UNIVERSIDADE ESTADUAL PAULISTA

INSTITUTO DE BIOCIÊNCIAS

CURSO DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

ÁREA DE CONSENTRAÇÃO: GENÉTICA

FÁBIO FERNANDES ROXO

Estudos macroevolutivos e de Biogeografia Histórica de membros das subfamílias Hypoptopomatinae, Neoplecostominae e Otothyrinae (Siluriformes: Loricariidae) e descrição de novas espécies dos gêneros *Hisonotus* e *Pareiorhina*

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BOTUCATU

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Dedico essa tese à minha mãe Cleisa, ao meu pai Luiz e minha irmã Daniela,
por serem minhas maiores fontes de inspiração.

Amo vocês!

Agradecimentos

Agradeço,

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Summary

Introduction

The rivers of the Neotropical region are hugely diverse, with more than 7000 estimated fish species extending from south of Mexico to south of Argentina, making it by far the most species-rich vertebrate fauna on earth (Lundberg et al. 2000; Berra 2001; Reis et al. 2003a; Lévêque et al. 2005; Lévêque et al. 2008; Petry 2008). Curiously, the evolutionary diversification of this amazing fish fauna occurred over a short time, a periods of tens of millions of years, and over a continental arena (Weitzman & Weitzman 1982; Vari 1988; Lundberg 1998; Roxo et al. 2012a).

The understanding of the historical origins of this singular fauna has been a challenge for generations of evolutionary biologists. However, the discovery of new fossils and new geological data bearing on paleoclimates and paleoenvironments has extended our knowledge of the temporal context and opened new perspectives on the conditions under for Neotropical fish diversification occurred (Lundberg & Chernoff 1992; Hoorn 1994; Hoorn et al. 1995; Räsänen et al. 1995; Casciotta & Arratia 1993; Gayet 2001; Gayet et al. 2002; Gayet & Meunier 2003; Lundberg & Aguilera 2003; Lundberg 2005; Hoorn 2006; Kaandorp et al. 2006; Sanchez-Villagra & Aguilera 2006; Wesselingh & Salo 2006; Hovikoski et al. 2007; Malabarba & Lundberg 2007; Sabaj-Perez et al. 2007; Malabarba & Malabarba 2008, 2010).

The fishes from Neotropical region belong to relatively few clades, and these clades are conspicuously absent from adjacent regions (Albert et al. 2011b). According to Reis et al. (2003) the Neotropical ichthyofauna includes 43 endemic families or subfamilies and almost all of which are present in Amazonia. This is a huge number compared to the 13 endemic families or subfamilies in North America (Albert et al. 2011a). As in most of the world's freshwater ecosystems, the Neotropical ichthyofauna is dominated by Ostariophysi fishes (i.e. Characiformes, Siluriformes, and Gymnotiformes), which constitute about 77% of the species (Albert et al. 2011a). Among these ostariophysan the most diverse is Characoidea (1,750 species), and the Loricarioidea (1,490 species) (Albert et al. 2011b).

South America rivers diversity

The huge diverse major aquatic habitat types recognized in Neotropical freshwaters in South America encompasses variations in stream gradient, altitude, soil type, rainfall level, temperature and vegetation cover (Olson et al. 1998). This region includes the greatest and most species-richness hydrographic systems in the world, being part of this the Amazon, Orinoco, La Plata, São Francisco basins, the Southeastern and Northeastern drainages into Atlantic and coastal drainages of the west Andes (Fig. 1).

The Amazonas River is the largest river in the world in length and volume of water, which has an year discharges of 16–20 % of the world's flowing freshwater into the sea, and which has a total river flow greater than the next eight world largest rivers combined (Richey et al. 1989ab; Goulding et al. 2003). The Orinoco river (also known as Orenoco) is one of the principal rivers of South America and is the third largest considering all continent with 880.000 km². The lowlands of the Amazon and Orinoco basins (5.3 million km²) are the center of diversity for most groups of Neotropical fishes (Albert et al. 2011b; Albert & Carvalho 2011), with about 2,200 fish species in Amazon and 1,000 species in the Orinoco basins (Lasso et al. 2004; Lasso et al. 2004).

The Guiana Shield (name derived from Amerindian word meaning "water" or "many waters"; Hammond 2005) is the smaller and more northern subunit of the Amazon Platform, elongated nearly east to west and roughly oval in shape. From its eastern margin along the Atlantic coast, it stretches across Brazil, French Guiana, Suriname, Guyana, and Venezuela, to southeastern Colombia in the west. Bounded by the Amazon basin to its south and the Orinoco river to its north and west, the Guiana Shield occupies some 2,288,000 km² and discharge approximately a quarter of South America's total volume of freshwater exported to the oceans (Hammond 2005). As a general pattern the ichthyologic fauna of Guyana rivers are poorly sampled and the better-sampled areas are the lowlands of Amazonas, Venezuela, the lower and upper Caroni of Venezuela, the Cuyuni of Venezuela, the Rupununi and Takutu of Guyana, and much of French Guiana. The most poorly sampled areas of Guyana are known to be the western high-lands, the Mazaruni, the Corantijne, and most rivers of the southern edge of the Guiana Shield (Lujan & Armbruster 2011).

The La Plata Basin is the fifth largest hydrographic systems in the world, and second in South America (Cox 1989; Potter 1997; Ribeiro 2006), encompassing more than 3 million km² in total area. The principal tributaries of this basin are the Paraná,

Paraguay, and Uruguay rivers. The streams of La Plata Basin (or Paraná-Paraguay system) originate from remote areas, including mountain and deserts in Argentina and Bolivia, the Pantanal wetlands of Paraguay, savannas and rainforests of central and southern Brazil, and the pampas of northern Uruguay (Brea & Zucol 2011). One of the main points of the fish fauna of this region is the fact that the Paraguayan freshwater fish fauna did not evolve in isolation from that of adjacent regions, mainly with tributaries headwaters of the Amazon basin (Pearson 1937). Carvalho & Albert (2011) bring an extensive list of fishes shared among Paraguay and Amazon basin and concluded that these basins shares 111 species, distributed in 31 families.

Another important hydrographic system of South America is the São Francisco basin, the fourth longest river in South America (after the Amazon, the Paraná and the Madeira) and overall in Brazil with 2,914 km. Menezes (1972) calculated a Simpson index of 39.3 for the species similarity between the La Plata and the São Francisco basins, a value higher than that calculated for the species similarity between the São Francisco and the coastal drainages (13.1). Menezes (1972) attributed the high number of species shared between the São Francisco and La Plata basins to dispersal across high-altitude swamps along the limits between the headwaters of the rio Paraná and the western tributaries of the São Francisco. Considering the extensive area of the La Plata basin only upper Paraná Basin shares a watershed divide with the São Francisco Basin. The upper Paraná is usually defined as including the Paraná watershed upstream from the now-flooded Sete Quedas waterfalls. The faunal similarity between the upper Paraná and the São Francisco is even greater if we consider only the Grande River drainage (81% of species from both basins occur in the Grande River drainages), which drains the southern slope of the São Francisco watershed divide and forms the Paraná River at the confluence with the Paranaíba drainage.

The drainages of southeast Brazil, comprising the Upper Rio Paraná Basin and coastal rivers (Paraiba do Sul and Ribeira de Iguape rivers), are among the most species-rich regions with regard to freshwater fish (Abell et al. 2008) are of great biogeographical significance, because of their highly endemic fish faunas (Ribeiro 2006). The basins constitute one of the main hydrographic basins of South America continent with a distinct fauna responsible to origin of important clades of Neotropical fishes (e.g. catfishes of subfamilies Neoplecostominae, Otothyrinae and Delturinae, in Chiachio et al. 2008 and Roxo et al. 2012a). Ribeiro (2006) proposed some interesting

biogeographic patterns exhibited by the freshwater ichthyofauna on the Brazilian crystalline shield and the Atlantic coastal drainages. The first pattern was defined by sister-group relationships between the endemic ichthyofauna of the Brazilian coastal drainages and adjacent shield that includes intermediate degree of inclusive and both sister-clades underwent some radiation. Ribeiro (2006) postulated that the members of genera *Lignobrycon*, *Rhinelepis*, *Spintherobolus*, and *Triportheus*, the tribes Aspidoradini and Glandulocaudini, and the subfamilies Cheirodontinae and Sarcoglanidinae are examples of this pattern.

Figure 1. Map showing the main hydrographic basins of South America.

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Another important river system of South America is the West Andean rivers system. They ichthyofaunas exhibit a highly distinct taxonomic composition, especially the high-altitude lakes and streams of the Andean plateaus above 4,000 m (e.g. catfishes of families Trichomycteridae and Astroblepidae; *Orestias* of family Cyprinodontidae). The rivers of Andes have several specialized forms as the *Chaetostoma* (Salcedo 2007) and *Creagrutus* (Vari & Harold 2001) from mountain rivers of the Andean piedmont and *Archolaemus* and *Sternarchorhynchus* (Albert 2001) and *Teleocichla* (Kullander 1988) from the rapids of the shield escarpments.

Molecular techniques

A central aim of research in modern historical biogeography is to understand the distributions of species and ecosystems in light of Earth history processes that shape landscape evolution (Cox & Moore 2005; Lomolino et al. 2006). The advent of gene sequencing techniques starting in the 1970s (Maxan & Gilbert 1977; Sanger et al. 1977; Gilbert 1981), and the development of molecular phylogenic statistical methodologies starting in the 1980s with the works of Joseph Felsenstein (Felsenstein 1981; Felsenstein 1985a,b; Felsenstein 1988) has led to a rapid proliferation of phylogenetic studies over the past decade (Nei & Kumar 2000). Part of the motivation for this large research efforts has been the desire to understand geographical circumstances that promote lineage diversification and the formation of species-rich clades and ecosystems (Brown & Lomolino 2000; Wiens & Donoghue 2004; Albert et al. 2011). Further, this revolution in phylogenetic methods has been accompanied by growing knowledge of the geological history of the Earth, and of techniques to date the divergences times of evolutionary lineages (Riddle et al. 2008).

Rivers captures

The distinguished taxonomic composition of the Neotropical ichthyofauna reflects its lengthy history linked with geological development, since freshwater fish evolution has been shaped by changes in the earth's surface involving changes in the courses of rivers and fluctuations in sea level, resulting in biota isolations. Indeed, by the standards of biogeography in a global context, the margins of the Neotropical

ichthyofauna region are remarkably sharp (Myers 1966; Miller 1966; Lomolino et al. 2006).

According to Ribeiro (2006), many cladogenetic events associated with tectonics and erosive processes (which are still active today across eastern South America) may be influencing ichthyofauna distribution and speciation. Among obligate freshwater organisms, lineage diversification is strongly affected by patterns of connectivity among portions of adjacent river basins (Smith 1981; Hocutt & Wiley 1986; Mayden 1988; Lundberg et al. 1998). River capture (also called stream capture or stream piracy) is a geomorphological process by which the flow of part of a stream or river drainage basin is diverted into that of a neighbouring basin. River capture is therefore an important process in landscape evolution that allows aquatic species to move, or disperse, between adjacent drainage basins. River capture may arise from the influence of several geomorphological processes, including tectonic uplift or tilting, damming by the actions of glaciers or landslides, denudation of watershed margins by erosion, or avulsion of watershed margins by sediment accumulation in fans and estuaries (Almeida & Carneiro 1998; Bishop 1995; Wilkinson et al. 2006) (Fig. 2).
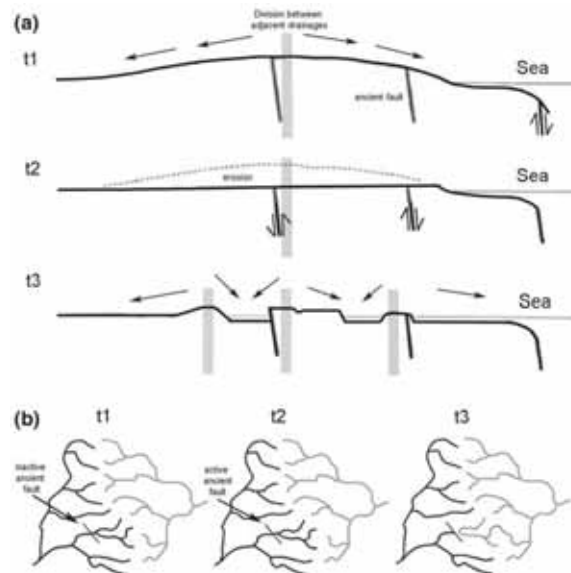

Figure 2. River capture in Roxo et al. (2012a) (a) Scheme activation of ancient faults and the erosive process in Serra do Mar formation (arrow indicate the direction of the flow); (b) Scheme of the headwater captures resulted of the activation ancient faults and erosive process. Modified from Almeida and Carneiro (1998) and Albert and Reis (2011).

Climate Oscillations

One of the prominent features of South America platform is how low it is compared with lands of other continents. About 50% of the total area of South America is below 250 m elevation (see fig. 1.4 in Albert & Reis 2011). As a comparative example the Africa has 15% of it lands below 250 m.

Periods of alternating marine transgressions and regressions can also exert strong influences on the distributions of lowland freshwater fish taxa in South America platform (Lovejoy et al. 2006; Albert & Reis 2011; Bloom & Lovejoy 2011). Marine transgressions caused by global climate oscillations or regional tectonic subsidence may cause sea levels flood low lying areas of the continental platforms, and the movement of shorelines converting lowland and coastal plains from freshwater to shallow marine ecosystems. Several authors (Lundberg et al. 1998, Albert et al. 2004, Albert & Crampton 2005, Albert et al. 2006, Ribeiro 2006; Sabaj-Perez et al. 2007) have hypothesized that marine transgressions could isolate and fragment populations promoting speciation in lowland South American fishes. By reducing the total amount of freshwater habitat, marine incursions may also be expected to result in extinction (Albert & Reis 2011). Marine incursions can also introduce marine fish taxa deep into the continental interiors and thus help some taxa transcend the ecophysiological osmotic barrier (Lovejoy et al. 2006, 2010; Bloom et al. 2011). Marine regression, in which sea levels fall relative to the land, expands lowland and coastal freshwater habitat, and can be expected to promote speciation and net diversification in Neotropical freshwater fishes (Lopez-Fernandes & Albert 2011; Lopez-Fernandes et al. 2013).

Vonhof et al. (2003), Brea and Zucol (2011), and Bloom and Lovejoy (2011) suggested that extinctions of aquatic taxa in the Orinoco and La Plata basins were certainly exacerbated by several protracted marine incursions during the Neogene that dramatically reduced the amount of freshwater habitat in these regions. A more recent marine incursion, approximately 6–5 Ma, was hypothesized by Hubert and Renno (2006) to have affected the distribution and diversity of characiform fishes in northeastern South America by isolating a series of upland freshwater refuges in respective eastern and western portions of the eastern Guiana Shield highlands. Further support for vicariance resulting from such an incursion is provided by Noonan and Gaucher (2005, 2006), who recovered a temporally and spatially congruent vicariance pattern in their molecular phylogenetic studies of *Dendrobates* and *Atelopus* frogs.

Roxo et al. (2012a) also raised the marine incursions and regressions hypothesis to help explain the great diversity of species distributed throughout the coastal drainages of Southeastern of Brazil and specifically the dispersal of Neoplecostominae genera through unconnected adjacent coastal drainages (Weitzman et al. 1998; Ribeiro 2006).

History of Loricariidae with emphases in the HNO-clade

Among Neotropical freshwater fish lineages, the Loricariidae represent the largest family with 869 valid species names (Eschmeyer & Fong 2013). Within Loricariidae the subfamilies Hypoptopomatinae, Neoplecostominae and Otothyrinae have a long and complex history of taxonomy and systematics, with morphological and molecular studies focusing on evolution of the subfamilies (Eigenmann & Eigenmann 1890; Regan 1904; Gosline 1947; Isbrücker 1980; Howes 1983; Schaefer 1987; Montoya-Burgos et al. 1998; Armbruster 2004; Pereira 2005; Reis et al. 2006; Chiachio et al. 2008; Cramer et al. 2008, 2011; Roxo et al. 2012a,b). However, the historical evolutionary relationships among endemic species from the southeast of Brazil and the mechanisms by which they diversified in space and time remain poorly understood mainly at subfamily level (Howes 1983; Schaefer 1987; Armbruster 2004).

After Eigenmann and Eigenmann (1890), Regan (1904) divided Loricariidae in five subfamilies: Argiinae, Hypoptopomatinae, Loricariinae, Neoplecostominae and Plecostominae. The subfamily Neoplecostominae was created to include the species *Neoplecostomus granosus* since the author considered the species of this genus close related to *Astroblepus*. Gosline (1945) presented the greatest revision until that moment recognizing 400 species in Loricariidae, a large increase in the family species number, and divided the family in four subfamilies: Plecostominae, Hypoptopomatinae, Loricariinae and Neoplecostominae. However, Gosline (1947) performed a new division considering six subfamilies: Plecostominae, Hypoptopomatinae, Loricariinae, Neoplecostominae, Astroblepinae (Arginae) and Lithogeninae.

