fig. 4 in Roxo et al. 2014).

Historical biogeography

Using the DEC model to estimate ancestral species ranges, Roxo et al. (2014) suggested that the ancestral lineages of the Hypoptopomatinae, Neoplecostominae and Otothyriinae subfamilies (the HNO-Clade) originated on the Atlantic Coast of southeastern Brazil (area A, see Fig. 5 in Roxo et al. 2014). However, in a geographic area dominated by headwater capture events, ancestral lineages would be expected to be more widely distributed in adjacent hydrographic systems (see e.g. Albert et al. 2011). Given this, Roxo et al. (2014) did not reject the hypothesis that the ancestral lineages of the HNO-clade were more widely distributed in South America during the early Cenozoic, including much of the modern Atlantic Coast (area A), upper Paraná (area B), Paraguay, lower Paraná and Uruguay (area C), and Amazon and Orinoco basins (area D).

Our ancestral range estimates found, using the DECj model and including *Microplecostomus forestii* sp. n. in the HNO phylogeny, that the ancestral lineages of these three subfamilies were widely distributed on the Atlantic Coast (area A) and in the Amazon and Orinoco basins (area D). While these two areas are not adjacent (i.e. they

do not share an endpoint or border), a number of studies have found evidence of the historical mixing of the faunas of the headwaters of the Amazon and Paraná rivers, and the drainage basins of the Atlantic Coast. The historical connection between the Paraguay and Amazon basins has been known for more than a century (e.g. Eigenmann and Eigenmann 1891; Jordan 1896; Eigenmann 1906; Pearson 1937; Carvalho and Albert 2011; Ribeiro et al. 2013), and may account for the geodispersal (*sensu* Albert and Reis 2011) from the Amazon drainage basins (in particular the Madeira, Tocantins and Xingu) located on the Brazilian Shield. Even so, geodispersal events in the reverse direction, i.e., from south to north should also be expected (Roxo et al. 2014), and the dispersal of the hypoptopomatine lineages (*sensu* Chiachio et al. 2008) is considered to be the result of historical connections among the Amazon, Orinoco and Paraguay basins (Albert et al. 2011; Roxo et al. 2014). A number of authors have proposed headwater capture as the likely mechanism determining the distribution of ancestral fish lineages in the Tietê, Paraíba do Sul, São Francisco and Ribeira de Iguape basins on the Brazilian Shield (Ab'Saber 1957; Ab'Saber 1998; Ribeiro 2006; Roxo et al. 2012c; 2014). The historical dispersal of ancestral fish lineages between areas A and D is thus quite conceivable.

All neoplecostomine lineages are found in southern and southeastern of South America, except *Microplecostomus forestii* sp. n. and *Pareiorhaphis regani* (Giltay, 1936), which are from the Amazon basin (area D). In a paper on the evolution of plants, Stebbins (1974) discussed the concepts of evolutionary museum and evolutionary cradle, which are used to define species occurrence patterns within an area. An evolutionary cradle is defined as an area of high speciation rates, while an evolutionary museum is an area with low extinction rates, where environmental conditions combine to preserve lineages over long periods of time. Roxo et al. (2014) suggested that in the Hypoptopomatinae, the *Lampiella gibbosa* (Miranda Ribeiro, 1908) and *Otocinclus affinis* (Steindachner, 1877b) lineages are relicts of the Atlantic Coast drainage basins, considering that other *Otocinclus* species are widely distributed in the lowland Amazon and Paraná-Paraguay basins. The new genus and species described here, *Microplecostomus forestii* sp. n., also appears to be a relict lineage of the Tocantins River basin (Amazon basin), given that all other neoplecostomine species, except *Pareiorhaphis regani*, are present in the upper Paraná, lower Paraná-Paraguay, and coastal drainage basins of the Brazilian Shield.

Comparative material

Chauliocheilos saxatilis Martins, Andrade, Rosa & Langeani, 2014: paratype, MZUSP 114758, 2, 38.9–40.2 mm SL, municipality of Itamarandiba, Minas Gerais state, Brazil, tributary of the Itamarandiba River.

Curculionichthys insperatus Britski & Garavello, 2003: LBP 4945, 5, 27.3–28.5 mm SL, 2 c&s, 28.2–29.9 mm SL, municipality of Botucatu, São Paulo state, Brazil, Tietê River basin.