Isbrücker (1980) in a revisionary study of the family Loricariidae contributed to the resolution of the taxonomy of the group and allowed subsequent description of a lot of new species. This author considered 600 species distributed for six subfamilies: Loricariinae, Hypoptopomatinae, Neoplecostominae, Hypostominae, Ancistrinae and Lithogeninae. Howes (1983) was the first author to perform a cladistics analysis in study of osteological and myological data of the Loricariidae and found that the family

had six monophyletic groups: Loricariinae, Hypoptopomatinae, Hypostominae, Neoplecostominae, Lithogeninae, and Chaetostominae. In this work *Hemipsilichthys* is included in the subfamily Chaetostominae (for Gosline, 1947 this genus was member of Neoplecostominae) and the condition of Hypostominae and Ancistrinae being monophyletic groups. Schaefer (1987), after a cladistic analysis, keep the same classification of Isbrücker (1980): Lithogeneinae, Neoplecostominae, Hypoptopomatinae, Loricariinae, Ancistrinae, and Hypostominae. The results of Schaefer (1987) suggested that Neoplecostominae should be sister group of all other remaining Loricariids, except Lithogeninae. Ancistrinae, Hypostominae and Loricariinae were recognized as monophyletic groups.

Montoya-Burgos et al. (1998) were the first to use molecular data in a phylogenetic study of the family Loricariidae (Fig. 3). This study was based in partial sequences of 12S and 16S rRNA genes obtained of 58 species representing twelve genera and five of the six subfamilies recognized previously by Isbrücker (1980) and Schaefer (1987), since Lithogeninae was not included in the analysis. The results suggested a division of species of Loricariidae into two groups, the higher and the lower. Additionally, they recognized a monophyletic Loricariidae, however no support to monophyly of the subfamilies investigated. They also support the enlarged concept of the Neoplecostominae proposed by Gosline (1947), except for the position of *Hemipsilichthys gobio* and *Pseudorinelepis agassizi*. The species *Hemipsilichthys splendens* (actually *Pareiorhaphis splendens*), *Hemipsilichthys* sp., *Isbrueckerichthys duseni*, *Kronichthys* sp., *Pareiorhina* sp. and Hypostominae unidentified were close related with Hypoptopomatinae species and *Neoplecostomus* sp. *Hemipsilichthys gobio* appeared as sister group of all other member of Loricariidae. Species of Hypoptopoma appeared close related with species of Neoplecostominae (i.e. sister of Hypoptopomatinae sp., *Pareiorhina* sp. *Pseudotocinclus tietensis*).

Based on morphological characters, Schaefer (1991, 1998) presented a phylogenetic analysis of the genera in the subfamily Hypoptopomatinae (recognized here sensu Chiachio et al. 2008, the new Hypoptopomatinae plus the new Otothyrinae), assigned new genera to the tribes Hypoptopomatini and Otothyrini, and provided phylogenetic diagnoses for the included genera plus an undescribed genus from Venezuela, subsequently described as *Niobichthys* (Schaefer & Provenzano 1998). The study of Schaefer (1991) was based in 55 osteological and myological features used to

construct a phylogenetic analysis of Loricariidae subfamily Hypoptopomatinae (genera *Acestridium*, *Hypoptopoma*, *Microlepidogaster*, *Otocinclus*, *Otothyris*, *Oxyropsis*, *Parotocinclus*, *Pseudotocinclus*, *Pseudotothyris*, *Schizolecis* and a new genus from
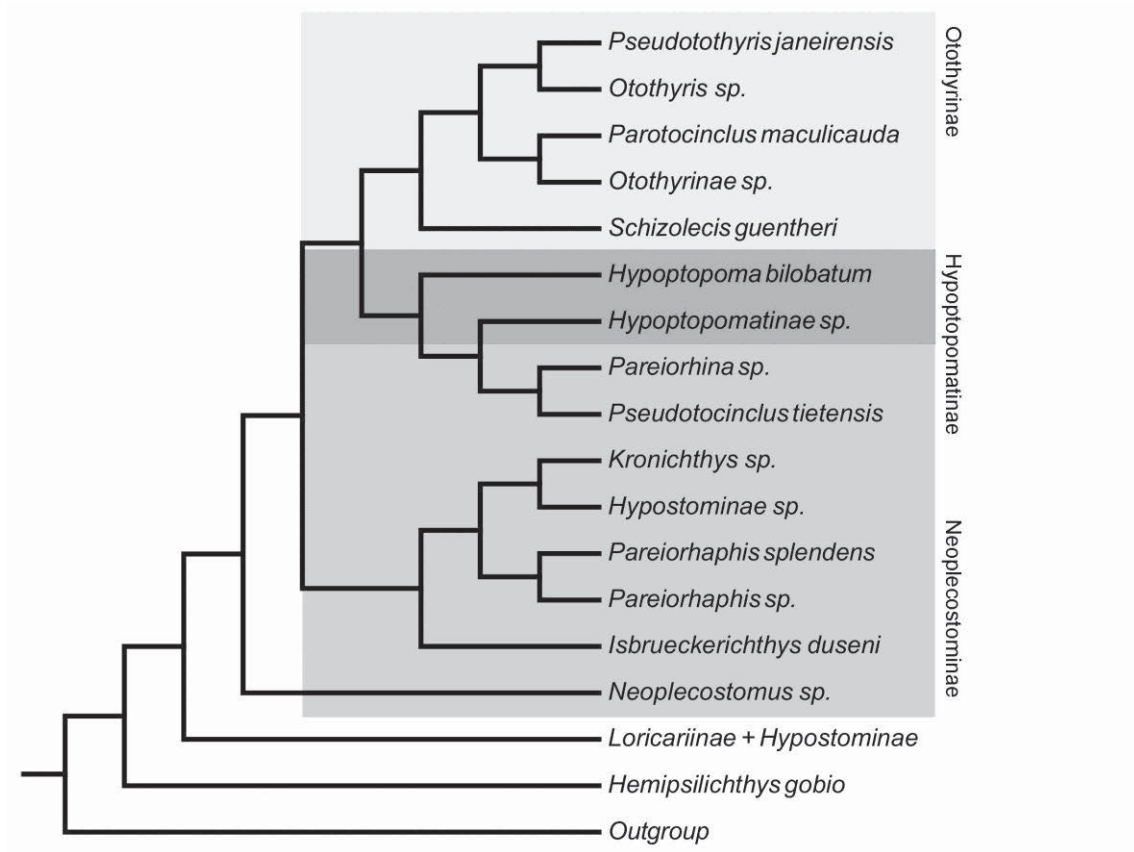
Figure 3. Phylogeny of Montoya-Burgos et al. (1998: modified from Fig. 3) based on Maximum Likelihood method using two mitochondrial genes (12S and 16S).

Venezuela). This work 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 Otothyrini to include the genera *Otothyris*, *Pseudotothyris*, *Parotocinclus*, *Schizolecis* and *Pseudotocinclus*. Schaefer (1998) (Fig. 4) 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 representing 17 genera of Hypoptopomatinae. The classifications remain the same of Schaefer (1991), except for the inclusion of *Microlepidogaster* in the Otothyrini. *Nannoptopoma* is the sister group of Hypoptopoma, and *Niobichthys* is the sister-group of all other Hypoptopomatini except

Otocinclus. Within Otothyrini the New taxon 3 from Tocantins and Xingu basin are sister-group of all others members of the tribe. *Microlepidogaster* was sister group of the clade composed of the genera *Schizolecis*, *Pseudotothyris* and *Otothyris*, and *Eurycheilichthys* was sister group of New taxon 2 from Coastal rivers of Sergipe State.
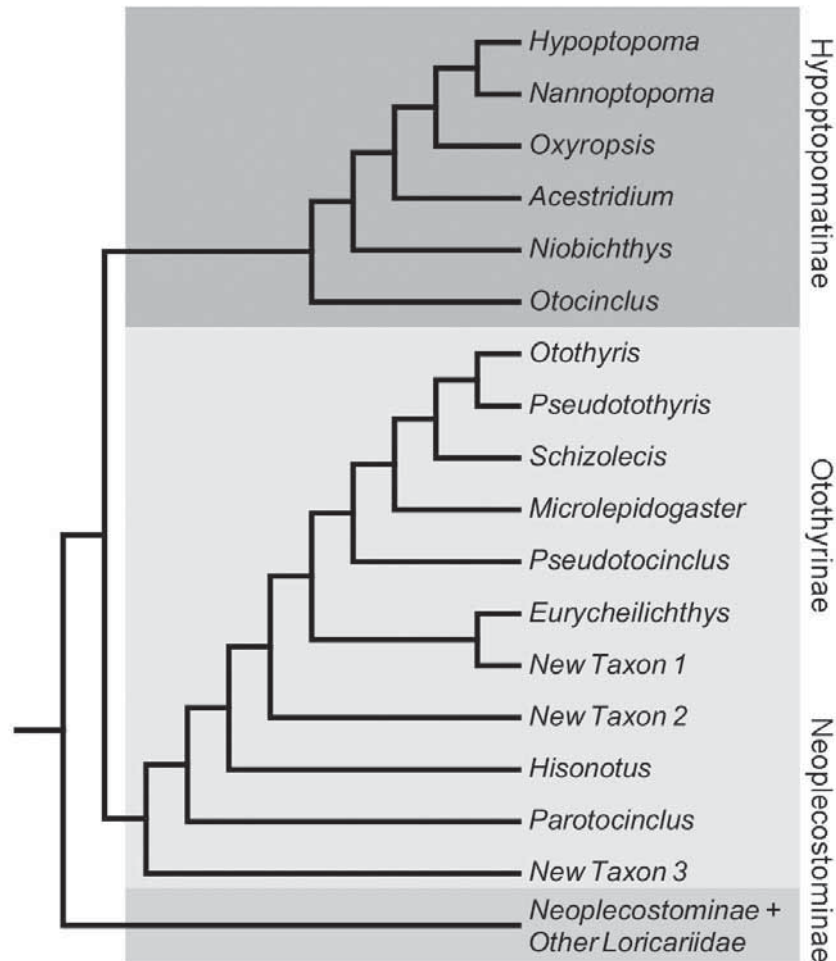
Figure 4. Phylogenetic interrelationships of the Hypoptopomatinae and Otothyrinae of Schaefer (1998) based on morphological characters.

Armbruster (2004) (Fig. 5) in a study of loricariids, included a great number of genera of Loricariidae, and found that *Hemipsilichthys gobio* (identified as *Upsilodus victori*) and *Delturus anguilicauda* were the sister group to all remaining loricariids, except *Lithogenes villosus*. A more basal position of *Hemipsilichthys gobio* within Loricariidae was previously recognized by Montoya-Burgos et al. (1998) based in molecular characters. Posteriorly, Reis et al. (2006) published a revision of *Delturus* and *Hemipsilichthys* (considering three valid species *H. gobio*, *H. papillatus*, and *H. nimius*)

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and included them in the new subfamily Delturinae. The remained species of *Hemipsilichthys* were included in the resurrected genus *Pareiorhaphis* by Pereira (2005) and as a member of Neoplecostominae. Armbruster (2004) also found that the subfamily Hypostominae is only monophyletic including Ancistrinae (recognized as tribe Ancistrini) and was divided in five tribes: Corymbophanini, Rhinelepini, Pterygoplichthini, Hypostomini and Ancistrini. Armbruster (2004) also maintained the genera *Hemipsilichthys* (*Pareiorhaphis*), *Isbrueckerichthys*, *Kronichthys*, *Neoplecostomus* and *Pareiorhina* in the subfamily Neoplecostominae, despite this subfamily did not appear monophyletic. In his analysis the relationship of Hypoptopomatinae members, and of its members with Neoplecostominae, remained uncertainly. *Kronichthys* was more related with species of Hypoptopomatinae and Otothyrinae than with species of Neoplecostominae and *Pareiorhaphis* was the sister group of all other members of Neoplecostominae, Hypoptopomatinae and Otothyrinae.
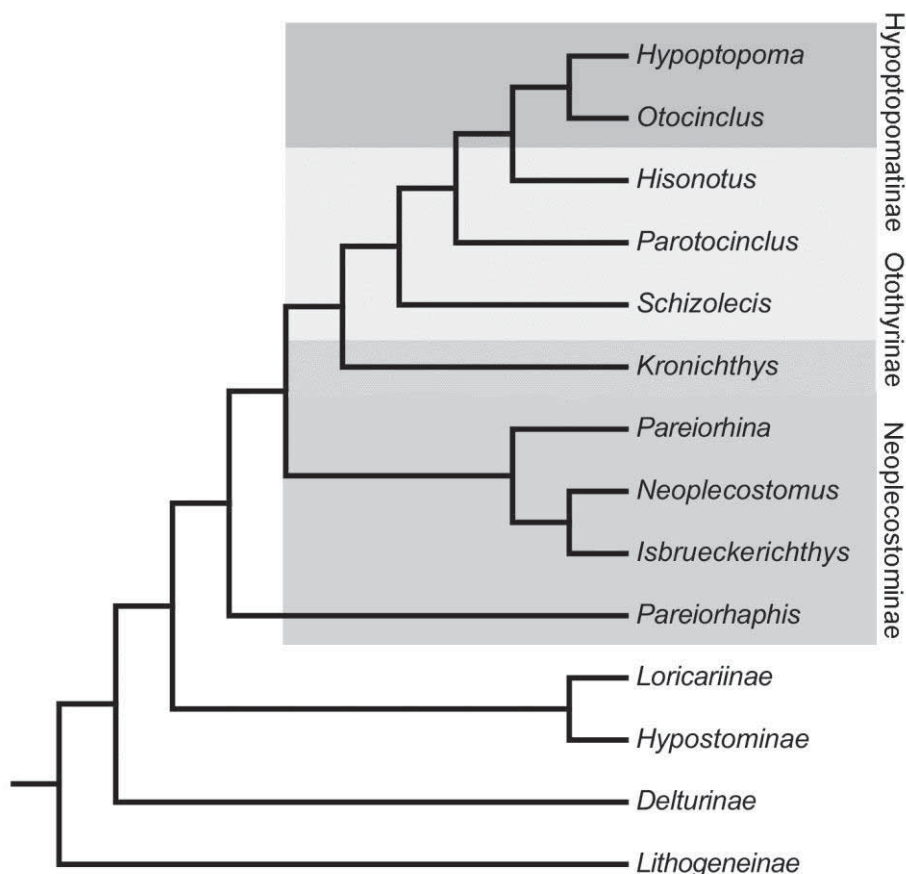
Figure 5. Phylogenetic interrelationships of the Loricariidae based on Parsimony method of morphological data, modified from Armbruster (2004).

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Chiachio et al. (2008) (Fig. 6), in a molecular study of Hypoptopomatinae (using partial sequences of the nuclear gene F-reticulon 4), found that the Hypoptopomatinae (sensu Schaefer et al. 1998) was not monophyletic and proposed the recognition of three monophyletic lineages named Hypoptopomatinae (former tribe Hypoptopomatini), Otothyrinae (former tribe Otothyrini) 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. (2012a,b). 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. Otothyrinae is composed for the genera *Hisonotus*, *Corumbataia*, *Schizolecis*, *Pseudotothyris*, *Microlepidogaster*, *Otothyropsis*, *Otothyris*, *Parotocinclus*, *Eurycheilichthys* and *Epactionotus*, and Hypoptopomatinae for the *Otocinclus*, *Lampiella*, *Macrotocinclus*, *Hypoptopoma*, *Nannoptopoma*, *Oxyropsis* and *Acestridium*. Within Otothyrinae the genus *Hisonotus* was not found as monophyletic, since there was species related with *Corumbataia*, others with *Epactionotus* and others with *Eurycheilichthys*.
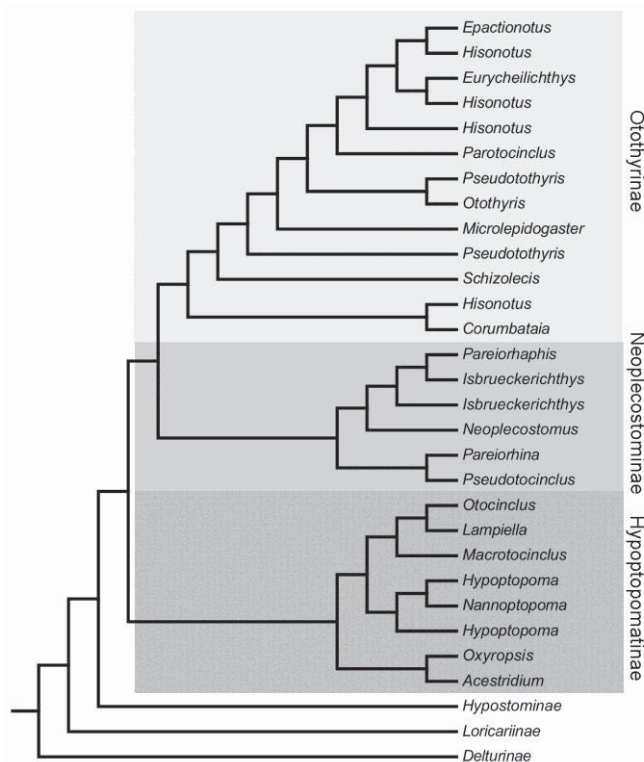
Figure 6. Phylogenetic interrelationships of the Loricariidae from Chiachio et al. (2008) based on partial sequence of F-Reticulon 4 nuclear gene.

Cramer et al. (2011) (Fig. 7) performed a phylogenetic analysis of Hypoptopomatinae, Neoplecostominae and Otothyrinae using one mitochondrial gene (COI) and three nuclear genes (RAG1, RAG2 and F-Reticulon 4) using a multiple approach of maximum parsimony, maximum likelihood and Bayesian methods. As a result the three subfamilies were not monophyletic, as well as several genera (*Hypoptopoma*, *Microlepidogaster*, *Hisonotus*, *Parotocinclus*, *Otothyris*, *Pareiorhaphis*, *Pareiorhina*, *Neoplecostomus* and *Isbrueckerichthys*). The close relationship between *Pseudotocinclus* and *Pareiorhina* previously recognized by Montoya-Burgos (1998) was corroborated by Cramer et al. (2011), and the clade composed of these two species formed sister group with all others species of Neoplecostominae, except some species of *Pareiorhaphis* that forms sister group of *Schizolecis* plus *Otothyris*. Within Otothyrinae the species *Gymnotocinclus anosteos* forms the sister group of *Corumbataia*.
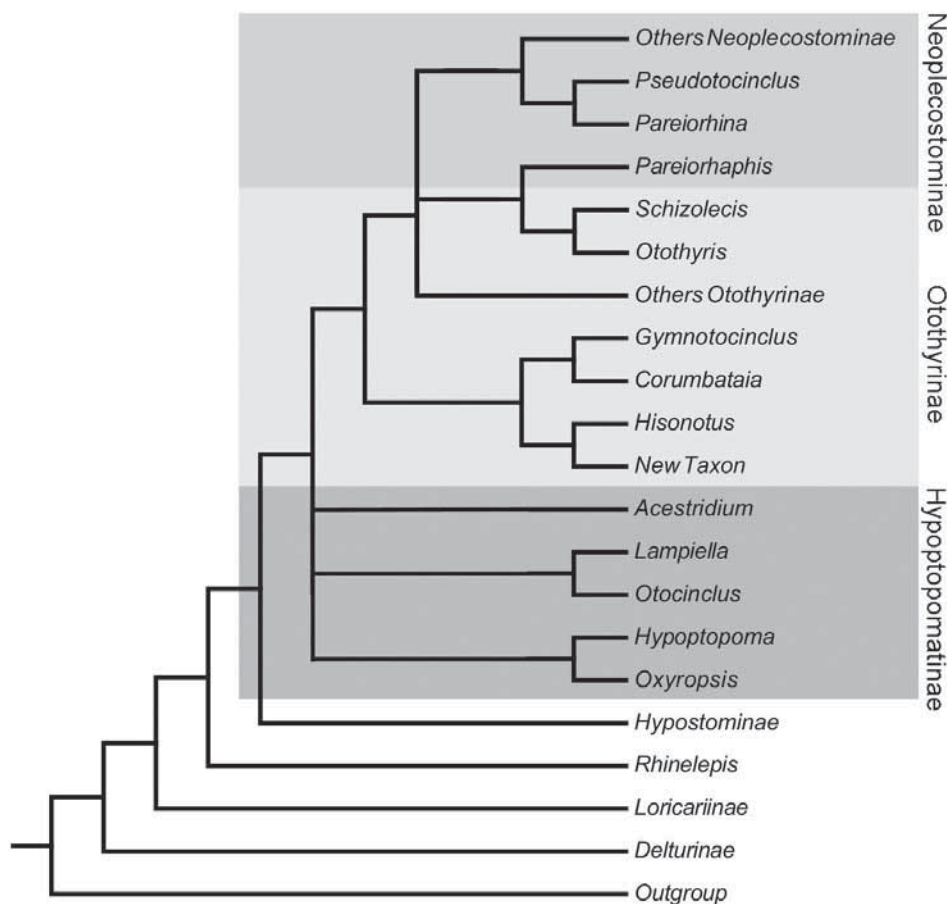
Figure 7. Phylogenetic interrelationships of the Loricariidae from Cramer et al. (2011) based on partial sequence of one mitochondrial (COI) and three nuclear (RAG1, RAG2 and (F-Reticulon 4) genes.

Roxo et al (2012a,b) (Fig. 8) performed a molecular phylogeny of the subfamily Neoplecostominae using a multiple approach a maximum parsimony, maximum likelihood and a bayesian methods with partial sequences of COI, CytB, 16S rRNA, 12S rRNA mitochondrial genes and F-reticulon 4 nuclear gene. 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*.

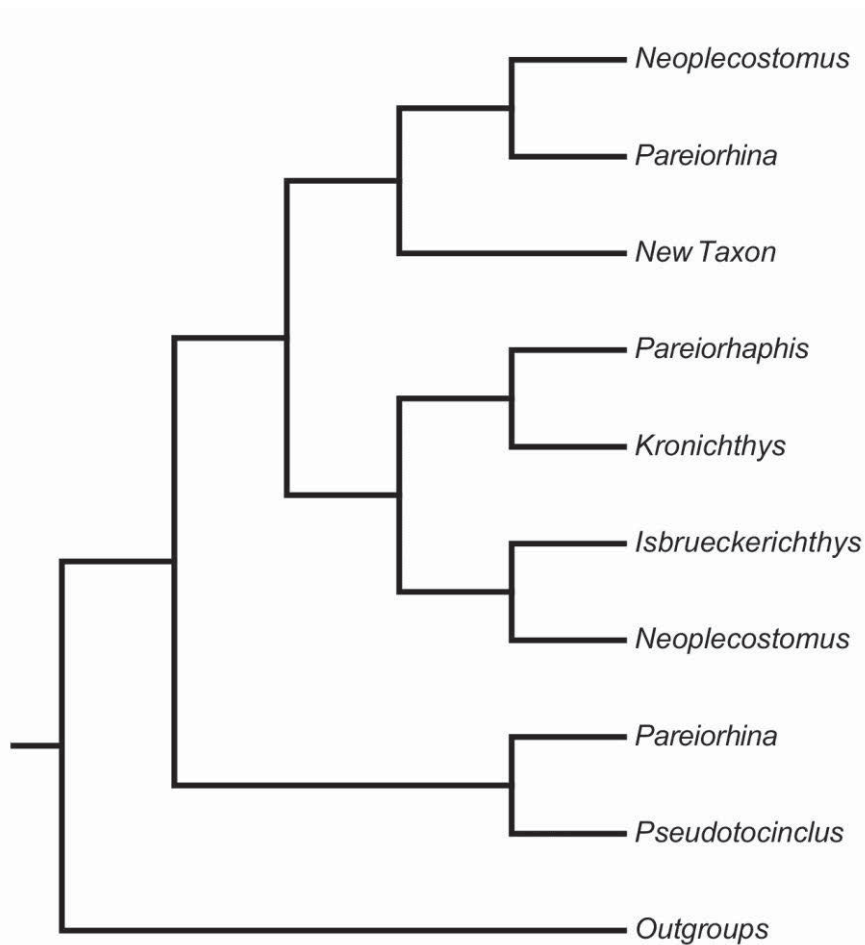**Figure 8.** Phylogenetic interrelationships of Neoplecostominae from Roxo et al. (2012a,b), based on partial sequence of 4 mitochondrial (16S, 12S, COI, CytB) and one nuclear gene (F-Reticulon 4).

The third is composed of the remaining species of the genera *Neoplecostomus*, except *N. ribeirensis*, *Pareiorhina carrancas*, *P.* cf. *carrancas*, *Pareiorhina* sp. 1 (possible new

species) and an undescribed taxon referred as New genus and species 2. Considering the results of these works, the two species identified as *Pareiorhina carrancas*, *P.* cf. *carrancas* and *Pareiorhina* sp. 1, should belong to a new genus (part of our clade C). Bockmann and Ribeiro (2003) in the description of the species suggested that its generic position could change after a better phylogenetic analysis of the Neoplecostominae. Also forming the sister group to *Isbrueckerichthys*, we found *Neoplecostomus ribeirensis*, resulting in a paraphyletic genus *Neoplecostomus*.

Objectives

The main objective of the present study was construct a species-dense molecular phylogeny of the loricariid catfish subfamilies Hypoptopomatinae, Neoplecostominae and Otothyrinae, using three mitochondrial and one nuclear gene markers and perform a combination of biogeographic and macroevolutionary analysis. Considering the specific points we will: (1) Test the hypotheses of occurrence of several historical river-capture events in Southeastern Brazil using parametric biogeographic methods and estimate ancestral geographic ranges; (2) Highlight the special role of river capture in the formation of the modern species richness and geographic distributions of the Hypoptopomatinae, Neoplecostominae and Otothyrinae; (3) Describe the major patterns of size evolution in Hypoptopomatinae, Neoplecostominae and Otothyrinae; (4) Evaluate different methods of ancestral reconstruction of continuous characters to tracking size evolution and used a maximum likelihood approach to estimate ancestral sizes; (5) Measures the rates of evolution in *darwins (d)* (Haldane 1949; Albert & Johnson 2011), in a phylogenetic context, to evaluate if lineages tend to increase in body size over evolutionary time (e.g. Cope's rule); and (6) described new species of the genera *Hisonotus* and *Pareiorhina*, as a resulted of collecting expeditions in different South America rivers.

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Chapter 1

2

3   **Description of two new species of *Hisonotus* (Ostariophysi:**

4   **Loricariidae) from rio Paraná-Paraguay basin, in Brazil**

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7   Running title: Two new species of *Hisonotus*

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10   **Abstract**

11   Two new species of *Hisonotus* from the rio Paraná and rio Paraguay basin from Brazil

12   are described. The most remarkable features of the new species are the odontodes

13   forming longitudinal aligned rows on head and trunk, a pair of rostral plates at the tip of

14   the snout, the v-shaped spinelet and the coloration of body. These features suggest a

15   close phylogenetic relationship with *H. bockmanni, H. insperatus*, *H. luteofrenatus* and

16   *H. piracanjuba*. Additionally, the two new species are distinguished from their

17   congeners by some characters related to head length and depth, orbital diameter,

18   suborbital depth, caudal peduncle depth, pectoral-fin spine length, snout length and

19   counts of teeth. The variation in number and shape of rostral plate, posterior rostrum

20   plates, infraorbitals and preopercle in the new species and in *Hisonotus insperatus* are

21   discussed.

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23   **Key words**

24   Cascudinhos; Freshwater; Hypoptopomatinae; Neotropical fishes; head plates

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Introduction

Hypoptopomatinae is composed of 19 genera and about 135 valid species (Eschmeyer and Fong 2013). This group includes *Hisonotus*, whose the type-species is *H. notatus* which was described by Eigenmann and Eigenmann (1889). These authors proposed the belly with large plates, eyes superior and humeral plate imperforate as diagnostic characters to the genus. After that, Regan (1904) studied the osteology of some members of Loricariidae and considered *Hisonotus* as a synonym of *Otocinclus*, until its resurrection by Schaefer (1998a) with the following combination of characters: reduced or absent snout plates anterior to the nostril, rostrum with enlarged odontodes, and thickened plates forming the lateral rostral margin. Some of the characters used to distinguish *Hisonotus* from the other Hypoptopomatinae genera, as rostrum with enlarged odontodes and thickened plates forming the lateral rostral margins are also present in some other species of Hypoptopomatinae, especially in species of *Microlepidogaster* (Britski and Garavello 2007).

The genus *Hisonotus* has 31 valid species (Eschmeyer 2013) and several have been discovered in the last years. Britski and Garavello (2007) described two species from the upper rio Tapajós, *H. chromodontus* and *H. luteofrenatus*. Carvalho and Reis (2009) performed the revision of *Hisonotus* from the upper rio Uruguay and described four new species. Carvalho et al. (2008) and Carvalho and Reis (2011) worked on the *Hisonotus* from the Laguna dos Patos system and described more seven new species showing the unexpected high species richness for the taxon. Subsequently Carvalho and Datovo (2012), Martins and Langeani (2012), and Roxo et al. (2013) described *Hisonotus piracanjuba*, *H. bockmanni* and *H. bocaiuva*, respectively. Herein, based on the recent collection efforts we present two new species of *Hisonotus*, one from the upper rio Paraná basin and the other as the first species of *Hisonotus* from rio Paraguay basin.

Material and methods

All measurements were taken from point to point to the nearest 0.1 mm with digital calipers from the left side of the fish. Body plate and osteology nomenclature follows Schaefer (1997) and measurements follow Carvalho and Reis (2009) as shown in Table I. Abbreviations used in the text followed Carvalho and Reis (2009) and were expressed as percent of standard length (SL), except for subunits of the head region that are expressed as percents of head length (HL). Specimens were cleared and double stained

67 (c&s) according to the method of Taylor and Van Dyke (1985). Vertebrae counts

68 included the five ones from the Weberian Apparatus. Given the lack of significant

69 differences between right and left side counts, only the left side series of plate and teeth

70 counts are included in the tables. All analyzed specimens were collected accordingly the

71 Brazilian laws, and are deposited under permanent scientific collection licenses. After

72 collection the animals were anesthetized using a 1% Benzocaine in water, fixed in 10%

73 Formaldehyde and preserved in 95% alcohol. All samples are deposited at the DZSJRP,

74 Departamento de Zoologia e Botânica, Universidade Estadual Paulista, São José do Rio

75 Preto; LBP, Laboratório de Biologia e Genética de Peixes, Universidade Estadual

76 Paulista, Botucatu; MCP, Museu de Ciências e Tecnologia, Pontifícia Universidade

77 Católica do Rio Grande do Sul, Porto Alegre; MZUSP, Museu de Zoologia,

78 Universidade de São Paulo, São Paulo; NUP, Coleção Ictiológica do Nupélia,

79 Universidade Estadual de Maringá, Maringá; ZMA, Zoologisches Museum, Universiteit

80 van Amsterdam, Amsterdam. Zoological nomenclature follows the International

81 Commission on Zoological Nomenclature (ICZN).

82

83 **Results**

84

85 *Hisonotus* **sp. 1**

86 **Figure 1; Table I**

87

88 Hisonotus sp. 4'' – Chapter 2, Figure 3 [phylogenetic relationships].

89

90 **Holotype.** MZUSP xx, 26.4 mm SL, female, Brazil, Paraná State, boundary between

91 municipalities of Cambira and Apucarana, ribeirão Cambira, affluent rio Ivaí, upper rio

92 Paraná basin, 23º38'54" S 51º29'58" W, coll. Zawadzki CH, de Paiva S, 29 October

93 2007.