- Gymnotocinclus anosteos* Carvalho, Lehmann A. & Reis, 2008: LBP 17125, 3, 18.8–33.0 mm SL, municipality of Alto Paraíso de Goiás, Goiás state, Brazil, Tocantins River basin.
- Hisonotus acuen* Silva, Roxo & Oliveria, 2014: holotype, MZUSP 115350, 25.9 mm SL, municipality of Querência, Mato Grosso state, Brazil, Xingu River basin; paratype, LBP 15755, 16, 19.5–26.0 mm SL, municipality of Ribeirão Cascalheira, Mato Grosso, Xingu basin.
- Hisonotus bocaiuva* Roxo, Silva, Oliveira & Zawadzki, 2013: holotype, MZUSP 112204, 24.2 mm SL, municipality of Bocaiúva, Minas Gerais state, Brazil, São Francisco River basin; paratype, LBP 9817, 9, 3 c&s, 18.3–23.2 mm SL, municipality of Bocaiúva, Minas Gerais state, Brazil São Francisco River basin.
- Hisonotus notatus* Eigenmann & Eigenmann, 1889a: LBP 18472, 7, 30.1–38.3 mm SL, municipality of Silva Jardim, Rio de Janeiro state, Brazil, coastal drainage basin.
- Isbrueckerichthys alipionis* (Gosline, 1947): LBP 7373, 17, 31.7–81.6 mm SL, municipality of Iporanga, São Paulo state, Brazil, Ribeira de Iguape River basin;
- Kronichthys subteres* Miranda Ribeiro 1908: LBP 515, 31, 28.4–61.9 mm SL, municipality of Iporanga, São Paulo state, Brazil, Ribeira de Iguape River basin.
- Lampiella gibbosa* (Miranda Ribeiro, 1908): LBP 7430, 5, 25.6–26.1 mm SL, municipality of Jacupiranga, São Paulo state, Brazil, Ribeira de Iguape River basin.
- Microlepidogaster arachas* Martins, Calegari & Langeani, 2013: LBP 10882, 3, 22.8–35.3 mm SL, municipality of Araxás, Minas Gerais state, Brazil, Paraná River basin.
- Nannoplecostomus eleonorae* Ribeiro, Lima & Pereira, 2012: LBP 19016, 51, 19.9–25.4 mm SL, municipality of Guarani de Goiás, Goiás state, Brazil, Tocantins River basin.
- Neoplecostomus microps* (Steindachner, 1877a): LBP 8036, 38, 41.3–65.0 mm SL, municipality of Piquete, São Paulo state, Brazil, Paraíba do Sul River basin.
- Neoplecostomus franciscoensis* Langeani, 1990: LBP 6489, 50, 42.8–55.9 mm SL, municipality of São Bartolomeu, Minas Gerais state, Brazil, São Francisco River basin.
- Neoplecostomus paranensis* Langeani, 1990: holotype, MZUSP 38572, 71.4 mm SL, municipality of Cajuru, Minas Gerais state, Brazil, Grande River basin.
- Otocinclus affinis* (Steindachner, 1877b): 19, 19.5–28.9 mm SL, municipality of Pocóné, Mato Grosso state, Brazil, Paraguay River basin.
- Otocinclus vittatus* Regan, 1904: 27, 18.2–21.7 mm SL, municipality of Cáceres, Mato Grosso state, Brazil, Paraguay River basin.
- Otothyropsis marapoama* Ribeiro, Carvalho & Melo, 2005: LBP 4698, 6, 23.9–36.3 mm SL, municipality of Marapoama, São Paulo state, Brazil, Tietê River basin.
- Pareiorhaphis splendens* (Bizerril, 1995): LBP 1117, 20, 32.0–100.0 mm SL, municipality of Morretes, Paraná state, Brazil, Atlantic Coast drainage basins.
- Pareiorhaphis steindachneri* (Miranda Ribeiro, 1918a): LBP 739, 6, 33.8–49.0 mm SL, municipality of Jaraguá do Sul, Santa Catarina state, Brazil, Atlantic Coast drainage basins.
- Pareiorhina brachyrhyncha* Chamon, Aranda & Backup, 2005: LBP 12240, 50, 26.4–36.9 mm SL, municipality of Pindamonhangaba, São Paulo state, Brazil, Paraíba do Sul River basin.

- Pareiorbina cepta* Roxo, Silva, Mehanna & Oliveira, 2012d: holotype, MZUSP 111095, 41.5 mm SL, municipality of São Roque de Minas, Minas Gerais state, Brazil, São Francisco basin, paratypes, LBP 10287, 13, 21.5–43.6 mm SL, municipality of São Roque de Minas, Minas Gerais, Brazil, Paraíba do Sul River basin.
- Pareiorbina rudolphi* (Miranda Ribeiro, 1911): LBP 8044, 18, 31.7–48.9 mm SL, municipality of Piquete, São Paulo state, Brazil, Paraíba do Sul River basin.
- Parotocinclus maculicauda* (Steindachner, 1877b): LBP 2869, 15, 20.2–44.7 mm SL, municipality of Miracatu, São Paulo state, Brazil, Ribeira de Iguape River basin.
- Plesioptopoma curvidens* Reis, Pereira & Lehmann A, 2012: LBP 17394, 39, 26.1–81.7 mm SL, municipality of Cristiano Ottoni, Minas Gerais state, Brazil, São Francisco River basin.
- Pseudotocinclus juquiaie* Takako, Oliveira & Oyakawa, 2005: LBP1081, 2, 29.0–31.9 mm SL, municipality of Juquitiba, São Paulo state, Brazil, Atlantic Coast drainage basins.
- Pseudotocinclus tietensis* (Ihering, 1907): LBP 2931, 3, 38.6–62.3 mm SL, municipality of Salesópolis, São Paulo state, Brazil, Tietê River basin.
- Schizolecis guntheri* (Miranda Ribeiro, 1918b): LBP 14335, 18, 18.3–35.3 mm SL, municipality of São Sebastião, São Paulo state, Brazil, Atlantic Coast drainage basins.

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Supplementary material 1

Table S1

Authors: Gabriel S. C. Silva, Fábio F. Roxo, Luz E. Ochoa, Claudio Oliveira

Data type: Microsoft Word document

Explanation note: Species included in the present study with vouchers and GenBank accession numbers.

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Supplementary material 2

Table S2

Authors: Gabriel S. C. Silva, Fábio F. Roxo, Luz E. Ochoa, Claudio Oliveira

Data type: Microsoft Word document

Explanation note: Models tested to estimate distribution ranges inherited by the descending lineages at each node of the tree.

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