94

95 **Paratypes.** All from Brazil, Paraná State. DZSJRP 18244, 3 males, 26.3-26.8 mm SL,

96 ribeirão Salto Grande, rio Ivaí basin, municipality of Maria Helena, 23°37'08" S

97 53°12'18" W, coll. Graça WJ, 30 December 2004. LBP 1325, 1 male, 23.4 mm SL, 6

98 females, 17.8-23.4 mm SL, ribeirão Keller, rio Ivaí basin, boundary between

99 municipalities of Marialva and Bom Sucesso, 23°38'30" S 51°51'32" W, coll. Oliveira

100 C, 15 October 2002. LBP 7358, 1 female, 28.4 mm SL, 1 unsexed, 12.4 mm SL,

101 ribeirão Keller, rio Ivaí basin, boundary between municipalities of Marialva and Bom

102 Sucesso, 23°38'30" S 51°51'33" W, coll. Devidé R, 15 October, 2002. LBP 13332, 1

103 male, 23.2 mm SL, 1 unsexed c&s, 23.7 mm SL, rio Mourão, rio Ivaí basin,

104 municipality of Campo Mourão, 24°02'23" S 52°16'22" W, coll. Zawadzki CH,

105 November 2010. LBP 13333, 1 male, 23.6 mm SL, 1 female, 25.4 mm SL, rio Mourão,

106 rio Ivaí basin, municipality of Campo Mourão, 24°02'23" S 52°16'22" W, coll. Pavanelli

107 CS, 4 December 2006. LBP 13334, 1 male, 24.9 mm SL, ribeirão Keller, rio Ivaí basin,

108 boundary between municipalities of Marialva and Bom Sucesso, 23°38'30" S 51°51'32"

109 W, coll. Zawadzki CH, November 2010. LBP 13335, 1 male, 26.0 mm SL, ribeirão

110 Salto Grande, rio Ivaí basin, municipality of Maria Helena, 23°37'08" S 53°12'18" W,

111 coll. Graça WJ, 30 December 2004. LBP 14917, 4 females, 28.8-29.6 mm SL, 2 males,

112 26.6-27.4 mm SL, ribeirão Cambira, rio Ivaí basin, boundary between municipalities of

113 Cambira and Apucarana, 23°58'54" S 51°29'58" W, coll. Zawadzki CH, de Paiva S, 29

114 November 2007. LBP 17578, 6 females, 27.7-30.4 mm SL, 4 males, 25.4-26.1 mm SL,

115 rio Mourão, rio Ivaí basin, boundary between municipalities of Engenheiro Beltrão and

116 Quintal do Sol, 23°49'41" S 52°11'43" W, coll. Zawadzki CH, Ruiz HB, Vieira RS, 01

117 April 2013. MCP 47860, 1 male, 25.6 mm SL, 1 female, 25.9 mm SL, ribeirão Salto

118 Grande, rio Ivaí basin, municipality of Maria Helena, 23°37'08" S 53°12'18" W, coll.

119 Graça WJ, 30 December 2004. NUP 3578, 7 females, 27.8-28.1 mm SL, 8 males, 24.7-

120 26.8 mm SL, 1 female c&s, 27.6 mm SL, 1 male c&s, 25.5 mm SL, ribeirão Salto

121 Grande, rio Ivaí basin, municipality of Maria Helena, 23°37'08" S 53°12'18" W, coll.

122 Graça WJ, 30 December 2004. NUP 7065, 1 male, 23.3 mm SL, 1 female, 25.4 mm SL,

123 1 c&s unsexed, 24.5 mm SL, rio Mourão, rio Ivaí basin, municipality of Campo

124 Mourão, 24°02'23" S 52°16'22" W, coll. Zawadzki CH, 7 April 2009. NUP 9839, 1

125 male, 25.3 mm SL, 1 female, 25.8 mm SL, 1 female c&s, 25.0 mm SL, collected with

126 holotype. ZMA 250.056, 2, 1 male, 26.1 mm SL, 1 female, 25.6 mm SL, rio Mourão,

127 rio Ivaí basin, municipality of Engenheiro Beltrão, 23°37'40.8" S 52°03'37.8" W, coll.

128 Zawadzki CH, Ruiz HB, Silva HP, 22 October 2012.

129

130 **Diagnosis.** *Hisonotus* sp. 1 can be distinguished from all congeners, except *Hisonotus*

131 *insperatus*, *H. luteofrenatus* and *Hisonotus* sp. 2 by having odontodes forming

132 longitudinal aligned rows on head and trunk, Fig. 2(A), (B) (*vs.* odontodes not forming

133 longitudinal aligned rows). Additionally, the new species can be distinguished from all

134 congeners, except *H. insperatus*, *H. luteofrenatus*, *Hisonotus* sp. 2, *H. piracanjuba*) by

135 having a pair of rostral plates at the tip of the snout (vs. a single rostral plate). Also

136 *Hisonotus* sp. 1 can be distinguished from congeners, except *H. bockmanni*, *H.*

137 *chromodontus*, *H. insperatus*, *H. luteofrenatus*, and *Hisonotus* sp. 2 by having a

138 functional v-shaped spinelet (*vs*. non-functional spinelet, square-shaped or absent

139 spinelet). The new species can be distinguished from *H. bockmanni* and *Hisonotus* sp. 2

140 by lacking an unusual contrasting dark geometric spots on anterodorsal region of body

141 (*vs*. presence of an unusual contrasting dark geometric spots on anterodorsal region of

142 body); from *H. insperatus* by having small odontodes forming rows on head and trunk,

143 Fig. 2(A), (B) (*vs*. large and conspicuous odontodes forming rows on head and trunk,

144 Fig. 2 (E), (F)), higher head depth 51.6-59.2% HL (*vs*. 44.3-48.7% HL) and higher

145 suborbital depth 20.9-25.5% HL (*vs*. 16.6-20.1% HL); from *H. luteofrenatus* by having

146 higher caudal peduncle depth 10.8-12.5% SL (*vs*. 8.9-10.2% SL) and lower snout length

147 46 9-52 2% HL (*vs*. 67.0-75.3% HL); from *Hisonotus* sp. 2 by having higher head depth

148 51.6-59.2% HL (*vs*. 42.4-47.7% HL), higher counts of premaxillary teeth 11-18 (*v*s. 6-

149 10), and higher counts of dentary teeth 11-15 (*v*s. 4-7); from *H. piracanjuba* by higher

150 caudal peduncle depth 10.8-12.5% SL (*v*s. 8.3-9.5% SL), and lower snout length 46.9-

151 52.2% HL (*vs*. 67.7-72.7% HL).

152

153 **Description.** Morphometric data presented in Table I. Maximum body length 28.4 mm

154 SL. Dorsal profile of head slightly convex to straight from upper part of rostrum to

155 posterior margin of nares, convex from eyes to posterior margin of parieto

156 supraoccipital, and straight to dorsal-fin origin. Dorsal profile of trunk slightly concave

157 and descending from dorsal-fin origin to end of dorsal-fin base, straight to caudal

158 peduncle. Ventral profile strongly concave from snout tip to opercular region; convex

159 from opercular region to anal-fin origin; concave to caudal peduncle end. Greatest body

160 depth at dorsal-fin origin (18.6-23.9% SL). Greatest body width at opercular region,

161 gradually decreasing towards snout and caudal fin. Cross-section of caudal peduncle

162 almost ellipsoid; rounded laterally and almost flat dorsally and ventrally.

163 Head rounded in dorsal view, snout round to slightly pointed. Dorsal and ventral

164 series of odontodes along anterior margin of snout completely covering its tip;

165 odontodes larger than remaining on head. Odontodes on head and trunk well defined

166 and arranged into longitudinal rows (character more prominent in head). Eyes

167 moderately small (13.9-17.6% HL), dorsolaterally positioned. Lips roundish and with

168 papillae uniformly distributed on base of dentary and premaxillary and slightly

169 decreasing distally. Lower lip larger than upper lip; its border fringed. Maxillary barbel

170 present; joined to lower lip by membrane to its half length. Teeth slender and bicuspid;

171 mesial cusp larger than lateral. Premaxillary teeth 11-18. Dentary teeth 11-15.

172 Dorsal-fin I,7; its origin slightly posterior to pelvic-fin origin. Tip of adpressed

173 dorsal fin almost reaching end of anal-fin base. Dorsal, pectoral and pelvic fins without

174 locking mechanism. Pectoral fin I,6; its tip almost reaching middle of pelvic-fin

175 unbranched ray length, when depressed. Pectoral axillary slit present between pectoral-

176 fin insertion and lateral process of cleithrum. Pectoral spine supporting odontodes on

177 ventral, anterior and dorsal surface. Pelvic fin i,5; its tip almost reaching anal-fin origin

178 when depressed in females and reaching anal-fin origin in males. Pelvic-fin unbranched

179 ray with dermal flap along its dorsal surface in males. Anal fin i,5; its tip reaching

180 seventieth to eightieth plate from its origin. Caudal fin i,14,i; forked shaped. Adipose fin

181 absent. Total vertebrae 27.

182 Body covered with bony plates except above lower lip, around pectoral and

183 pelvic-fin origins and on dorsal-fin base. Cleithrum and coracoid totally exposed.

184 *Arrector fossae* partially to completely enclosed by ventral lamina of coracoids.

185 Abdomen entirely covered by plates (Fig. 3A); abdomen covered by lateral plate series

186 with large elongate plates, formed by two lateral rows, approximately of same size;

187 median plates formed by two patterns of plate distributions; first, median plate series

188 not reaching anal shield plates and both lateral plate series reaching themselves at

189 middle of abdomen; second, median plate series reaching anal shield and both lateral

190 plate series not reaching themselves at middle of abdomen; anal plates series covered by

191 large plates squared and triangular shaped. Lateral of body entirely covered by plates

192 (Fig. 3B); mid-dorsal plates poorly developed and reaching middle of dorsal-fin base;

193 median plates not interrupted in median portion of body; mid-ventral plates reaching

194 end of dorsal-fin base.

195 Parts of dorsal head bone plates presented in Fig. 3(C). Snout tip formed by pair

196 of rostral square-shaped plates (r). Nasal (n) rectangular, forming anterior medial nostril

197 margin contacting posteriorly with frontals (f) and anteriorly and laterally with pre-

198 nasals (pn). Pre-nasals (pn) positioned posteriorly to rostral plates (r); formed by two

199 large square-shaped plates, one small and triangular and one elongated and rectangular

200 between nares. Top of head composed by compound pterotic (cpt), parieto

201 supraoccipital (soc) and frontal (f), largest bones of head, and prefrontal (pf) and

202 sphenotic (sp). Compound pterotic (cpt) covered with few and small fenestra randomly

distributed. Lateral surface of head presented in Fig. 3(D). Posterior rostrum plates pr1-pr2 smallest, and rectangular shaped; pr4-pr3 largest, first rectangular and second squared-shaped. Complete infraorbital plate series (io1-io5), present just above posterior rostrum series, all covered by latero-sensory canal system; io2 largest and io5 smallest; io3, io4 and io5 forming inferior orbital margin of eyes. Preopercle (pop) elongated and rectangular shaped, covered by latero-sensory canal; Preopercle present under pr4, io4 and io5, and upper cp1, cp2 and op. Subocular cheek plates (cp1-cp2) and opercle (op) form posterior lateral margin of head.

Coloration in alcohol. Pale yellowish ground color. Dorsal surface of head dark brown, except for pale yellowish areas on snout tip, lateral margin of head and tip of parieto supraoccipital. Three dark brownish saddles crossing dorsum, reaching longitudinal dark stripe on side of trunk: first below dorsal-fin origin, second at typical adipose-fin region, and third at end of caudal peduncle. Ventral region of anal-fin origin with small spots with chromatophores. Caudal fin hyaline with two black bars; first on caudal-fin origin, second on middle of caudal fin (Fig. 1).

Sexual dimorphism. Adult males are distinguished from females by bearing a papilla in urogenital opening (*vs.* absent in females); by longer pelvic fin that extends beyond anal-fin origin (*vs.* pelvic fin not reaching anal-fin origin in females); and by pectoral and pelvic-fin unbranched ray supporting dermal flap on their proximal dorsal surface in males. Both sex present membrane in anal opening; however this membrane is more developed in females (Fig. 4A) than in males (Fig. 4D).

Distribution. The species is known from four small to medium streams, the ribeirão Salto Grande, ribeirão Keller, rio Mourão, and the ribeirão Cambira, all tributaries from the rio Ivaí in the upper rio Paraná basin (Fig. 5A).

Hisonotus **sp. 2*, sp. n.**
Figure 6; Table I

Hisonotus sp. 6'' – Chapter 2, Figure 3 [phylogenetic relationships].

Holotype. MZUSP xx, 26.2 mm SL, female, Brazil, Mato Grosso State, municipality of Santo Afonso, riacho Águas Claras, affluent rio Sepotuba, rio Paraguay basin, 14°21'03" S 57°33'07" W, coll. Troy WP, 14 September 2010.

Paratypes. All from Brazil, Mato Grosso State, rio Sepotuba basin. DZSJRP 18245, 2 females, 19.9-24.3 mm SL, collected with holotype. LBP 13347, 2 females, 18.9-19.6 mm SL, collected with holotype. LBP 13351, 9, 14.7-24.3 mm SL, riacho Águas Claras, Santo Afonso, 14°21'03" S 57°33'07" W, coll. Troy WP, April 2012. LBP 13352, 1, 23.7 mm SL, riacho Águas Claras, Santo Afonso, 14°21'03" S 57°33'07" W, coll. Troy WP, April 2012. LBP 17532, 1 male 22.6 mm SL, 4 female 19.5-23.8 mm SL, 1 unsexed not measured, riacho Maracanã, boundary between municipalities of Santo Afonso and Nova Marilândia, 14°22'40" S 57°35'11" W, coll. Troy WP, Paliga T, Silva VM, 03 April 2010. NUP 10928, 2 males, 23.2-24.2 mm SL, 2 c&s, 23.6-24.2 mm SL, 1 unsexed not measured, collected with holotype. NUP 10976, 3 unsexed, 16.7-20.5 mm SL, riacho São Jorge, municipality of Santo Afonso, 14°27'26" S 57°34'34" W, coll. Zawadzki CH, Troy WP, 19 August 2010.

Diagnosis. *Hisonotus* sp. 2 can be distinguished from all congeners, except *H. bockmanni*, by its unusual contrasting dark geometric spots on anterodorsal region of body (see coloration section) (*vs.* absence of geometric spots). Additionally, the new species can be distinguished from all congeners, except *H. insperatus*, *H. luteofrenatus*, *Hisonotus* sp. 1., *H. piracanjuba*) by having a pair of rostral plates at the tip of the snout (vs. a single rostral plate). Also *Hisonotus* sp. 2 can be distinguished from all congeners, except *Hisonotus insperatus*, *H. luteofrenatus* and *Hisonotus* sp. 1 by having odontodes forming longitudinal aligned rows on head and trunk, Fig. 2(A), (B) (*vs.* odontodes not forming longitudinal aligned rows). The new species can be distinguished from *H. bockmanni* by having continuous perforated median plate series (*vs.* median plate series of perforated plates discontinuous, that is, with a gap of unperforated plates), by lacking unpaired plates between contra-lateral dorsal series (*vs.* having two tiny unpaired plates between contra-lateral dorsal series, placed eight plates posterior to dorsal fin – Carvalho and Datovo 2012; Fig. 4), and by having caudal fin with anterior half dark pigmented medially (*vs.* caudal fin with anterior half hyaline); from *H. insperatus* by a great pectoral-fin spine length, 27.0-30.1% in SL (*vs.* 20.6-25.9%); from *H. luteofrenatus* by a greater head length 36.1-41.7% SL (*vs.* 28.8-33.3%), lower orbital

270 diameter 11.0-14.1% HL (*vs.* 15.0-18.1%) and lower snout length 50.7-57.1% HL (*vs.*

271 67.0-75.3%); from *Hisonotus* sp. 1 by lower head depth 42.4-47.7% HL (*vs.* 51.6-

272 59.2%), lower counts of premaxillary teeth 6-10 (*vs.* 11-18) and lower counts of dentary

273 teeth 4-7 (*vs.* 11-15); from *H. piracanjuba* by higher head length 36.1-41.7% SL (*vs.*

274 27.9-32.2), higher caudal peduncle depth 10.2-11.3% SL (*vs.* 8.3-9.5%), lower counts of

275 premaxillary teeth 6-10 (*vs.* 14-22) and by lower counts of dentary teeth 4-7 (*vs.* 12-19).

276

277 **Description.** Morphometric data presented in Table I. Maximum body length 26.2 mm

278 SL. Lateral profile of head convex; straight from upper part of rostrum to posterior

279 margin of nares, slightly curved from eyes to posterior margin of parieto supraoccipital,

280 almost straight to dorsal-fin origin. Dorsal profile of trunk slightly concave, descending

281 from base of dorsal-fin origin to end of dorsal-fin base, straight to caudal peduncle.

282 Ventral profile slightly concave from snout tip to pectoral-fin origin, convex to anal-fin

283 origin, slightly concave to caudal peduncle. Greatest body depth at dorsal-fin origin

284 (16.9-20.7% SL). Greatest body width at opercular region, gradually decreasing towards

285 snout and caudal fin. Cross-section of caudal peduncle almost ellipsoid; rounded

286 laterally and almost flat dorsally and ventrally.

287 Head rounded in dorsal view. Snout slightly pointed, its tip rounded, elongated

288 (51.5-57.1% HL) and depressed in front of each nostril in dorsal surface. Dorsal and

289 ventral series of odontodes completely covering anterior margin of snout; odontodes of

290 snout , similar in size to remaining ones found on head. Snout tip lacking free-band.

291 Odontodes on head and trunk well defined and arranged into longitudinal rows

292 (character more prominent in head). Eyes small (11.0-14.1% HL), dorsolaterally

293 positioned. Lips roundish and papillose; uniformly distributed on base of dentary and

294 premaxillary and slightly decreasing distally. Lower lip larger than upper lip; its border

295 strongly fringed. Maxillary barbel present. Teeth slender and bicupid; mesial cusp larger

296 than lateral. Premaxillary teeth 6-10. Dentary teeth 5-6.

297 Dorsal fin I,7; its origin slightly anterior to pelvic-fin origin. Tip of adpressed

298 dorsal-fin rays surpassing end of anal-fin base. Dorsal, pectoral and pelvic fins without

299 locking mechanism. Pectoral fin I,6; its tip reaching half of pelvic-fin length, when

300 depressed. Pectoral axillary slit present between pectoral-fin insertion and lateral

301 process of cleithrum. Pectoral spine supporting odontodes anteroventrally. Pelvic fin i,5;

302 its tip almost reaching anal-fin origin when depressed in females and reaching anal-fin

303 origin in males. Pelvic-fin unbranched ray with dermal flap along its dorsal surface in

304   males. Anal fin i,5; its tip reaching eightieth to ninetieth plate from its origin. Caudal fin

305   i,14,i; emarginated shaped. Adipose fin absent. Total vertebrae 27.

306   Body covered with bony plates except on ventral part of head, around pectoral

307   and pelvic-fin origin and on dorsal-fin base. Cleithrum and coracoid totally exposed.

308   *Arrector fossae* partially enclosed by ventral lamina of coracoids. Abdomen entirely

309   covered by plates (Fig. 7A), abdomen formed by lateral plate series with elongate and

310   large plates, formed by two lateral plates series, similar in size; median plates formed by

311   one to three plates series reaching anal shield. Lateral of body entirely covered by plates

312   (Fig. 7B); mid-dorsal plates poor developed, reaching middle of dorsal-fin base; median

313   plates not interrupted in median portion of body; mid-ventral plates reaching end of

314   dorsal-fin base.

315   Parts of dorsal head bone plates presented in Fig. 7(C). Snout tip formed by pair

316   of rostral square-shaped plates (r). Nasal (n) almost rectangular forming anterior medial

317   nostril margin in contact posteriorly with frontals (f) and anteriorly and laterally with

318   pre-nasals (pn). Pre-nasals (pn) positioned posteriorly of rostral plates (r), formed by

319   two large and one small square-shaped plates, and one elongate rectangular shaped

320   between nares. Top of head composed by compound pterotic (cpt), parieto

321   supraoccipital (soc) and frontal (f), largest bones of head, and prefrontal (pf) and

322   sphenotic (sp). Compound pterotic (cpt) fenestrated randomly distributed. Lateral

323   surface of head presented in Fig. 3(D). Posterior rostrum plates pr1-pr2 small, and

324   rectangular shaped; pr4-pr3 largest, first rectangular and second square-shaped.

325   Infraorbital plate series complete (io1-io5), present just above posterior rostrum series,

326   all covered by latero-sensory canal system; io2 largest and io5 smallest; io3, io4 and io5

327   forming inferior orbital margin of eyes; one little plate in left side of head present

328   between io1 and io2 (Fig. 7D, red arrow); which is absent in right side. Preopercle (pop)

329   elongated and rectangular shaped, covered by latero-sensory canal; Preopercle present

330   under io4 and io5, and upper cp1, cp2 and op. Subocular cheek plates (cp1-cp2) and

331   opercle (op) form posterior lateral margin of head.

332

333   **Coloration in alcohol.** Ground color of dorsal and ventral region of head and trunk pale

334   yellowish. Conspicuous longitudinal dark stripe enlarging from rostral plates to anterior

335   corner of eyes, straightening and bordering to inferior corner of eyes, enlarging again

336   through compound pterotic and lateral series of plates to caudal-fin. Another

337   conspicuous longitudinal dark stripes medially starting at pre-nasal plate region

338   enlarging to supraoccipital region. This pattern form hyaline v-shaped mark from rostral

339   plate passing through nares to orbital margins. Longitudinal dark stripe from superior

340   portion of sphenotic through mid-dorsal plates to posterior margin of dorsal-fin base.

341   Dark blotch on compound pterotic overlap this mid-dorsal longitudinal dark stripe. Dark

342   saddle on middle portion of predorsal region reaching mid-dorsal longitudinal dark

343   stripe. This unusual combination of color characteristics forms geometric spots on

344   anterodorsal region of body. Three dark saddles crossing posterodorsal region of body,

345   reaching longitudinal stripe on laterals of trunk: first at middle of dorsal fin, second at

346   typical adipose-fin region, and third at end of caudal peduncle. Saddles inconspicuous

347   in some specimens. Ventral region of body almost complete pale yellowish, except few

348   dark spots on caudal peduncle and dark ring at anal-fin origin. Dorsal, pectoral, and

349   pelvic fins with dark chromatophores forming irregular sets of bands: three on dorsal

350   and pectoral fin, and one on pelvic fin. Anal fin with few and sparse chromatophores,

351   sometimes forming bands. Caudal fin hyaline, except for dark spot on origin of rays,

352   and dark band on middle of rays (Fig. 6).

353

354   **Sexual dimorphism.** Adults males are distinguished from females by bearing papilla in

355   urogenital opening (*vs.* absent in females); by longer pelvic fin that extends beyond

356   anal-fin origin (*vs.* pelvic fin not reaching anal-fin origin in females); and by pelvic-fin

357   unbranched ray supporting dermal flap along its dorsal surface in males. Both sex

358   present membrane in anal opening; however, this membrane is more developed in

359   females (Fig. 4B) than in males (Fig. 4E).

360

361   **Distribution.** The species is known from three small tributaries the riacho Águas

362   Claras, riacho Maracaña and riacho São Jorge, both draining to the rio Sepotuba, in the

363   upper rio Paraguay basin (Fig. 5B).

364

365   **Discussion**

366   *Hisonotus* sp. 1 is a similar species to *H. insperatus* and *H. piracanjuba* both species

367   from upper stretches of the rio upper rio Paraná basin, while *Hisonotus* sp. 2 is more

368   similar to *H. bockmanni* from the rio Tapajós basin. *Hisonotus insperatus*, *H.*

369   *chromodontus*, *H. luteofrenatus*, and *Hisonotus* sp. 1 have conspicuous odontodes

370   forming well defined and widely spaced rows of odontodes on head and trunk, the main

371   character used to distinguish theses species, while *Hisonotus* sp. 2 have smaller

372   odontodes, not conspicuous and forming closely spaced rows (Fig. 2). Additionally,

373   *Hisonotus insperatus, Hisonotus* sp. 1 and *H. piracanjuba* have a deep head with snout

374   tip raising abruptly to interorbital region in lateral view, resulting in fishes with a short-

375   snouted profile. In *H. bockmanni, H. chromodontus, H. luteofrenatus* and *Hisonotus* sp.

376   2, the snout tip raise slowly to interorbital region in lateral view, resulting fishes with a

377   long-snouted profiles. The two snout patterns fit to geographic patterns since *H.*

378   *insperatus, Hisonotus* sp. 1 and *H. piracanjuba* inhabit the upper rio Paraná while

379   *Hisonotus* sp. 2 is from the upper rio Paraguay and *H. bockmanni, H. chromodontus* and

380   *Hisonotus* sp. 2 are from the upper rio Tapajós. Such patterns could probably reflect an

381   ancient exclusive ancestral to the three latter in the region. *Moenkhausia cosmops* Lima,

382   Britski and Machado 2007*, Leporinus octomaculatus* Britski and Garavello, 1993*,*

383   *Moenkhausia phaeonota* Fink, 1979, *Hyphessobrycon vilmae* Géry, 1966, and

384   *Aequidens rondoni* Miranda-Ribeiro, 1918, *Parodon nasus* Kner, 1859, *Hemiodus*

385   *semitaeniatus* Kner, 1858, are examples of fish occurring in the upper rio Paraguay

386   basin, as well as in the upper rio Tapajós basin. There is also *Batrochoglanis melanurus*

387   Shibatta and Pavanelli 2005, which occurs at the upper rio Paraguay and appears to

388   posses its sister-taxa at the rio Tapajós basin. According to Hubert and Renno (2006)

389   and Lima et al. (2007) these examples can infer that a dispersion route has taken place

390   between the upper rio Tapajós and the upper rio Paraguay basins. Yet, both snout

391   profile patterns are quite different from all the remaining *Hisonotus* species.

392   Carvalho and Datovo (2012) in description of *H bockmanni* recognized a

393   functional V-shaped spinelet as a character shared among *H. bockmanni, H.*

394   *chromodontus, H. insperatus* and *H. luteofrenatus*, a character that is also present in the

395   two new species *Hisonotus* sp. 1 and *Hisonotus* sp. 2. Carvalho and Datovo (2012) in

396   personal communication with Roberto E. Reis suggested that this is apparently a unique

397   condition within *Hisonotus*, and previously recognized that these species could compose

398   a new monophyletic genus within the Hypoptopomatinae.

399   *Hisonotus* sp. 2 have an unusual coloration pattern with contrasting dark stripes

400   and bands converging to form geometric spots on anterodorsal region of body which is

401   even more similar in coloration to species of *Otocinclus* than to *Hisonotus*. However,

402   *Hisonotus* sp. 2 is morphologically similar to nominal species already assigned to

403   *Hisonotus*, rather than to any other Hypoptopomatinae species. Additionally, *Hisonotus*

404   sp. 2 and *Hisonotus* sp. 1 present one of the diagnostic character used to define

405   *Hisonotus* in its resurrection by Schaefer (1998a), the enlarged odontodes on rostrum.

Thus, concerning the shared aforementioned characters cited above with *H. bockmanni*, *Hisonotus insperatus*, *H. luteofrenatus*, *Hisonotus* sp. 1, *Hisonotus* sp. 2 and *H. piracanjuba* they may suggest a close phylogenetic relationship among these species.

Considering several studies on loricariid bones, osteological characters are known to be conservative within Hypoptopomatinae species compared to external anatomy (Schaefer 1987, 1997, 1998b; Garavello 1977; Mo 1991; de Pinna 1998; Diogo et al. 2001; Ribeiro et al. 2005). Britski and Garavello (2003) used the presence of a pair of rostral plates in snout tip as a diagnostic character to distinguish *Hisonotus insperatus* from its congeners and Martins and Langeani (2012) to distinguished *H. piracanjuba*. This character is presented in both *Hisonotus* sp. 1 and *Hisonotus* sp. 2. However, our results showed that the number and shape of head plates can be considerably variable among specimens of a given species. We analyzed 18 stained and cleared specimens of *Hisonotus insperatus* from rio Capivara and rio Araquá from Botucatu, São Paulo State (Table II). Three individuals of *H. insperatus* presented a single rostral plate, instead of a pair of rostral plates (Table II), however, all specimens of *Hisonotus* sp. 1 and *Hisonotus* sp. 2 presented a pair of rostral plate. Variation in shape and number was further found in other head plates as the posterior rostrum plates, infraorbitals and preopercle plate (red arrows in Fig. 8). The fourth infraorbital in the right side is split in specimen of Fig. 8(C), what does not happen in the left side of the same specimen. This variation was also found in one paratype of *Hisonotus* sp. 1 (NUP 9839, 23 7 mm SL) in both sides of the specimen. Also, the first infraorbital of both sides in the specimen of Fig. 8(A), (B) reach the ventral margin of the rostrum, among the second and third posterior rostrum plates, what does not happen in specimen of Fig. 8(C), (D). Additionally, the size of the first infraorbital is variable among the specimens of *Hisonotus insperatus* and *Hisonotus* sp. 1. A similar pattern of variation was observed on posterior rostrum plates. In first and second posterior rostrum plates on the left side of the specimen of Fig. 8(C), (D), it appear to be split, what does not happen in the right side. Thus, the left side present six posterior rostrum plates series and the right side present just four plates. Finally, an extra plate is found among preopercle and compound pterotic perforated to infraorbital canal of the specimen of Fig. 8(C), (D).

Comparative material

All from Brazil, except when noticed: *Hisonotus aky*: MHNG 2643.039, 2, 33.1-34.2 mm SL, paratypes, arroio Fortaleza, Argentina; *Hisonotus bocaiuva*: MZUSP 112204,

440 male, 24.2 mm SL, holotype, córrego Cachoeira, Bocaiúva, Minas Gerais; LBP 9817, 9,

441 3 c&s, 18.3-23.2 mm SL, paratypes, córrego Cachoeira, Bocaiúva, Minas Gerais;

442 *Hisonotus carreiro*: MCP 40943, 3, 33.6-35.8 mm SL, arroio Guabiju, Guabiju, Rio

443 Grande do Sul; *Hisonotus charrua*: LBP 4861, 1, 35.9 mm SL, arroio Guaviyú, Artigas,

444 Uruguai; MHNG 2650.051, 1, 34.2 mm SL, paratype, arroio Aspinillar, Uruguay;

445 *Hisonotus chromodontus*: LBP 7964, 25, 24.0-28.3 mm SL, 3 females c&s, 26.5-28.9

446 mm SL, 1 male c&s 24.9 mm SL, rio dos Patos, Nova Mutum, Mato Grosso; LBP

447 12278, 2, 26.7-28.7 mm SL, 1 unsexed c&s, 26.7 mm SL, rio Sumidouro, Tangará da

448 Serra, Mato Grosso; MZUSP 45355, holotype, 25.9 mm SL, affluent rio Preto,

449 Diamantino, Mato Grosso; *Hisonotus depressicauda*: MZUSP 5383, 24.4 mm SL,

450 paralectotype (designated by Britski, 1969), Sorocaba; *Hisonotus francirochai*: LBP

451 13923, 22, 25.7-35.7 SL, córrego sem nome, Capitinga, Minas Gerais; MZUSP 3258,

452 29.4 mm SL, lectotype (designated by Britski 1969), rio Grande, São Paulo; *Hisonotus*

453 *heterogaster*: LBP 3335, 39, 20.8-30.1 mm SL, arroio sem nome, rio Grande, Rio

454 Grande do Sul; *Hisonotus insperatus*: LBP 1299, 3, 23.5-29.6 mm SL, 1 female c&s,

455 24.8 mm SL, rio Araquá, Botucatu, São Paulo; LBP 1316, 2, 24.1-27.4 mm SL, 1

456 female c&s, 24.7 mm SL, 1 male c&s, 23.9 mm SL, rio Araquá, Botucatu, São Paulo;

457 LBP 1344, 2, 22.9-24.9 mm SL, rio Araquá, Botucatu, São Paulo; LBP 1373, 1, 25.8

458 mm SL, rio Araquá, Botucatu, São Paulo; LBP 1405, 2, 22.2-27.3 mm SL, rio Araquá,

459 Botucatu, São Paulo; LBP 4699, 17, 19.6-26.9 mm SL, 4 females c&s, 20.3-26.8 mm

460 SL, 3 males c&s, 24.3-26.1 mm SL, ribeirão Cubatão, Marapoama, São Paulo; LBP

461 4945, 5, 27.3-28.5 mm SL, 2 females c&s, 28.2-29.9 mm SL, Botucatu, São Paulo; LBP

462 6770, 5, 25.1-28.2 mm SL, 3 females c&s, 20.0-27.0 mm SL, ribeirão Cubatão,

463 Marapoama, São Paulo; LBP 13336, 1 female c&s, 26.0 mm SL, rio Capivara,

464 Botucatu, São Paulo; LBP 13337, 2 females c&s, 27.4-28.6 mm SL, rio Araquá,

465 Botucatu, São Paulo; MZUSP 22826, paratype, 1, 25.4 mm SL, córrego Água Tirada,

466 Três Lagoas, Mato Grosso; MZUSP 24832, paratype, 1, 23.8 mm SL, rio Corumbataí,

467 Corumbataí, São Paulo; MZUSP 78957, holotype, 29.6 mm SL, rio Capivara, Botucatu,

468 São Paulo; MZUSP 78960, paratypes, 31, 12.6-26.0 mm SL, 5 c&s, 22.7-24.7 mm SL,

469 rio Pardo, Botucatu, São Paulo; MZUSP 78965, paratypes, 10, 15.6-28.6 mm SL, 3

470 c&s, not measured, rio Araquá, Botucatu, São Paulo; MZUSP 78968, paratypes, 5,

471 24.1-27.3 mm SL, córrego da Figueira, Lins, São Paulo; *Hisonotus iota*: LBP 13072, 5,

472 32.3-33.0 mm SL, rio Chapecó, Coronel Freitas, Santa Catarina; *Hisonotus laevior*:

473 LBP 3377, 1, 25.2 mm SL, arroio dos Corrientes, Pelotas, Rio Grande do Sul; LBP

474   6037, 8, 33.4-47.0 mm SL, rio Maquiné, Osório, Rio Grande do Sul; LBP 13187, 7,

475   19.4-45.8 mm SL, Córrego sem nome, Camaquá, Rio Grande do Sul; *Hisonotus*

476   *leucofrenatus*: LBP 2085, 7, 38.3-50.6 mm SL, rio Sagrado, Morretes, Paraná; LBP

477   6837, 36, 35.1-43.5 mm SL, rio Fau, Miracatu, São Paulo; *Hisonotus leucophrys*: LBP

478   13065, 6, 17.2-33.6 mm SL, rio Ariranhas, Xavantina, Santa Catarina; LBP 13073, 1,

479   36.8 mm SL, rio Guarita, Palmitinho, Rio Grande do Sul; *Hisonotus luteofrenatus*:

480   MZUSP 62593, holotype, 28.6 mm SL, córrego Loanda, Cláudia, Mato Grosso;

481   MZUSP 62594, paratype, 8, 22.4-30.5 mm SL, riacho Selma, Sinop, Mato Grosso;

482   MZUSP 95940, 3, 26.1-28.5 mm SL, affluent rio Teles Pires, Itaúba, Mato Grosso;

483   *Hisonotus maculipinnis*: BMNH 1909.4.2.19-22, 1, 27.0 mm SL, syntype, rio de La

484   Plata, Argentina; *Hisonotus megaloplax*: LBP 13108, 6, 36.4-37.8 mm SL, Córrego sem

485   nome, Saldanha Marinho, Rio Gande do Sul; *Hisonotus montanus*: LBP 13051, 3, 26.4-

486   27.2 mm SL, rio Goiabeiras, Vargem, Santa Catarina; LBP 13055, 5, 24.8-31.9 mm SL,

487   rio Canoas, Vargem, Santa Catarina; *Hisonotus nigricauda*: BMNH 1891.3.16.53-62, 1,

488   32.0 mm SL, syntype, Rio Grande do Sul; LBP579, 16, 34.1-40.1 mm SL, rio Guaíba,

489   Eldorado do Sul, Rio Grande do Sul; *Hisonotus notatus*: LBP 3472, 20, 21.0-34.3 mm

490   SL, rio Aduelas, Macaé, Rio de Janeiro; LBP 10742, 25, 24.4-43.3 mm SL, rio Macabu,

491   Conceição de Macabu, Rio de Janeiro; *Hisonotus paulinus*: BMNH 1907.7.6.9, 28.4

492   mm SL, holotype, rio Piracicaba, São Paulo; *Hisonotus piracanjuba*: NUP 5059, 1, 24.7

493   mm SL, córrego Posse, Anápolis, Goiás; NUP 10979, 3, 21.4-21.8 mm SL, ribeirão

494   Bocaina, Piracanjuba, Goiás; *Hisonotus prata*: MCP 40492, 18, 19.5-33.2 mm SL, rio

495   da Prata, Nova Prata, Rio Grande do Sul; LBP 9918, 14, 21.7-32.6 mm SL, Laguna dos

496   Patos system, Nova Prata, Rio Grande do Sul; *Hisonotus ringueleti*: FMNH 108806, 2,

497   25.7-32.2 mm SL, rio Quaraí basin, Uruguay; LBP 13148, 1, 24.5 mm SL, arroio Putiá,

498   Uruguaiana, Rio Grande do Sul. *Microlepidogaster arachas*: LBP 10882, 3, 22.8-35.3

499   mm SL, rio Paraná basin, Araxás, Minas Gerais; *Microlepidogaster dimorpha*: LBP

500   10683, 2, 28.8-35.6 mm SL; rio Paraná basin, Uberaba, Minas Gerais; *Otothyris*

501   *travassosi*: LBP 1971, 13, 14.0-27.2 mm SL; coastal drainage, Canavieiras, Bahia;

502   *Otothyropsis marapoama*: LBP 4698, 6, 23.9-36.3 mm SL; rio Tietê basin, Marapoama,

503   São Paulo. *Parotocinclus* cf. *bahiensis*: LBP 7182, 3, 27.9-35.6 mm SL; rio Paraguaçu

504   basin, Lençois, Bahia. *Parotocinclus maculicauda*: LBP 2869, 15, 20.2-44.7 mm SL, rio

505   Ribeira do Iguape basin, Miracatu, São Paulo; *Parotocinclus polyocrhus*: LBP 12272, 2,

506   21.2-22.6 mm SL, ribeirão Ínsula, Barra do Garça, Mato Grosso; *Parotocinclus prata*:

507 LIRP 1136, 38, 19.8-41.9 mm SL; rio São Francisco basin, Presidente Oligário, Minas

508 Gerais.

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606 **Table I.** Morphometrics and meristics of *Hisonotus* sp. 1 and *Hisonotus* sp. 2. SD =

607 Standard deviation.

	Hisonotus sp. 1 n = 27				*Hisonotus* sp. 2 = 15			
	Holotype	**Low-High**	**Mean**	**SD**	**Holotype**	**Low-High**	**Mean**	**SD**
Standard length (*LS*)	26.4	22.8-28.4	24.4	1.43	26.2	18.0-26.2	22.7	2.99
Percents of Standard length (*LS*)								
Head length	36.5	35.6-41.1	37.7	1.41	39.2	36.1-41.7	39.4	1.44
Predorsal length	46.8	45.3-52.1	48.3	1.51	47.9	46.9-51.8	49.0	1.54
Dorsal-fin spine length	22.4	22.4-28.3	24.5	1.62	25.4	25.2-27.0	26.2	0.50
Anal-fin unbranched ray length	18.7	16.3-21.3	19.2	1.34	18.2	17.4-21.4	19.8	0.87
Pectoral-fin spine length	23.6	21.6-27.6	24.7	1.57	27.5	27.0-30.1	28.2	0.53
Pelvic-fin unbranched ray length	18.4	16.8-23.2	20.6	1.45	18.7	18.0-21.1	19.7	0.98
Cleithral width	24.6	23.8-26.8	25.3	0.89	23.5	22.2-24.3	23.3	0.49
Thoracic length	18.4	17.6-21.6	19.0	0.80	18.8	16.1-19.8	17.8	1.12
Abdominal length	21.9	17.9-22.3	20.5	1.24	21.5	16.2-21.6	19.0	1.82
Body depth at dorsal-fin origin	21.1	18.6-23.9	21.6	1.25	18.8	16.9-20.7	18.1	1.30
Caudal-peduncle length	28.3	26.3-31.5	29.3	1.18	27.5	25.3-29.8	27.7	1.61
Caudal-peduncle depth	10.5	10.8-12.5	11.4	0.64	10.6	10.2-11.3	10.7	0.27
Percents of Head Length (*LH*)								
Snout Length	50.7	46.9-52.2	49.6	1.49	51.5	50.7-57.1	53.7	1.50
Orbital diameter	15.9	13.9-17.6	15.6	0.93	12.8	11.0-14.1	12.5	0.88
Interorbital width	35.2	32.1-37.1	34.9	1.52	32.8	32.4-36.0	34.2	1.21
Head depth	54.7	51.6-59.2	55.4	2.17	45.3	42.4-47.7	44.8	1.99
Suborbital depth	24.7	20.9-25.5	24.1	1.26	20.8	17.4-22.0	20.0	0.85
Mandibular ramus	11.2	6.8-12.9	10.7	1.12	6.0	6.0-8.0	6.8	0.57
Meristics	**Holotype**	**Low-High**	**Mode**	**SD**	**Holotype**	**Low-High**	**Mode**	**SD**
Left premaxillary teeth	13	11-18	14	2.0	10	6-10	8	1.37
Left dentary teeth	14	11-15	13	1.22	6	4-7	6	0.42
Left lateral scutes	24	24-25	24	0.64	24	24-25	24	0.48

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610 **Table II.** Table showing the rostral plates variation found in species of *Hisonotus*

611 *insperatus*.

	Voulcher No.	**Species**	**Sex**	**Standard length (SL)**	**Rostral plates No.**
1	LBP 1299	*Hisonotus insperatus*	female	24.8 mm SL	1
2	LBP 1316	*Hisonotus insperatus*	female	24.7 mm SL	2
3	LBP 1316	*Hisonotus insperatus*	male	23.9 mm SL	2
4	LBP 4699	*Hisonotus insperatus*	female	20.3 mm SL	2
5	LBP 4699	*Hisonotus insperatus*	female	22.0 mm SL	2
6	LBP 4699	*Hisonotus insperatus*	female	25.3 mm SL	2
7	LBP 4699	*Hisonotus insperatus*	female	26.8 mm SL	2
8	LBP 4699	*Hisonotus insperatus*	male	24.3 mm SL	2
9	LBP 4699	*Hisonotus insperatus*	male	25.0 mm SL	2

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10	LBP 4699	*Hisonotus insperatus*	male	26.1 mm SL	2
11	LBP 4945	*Hisonotus insperatus*	female	28.2 mm SL	2
12	LBP 4945	*Hisonotus insperatus*	female	29.9 mm SL	2
13	LBP 6770	*Hisonotus insperatus*	female	20.3 mm SL	2
14	LBP 6770	*Hisonotus insperatus*	female	20.0 mm SL	2
15	LBP 6770	*Hisonotus insperatus*	female	27.0 mm SL	1
16	LBP 13336	*Hisonotus insperatus*	female	26.0 mm SL	1
17	LBP 13337	*Hisonotus insperatus*	female	27.4 mm SL	2
18	LBP 13337	*Hisonotus insperatus*	female	28.6 mm SL	2

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Figure 1. *Hisonotus* sp. 1, holotype, MZUSP xx**,** female, 26.4 mm SL, from ribeirão Cambira, affluent rio Ivaí, upper rio Paraná basin, boundary between municipalities of Cambira and Apucarana, Paraná State, Brazil.
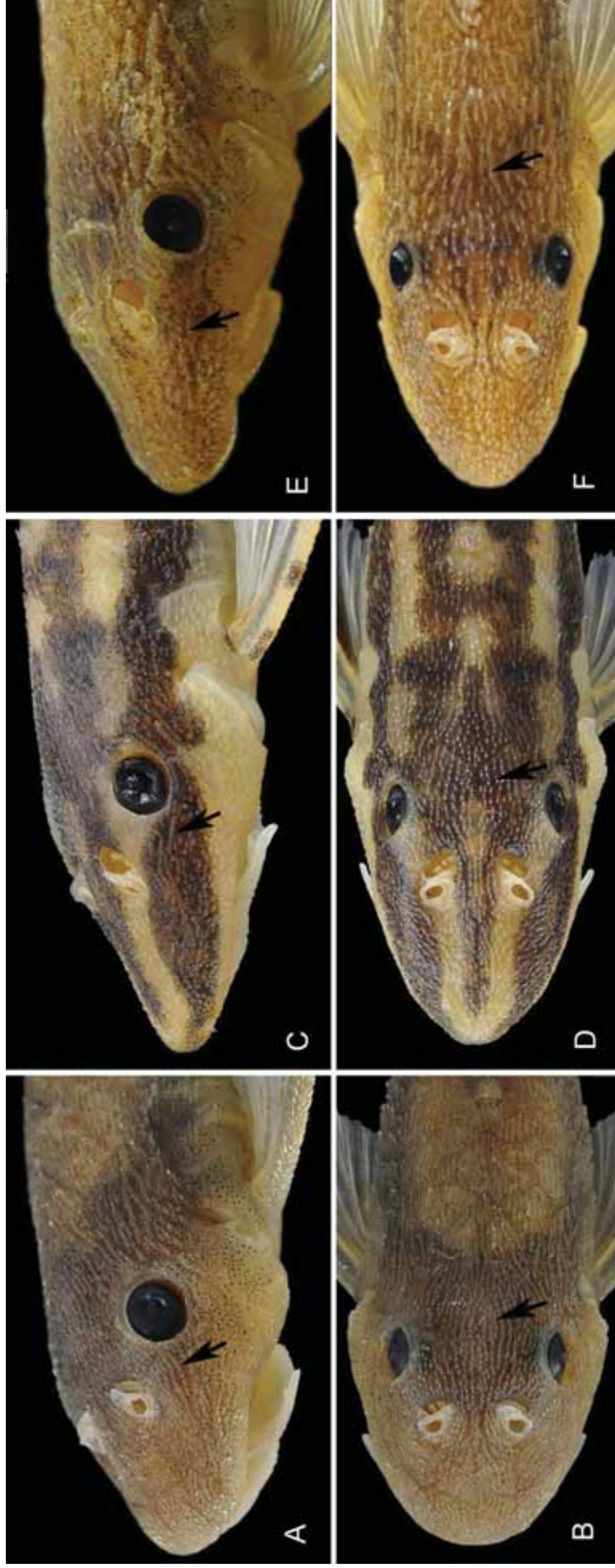
Figure 2. Picture showing the well-defined series of odontodes on the head. (A) *Hisonotus* sp. 1, paratype, NUP 9839, female, 25.8 mm SL, lateral view showing the small odontodes series; (B) *Hisonotus* sp. 1, paratype, NUP 9839, female, 25.8 mm SL, dorsal view showing the small odontodes series; (C) *Hisonotus* sp. 2, paratype, NUP 10928, male, 24.2 mm SL, lateral view showing the small odontodes series; (D) *Hisonotus* sp. 2, paratype, NUP 10928, male, 24.2 mm SL, dorsal view showing the small odontodes series. (E) *Hisonotus insperatus*, LBP 1316, 24.7 mm SL, lateral view showing the large and conspicuous series of odontodes; (F) *Hisonotus insperatus*, LBP 1316, 24.7 mm SL, dorsal view showing the large and conspicuous series of odontodes.
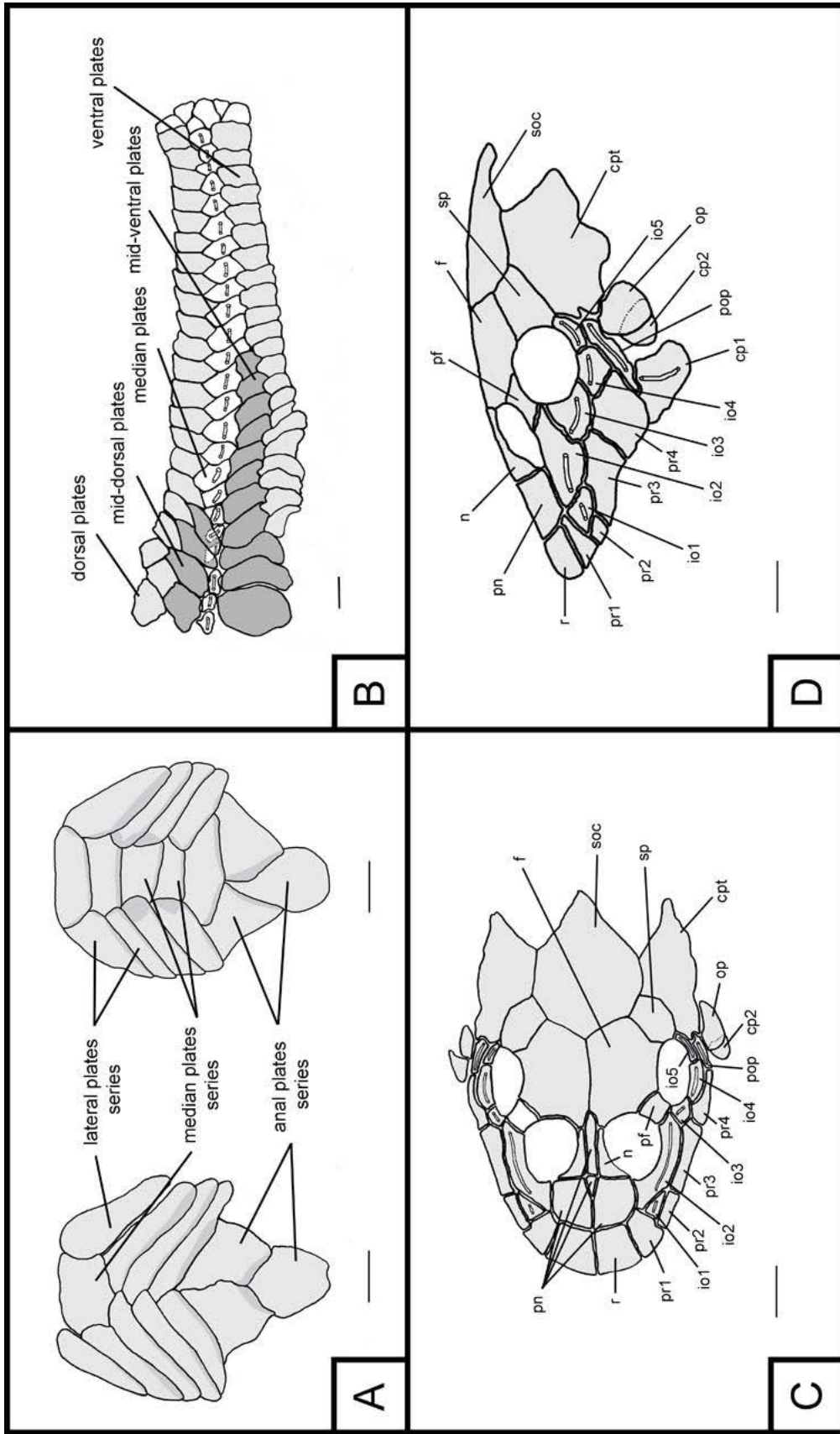
Figure 3. *Hisonotus* sp. 1, paratype, NUP 7065, unsexed, 24.5 mm SL; (A) Ventral view of abdominal region showing the two pattern of abdominal plates; (B) Trunk lateral dermal plate pattern; (C) Dorsal view of the head showing bone edges; (D) Lateral view of left side of head showing bone edges. Scale bars = 1 mm.

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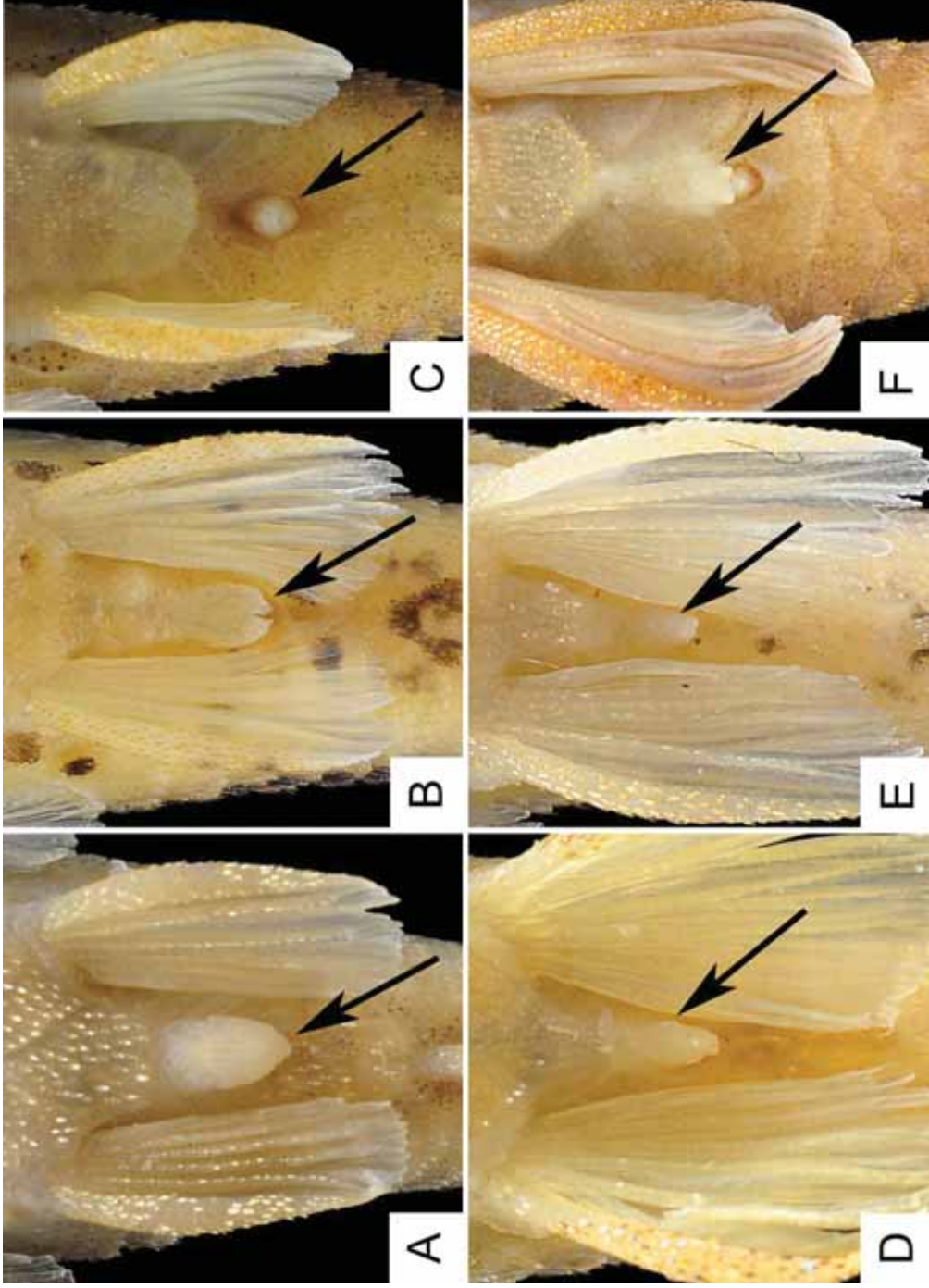
Figure 4. Ventral view of abdominal region, arrows indicate anus membrane. (A) *Hisonotus* sp. 1, MZUSP xx, holotype, female, 26.4 mm SL; (B) *Hisonotus* sp. 2, MZUSP xx, holotype, female, 26.2 mm SL; (C) *H. chromodontus*, LBP 7964, female, 28.1 mm SL; (D) *Hisonotus* sp. 1, NUP 3578, male, 27.1 mm SL; (E) *Hisonotus* sp. 2, NUP 10928, male, 24.2 mm SL; (F) *H. chromodontus*, LBP 12278, male, 26.7 mm SL.

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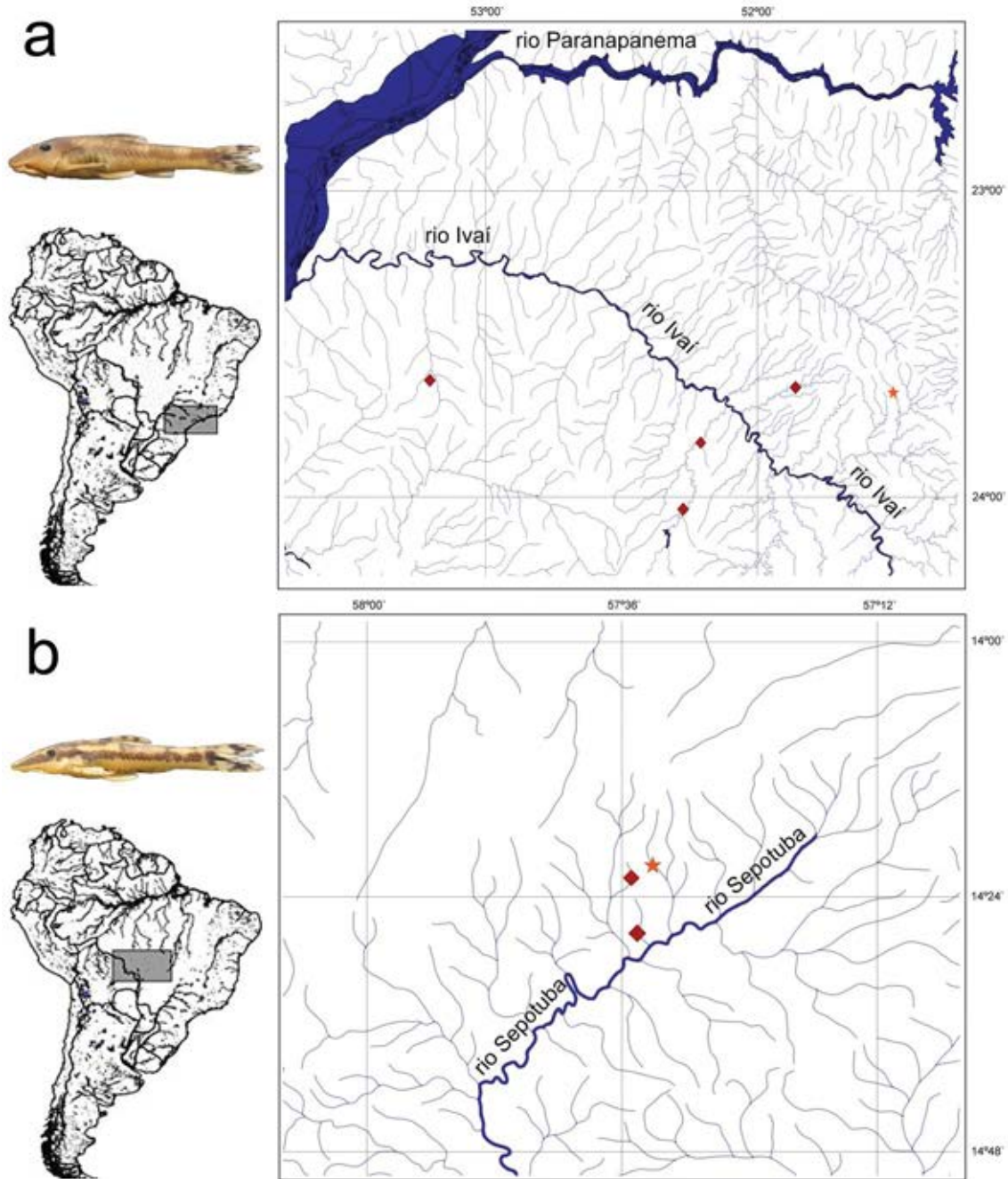
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649 **Figure 5.** (a) Map of the distribution of (a) *Hisonotus* sp. 1. Star = holotype locality,
650 ribeirão Cambira. Diamonds = paratype localities; (b) *Hisonotus* sp. 2. Star = holotype
651 locality, riacho Águas Claras. Diamond = paratypes locality.
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653
654 **Figure 6.** *Hisonotus* sp. 2, holotype, MZUSP xx, female, 26.2 mm SL, riacho Águas
655 Claras, affluent rio Sepotuba, rio Paraguay basin, municipality of Santo Afonso, Mato
656 Grosso.
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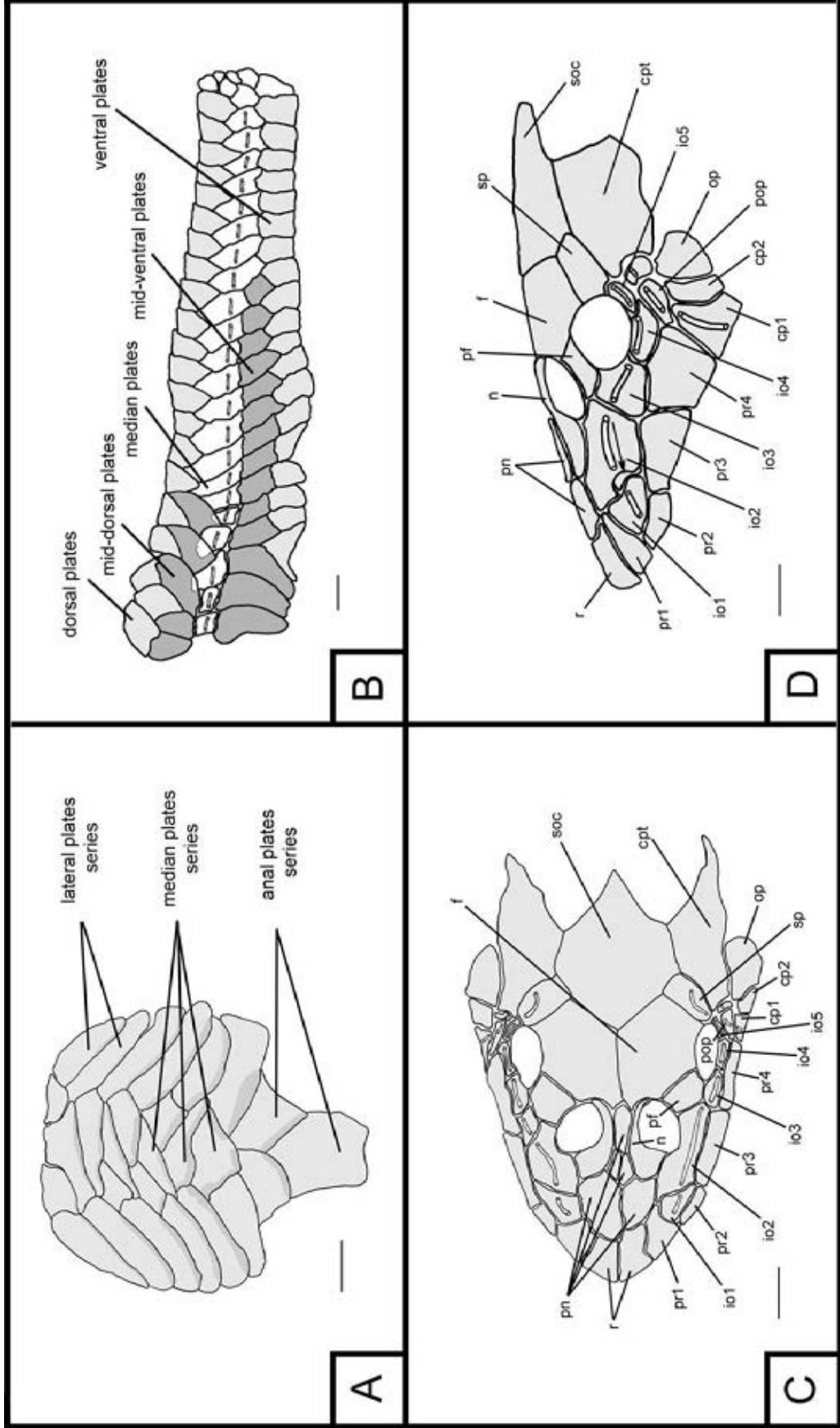
Figure 7. *Hisonotus* sp. 2, paratype, NUP 10928, male, 24.2 mm SL; (A) Ventral view of abdominal region showing the abdominal plates; (B) Trunk lateral dermal plate pattern; (C) Dorsal view of the head showing bone edges; (D) Lateral view of left side of head showing bone edges. Black arrows (D) indicate an extra plate absent in the right side of this same specimen. Scale bars = 1 mm.

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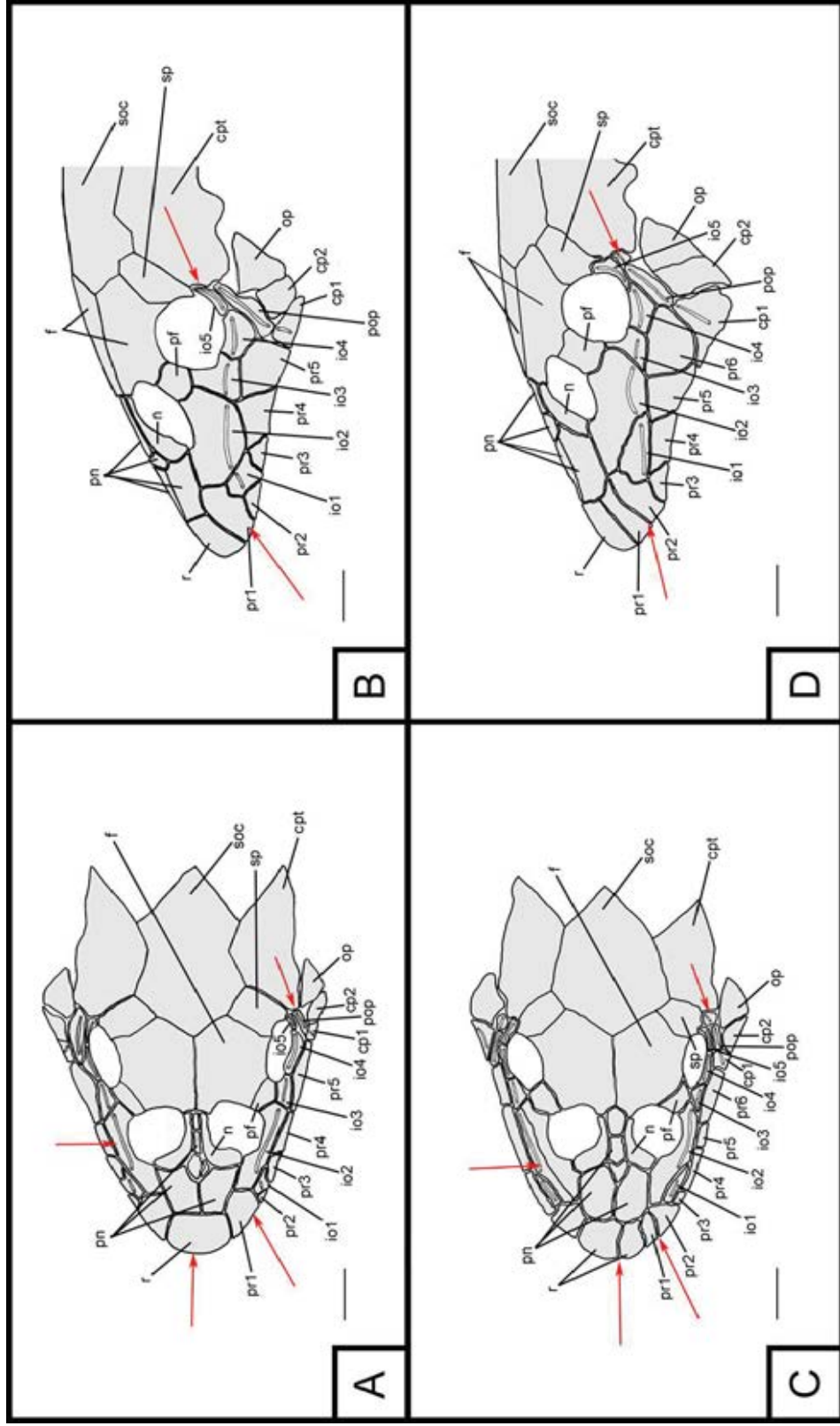
Figure 8. (A) Dorsal view of the head showing bone edges of *Hisonotus insperatus*, LBP 13336, female, 26.0 mm SL, from rio Capivara, Botucatu, São Paulo State; (B) Lateral view of left side of head showing bone edges of *H. insperatus*, LBP 13336, female, 26.0 mm SL, from rio Capivara, Botucatu, São Paulo State; (C) Dorsal view of the head showing bone edges of *H. insperatus*, LBP 13337, female, 28.6 mm SL, from rio Araquá, Botucatu, São Paulo State; (D) Lateral view of left side of head showing bone edges of *H. insperatus*, LBP 13337, female, 28.6 mm SL, from rio Araquá, Botucatu, São Paulo State. Red arrows indicate the differences found in osteology between samples. Scale bars = 1 mm.

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Chapter 2

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3   **River capture promotes diversification in the Neotropical cascudinhos**

4   **Hypoptopomatinae, Neoplecostominae and Otothyrinae (Siluriformes:**

5   **Loricariidae)**

6

7   **Abstract**

8   The main objective of this study is estimate a species-dense, time-calibrated molecular

9   phylogeny of Hypoptopomatinae, Neoplecostominae and Otothyrinae, which together

10   comprise a armoured catfishes group widely distributed across the South American, to

11   place the origin of major clades, and to demonstrate the role of river capture on patterns

12   of diversification in these taxa. We used a maximum likelihood and Bayesian methods

13   to estimate a time-calibrated phylogeny of 114 loricariid species, using three

14   mitochondrial and one nuclear gene to generate a matrix of 4,500 base pairs, and

15   parametric biogeographic to estimate ancestral geographic ranges and to infer the

16   effects of river capture events on the geographic distributions of these taxa. Our analysis

17   revealed that Hypoptopomatinae, Neoplecostominae and Otothyrinae are recovered as

18   monophyletic with strong statistical support, and Neoplecostominae is found to be more

19   closely related to Otothyrinae than to Hypoptopomatinae. Our time-calibrated

20   phylogeny and ancestral area reconstructions indicate an origin of Hypoptopomatinae,

21   Neoplecostominae and Otothyrinae during the Lower Eocene in the Atlantic Coastal

22   Drainages, from which it is possible to infer several dispersal events to adjacent river

23   basins during the Neogene. As conclusion we infer a strong influence of river capture

24   in: (1) the accumulation of modern clade species-richness values; (2) the formation of

25   the modern basin-wide species assemblages, and; (3) the presence of many low-

26   diversity, early-branching lineages restricted to the Atlantic Coastal Drainages. We

27   further infer the importance of headwater stream capture and marine transgressions in

28   shaping patterns in the distributions of Hypoptopomatinae, Neoplecostominae and

29   Otothyrinae throughout South America.

30   **Keywords:** freshwater, Neotropics, molecular systematics, parametric biogeography,

31   river capture, relaxed clock, geodispersal

32

33   **Introduction**

A central aim of research in modern historical biogeography is to understand the distributions of species and ecosystems in light of Earth history processes that shape landscape evolution (Cox & Moore 2005; Lomolino *et al.* 2010). The effort to understand biotic diversification in light of Earth history processes has made rapid progress over the past decade in the study of Neotropical freshwater fishes. The continental fishes of tropical South America represent about one in five of all the world's fish species, or 10% of all vertebrate species (Vari & Malabarba 1998; Albert *et al.* 2011). The evolutionary and ecological reasons for the origins and maintenance of this high diversity remains incompletely understood. However the role of watersheds boundaries as dispersal filters is increasingly being recognized as an important landscape feature of river drainage networks, that serves to isolate lineages and promote diversification (Waters *et al.* 2006; Winemiller *et al.* 2008; Albert & Crampton 2010).

From a macroevolutionary perspective, the total number of species lineages that inhabit a biogeographic region is the accumulated result of speciation and dispersal events, which in combination act to increase the total species count, and extinction events, which act to reduce the species count (Stanley 1998; Jablonski *et al.* 2006). In this literature care has been taken to distinguish the terms 'dispersal' and 'dispersion', as two closely related but distinct phenomena (Platnick 1976; Armstrong 1977; Lomolino *et al.* 2010). 'Dispersal' refers to the colonization of new areas outside an established species range (i.e. species range expansion), whereas 'dispersion' refers to movements of individual organisms within an established species range. This distinction is important because 'dispersal', but not 'dispersion', is the macroevolutionary process that affects patterns at and above the species level. This distinction also specifies the biological meaning of the parameter called 'dispersal' in the Dispersal-Extinction-Cladogenesis (DEC) model of geographic range evolution (Ree *et al.* 2005; Ree & Smith 2008). Under this definition, dispersal can arise from either 'biotic dispersal', meaning the movement of organisms to new geographic areas outside the species' ancestral range, or from 'geodispersal', meaning the erosion of barrier(s) (i.e. merging) of adjacent geographic areas (Lieberman & Eldredge 1996; Lieberman 2008). In other words, a geodispersal event can connect formerly separated areas and affect geographic ranges without necessarily involving biotic dispersal.

Among obligate freshwater organisms, lineage diversification is strongly affected by patterns of connectivity among portions of adjacent river basins (Smith 1981; Hocutt & Wiley 1986; Mayden 1988; Lundberg *et al.* 1998). River capture (also

68   called stream capture or stream piracy) is a geomorphological process by which the

69   flow of part of a stream or river drainage basin is diverted into that of a neighbouring

70   basin. River capture is an important process in landscape evolution that allows aquatic

71   species to move, or disperse, between adjacent drainage basins. River capture may arise

72   from the influence of several geomorphological processes, including tectonic uplift or

73   tilting, damming by the actions of glaciers or landslides, denudation of watershed

74   margins by erosion, or avulsion of watershed margins by sediment accumulation in fans

75   and estuaries (Almeida & Carneiro 1998; Bishop 1995; Wilkinson *et al.* 2006, 2010). In

76   reviewing the geological history of eastern South America, Ribeiro (2006) concluded

77   that river capture affected the distributional ranges of many fish taxa on the Brazilian

78   shield.

79   The biogeographic consequences of river capture for an *in situ* aquatic biota are

80   unique. River capture simultaneously separates portions of river basins that were

81   formerly connected (i.e., vicariance) and connects portions of river basins that were

82   formerly isolated (i.e., geodispersal). In effect, river capture acts to move the physical

83   location of watershed barriers (Albert & Crampton 2010). The consequences of river

84   capture can be profound for species such as obligate freshwater fishes and amphibians

85   that are restricted to river basins, and for which watershed boundaries strongly limit

86   dispersal (Grant *et al.* 2007; Muneepeerakul *et al.* 2008; Bertuzzo *et al.* 2009). As in all

87   vicariance events, the separation of formerly adjacent river basin segments promotes

88   allopatric divergence and speciation. However, in addition, and unlike many vicariance-

89   only events, *river capture always results in both the separation and the merging of*

90   *adjacent river basin segments* (Albert *et al.* 2011; Carvalho & Albert 2011). In other

91   words, in the special case of river capture, vicariance and geodispersal are near

92   simultaneous and complementary biogeographic processes. Further, both vicariance and

93   geodispersal may result in concordant biogeographic patterns among many lineages that

94   constitute a regional biota, (see fig. 10 in Lieberman 2008; Albert & Carvalho 2011).

95   Importantly, in the case of river capture, and under the widely-used convention that

96   geographic range is defined in terms of the river basin(s) a species occupies (e.g. Reis *et*

97   *al.* 2003; Abell *et al.* 2008), geodispersal can result in geographic range expansion

98   without necessarily involving biotic dispersal (Ribeiro *et al.* 2013).

99   Among Neotropical freshwater fishes, members of the family Loricariidae,

100 armoured catfishes, represent an excellent model to study the effects of landscape

101 evolution on lineage diversification. Loricariids inhabit most aquatic habitats and

geographic regions of tropical South and Central America. About 869 loricariid species are currently recognized as valid (Eschmeyer & Fong 2013), which makes this taxon the second-most species-rich family of Neotropical freshwater fishes (after Characidae). Loricariids also exhibit a broad range of ecological tolerances and geographic distributions. Many species are extreme habitat or trophic specialists (Langeani 1990; Armbruster 1998; Covain & Fish-Miller 2007; Nelson *et al.* 1999; Sabaj 1999; Armbruster 2004), and many species are highly endemic, with small geographic ranges (Bizerril 1994; Ribeiro 2006; Albert & Carvalho 2011).

Within the Loricariidae the three subfamilies, Hypoptopomatinae, Neoplecostominae and Otothyrinae, have long been recognized together as natural group using morphological and molecular data (Schaefer 1991, 1998; Armbruster 2004; Chiachio *et al.* 2008; Cramer *et al.* 2008, 2011). Separated these three subfamilies were hypothesized to form monophyletic groups Chiachio *et al.* (2008) using molecular data. No formal infrafamily name has yet been applied to this clade, which we will refer to here as the HNO-clade. Each of these three subfamily-level clades is also species-rich, and the HNO-clade as a whole has 182 nominal species currently recognized (Eschmeyer & Fong 2013). Each of these subfamilies exhibits a wide geographic distribution throughout tropical cis-Andean South America, and has a lengthy and complex taxonomic histories, including studies using both morphological and molecular datasets (Eigenmann & Eigenmann 1890; Regan 1904; Gosline 1947; Isbrücker 1980; Howes 1983; Schaefer 1987; Montoya-Burgos *et al.* 1998; Armbruster 2004; Reis *et al.* 2006; Chiachio *et al.* 2008; Cramer *et al.* 2008, 2011; Roxo *et al.* 2012a,b).

Here we present a time-calibrated phylogenetic analysis of the loricariid catfish subfamilies Hypoptopomatinae, Neoplecostominae and Otothyrinae, using a combination of three mitochondrial and one nuclear gene markers, and the most species-dense taxon sampling of these groups to date. We then use parametric biogeographic methods to estimate ancestral geographic ranges, and to document several historical river-capture events in the region of Southeastern Brazil. Our results highlight the special role of river capture in the formation of the modern species richness and geographic distributions of the Hypoptopomatinae, Neoplecostominae and Otothyrinae.

Material and Methods

Taxon Sampling

Diplomystes mesembrinus (Diplomystidae) was used as a distant outgroups to root all phylogenies. Diplomystidae has been alternatively been reported as the sister group to all other catfishes, or as the sister group to Siluroidea, a clade of catfishes that excludes Loricarioidea (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, subfamily Delturinae), *Rineloricaria lanceolata*, *Spatuloricaria* sp. 1 (Loricariidae, subfamily Loricariinae), *Hypostomus ancistroides*, *H. nigromaculatus* and *H. microstomus* (Loricariidae, subfamily Hypostominae) were included in the analysis as additional outgroups (see table S1 to all species names and localities and table S2 to taxonomic summary of ingroup species).

All fishes examined were collected in accordance with Brazilian laws, under a permanent scientific collection license in the name of Dr. Claudio Oliveira. After collection, animals were anesthetized with benzocaine, and a piece of muscle tissue was extracted from the right side of the body and preserved in 95% ethanol. Voucher specimens were fixed in 10% formalin for two weeks, and then transferred to 70% ethanol for permanent storage.

Vouchers of all samples were deposited in the collection of the Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil, Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul (MCP), Porto Alegre, Rio Grande do Sul, Brazil; Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUP), Universidade Estadual de Maringá, Paraná, Brazil, or the Museum of Natural History of the City of Geneva (MHNG), Geneva, Switzerland.

DNA Extraction and Sequencing

Total DNA was extracted from ethanol preserved muscle samples with the DNeasy Tissue Kit (Qiagen), following manufacturer's instructions. Partial sequences of the genes 16S rRNA, cytochrome *b* (Cytb), cytochrome c oxidase subunit I (COI) and F-reticulon 4 were amplified using polymerase chain reaction (PCR) with the primers described in Table S3. Amplifications were performed in a total volume of 12.5

169 μl with 1.25 μl of 10X buffer (10 mM Tris-HCl+15 mM MgCl2), 0.5 μl dNTPs (200

170 nM of each), 0.5 μl each 5 mM primer, 0.05 μl Platinum® *Taq* Polymerase (Invitrogen),

171 1 μl template DNA (12 ng), and 8.7 μl ddH2O. The PCR reactions consisted of 30 - 40

172 cycles, 30 s at 95°C, 15-30 s at 48-58°C (according to primer and species), and 45 - 90 s

173 at 72°C (according to gene primers). Nested-PCRs were used to amplify the nuclear

174 markers; the first amplification was performed using the primers Freticul4-D and

175 Freticul4-R with a total volume of 12.5 μl for 30 - 40 cycles (30 s at 95°C, 30 s at 48°C,

176 and 135 s at 72°C); the second amplification was performed using the primers Freticul4

177 D2 and Freticul4 R2 with a total volume of 12.5 μl for 30 - 40 cycles (30 s at 95°C, 30 s

178 at 53 - 54°C, and 135 s at 72°C). All PCR products were first visually identified on a

179 1% agarose gel and then purified using ExoSap-IT® (USB Corporation) following

180 instructions of the manufacturer. The purified PCR products were sequenced using the

181 "Big DyeTM Terminator v 3.1 Cycle Sequencing Ready Reaction Kit" (Applied

182 Biosystems), purified again by ethanol precipitation and loaded on an automatic

183 sequencer 3130-Genetic Analyzer (Applied Biosystems) in the Instituto de Biociências,

184 Universidade Estadual Paulista, Botucatu, São Paulo.

185

186 *Sequence and Phylogenetic Analysis*

187   All individual sequences for each species were initially analysed using the

188 software program BioEdit 5.0.9 (Hall 1999) and consensus sequences were obtained.

189 All sequences for each gene were independently aligned using MUSCLE (Edgar 2004)

190 under default parameters and the alignments inspected by eye for any obvious

191 misalignments. Only alignment errors were corrected, where indels of 1 bp were added

192 to introns of the reticulon gene. A quality control step was included in our workflow to

193 detect potential cases of sequencing errors due to contamination or paralogy.

194 Alignments for each gene were initially analysed by Maximum Likelihood (ML)

195 (Stamatakis *et al.* 2008) using the web servers RAxML BlackBox (Stamatakis 2006) for

196 a previous phylogenetic analysis, and controlling potential sequencing errors involving

197 pseudogenes, paralogous copies or even laboratory cross-contamination or mistakes

198 during the sequencing process.

199   Sequences that were found misplaced in the resulting gene tree (as, for example,

200 species of one subfamily grouped with species of an obviously non-related subfamily)

201 were re-sequenced. Nucleotide variation, substitution patterns, and genetic distances

202 were examined using MEGA 5.0 (Tamura *et al.* 2007). To evaluate the occurrence of

203 substitution saturation for each gene separately, we estimated the index of substitution

204 saturation (Iss) in DAMBE 5.2.31 (Xia & Xie 2001), as described by Xia *et al.* (2003)

205 and Xia and Lemey (2009) and the rate of transitions/transversions evaluated in the

206 software DAMBE 5.2.31 (Xia & Xie 2001). The Iss estimation was performed without

207 taking into account gaps because unresolved sites reduce the ability of the method to

208 test for phylogenetic signal. The best-fit partitioning schemes and the best nucleotide

209 evolution model for each partition were evaluated in the software Partition Finder

210 (Lanfear *et al.* 2012) under the information-theoretic measure of Akaike Information

211 Criterion (AICc).

212 Maximum Likelihood analyses were performed using RAxML Web-Servers

213 (Stamatakis *et al.* 2008). RAxML implements a faster algorithm of heuristic searches

214 with bootstrap pseudoreplicates (RBS). Bootstrap (BS) resampling (Felsenstein 1985)

215 was applied to assess support for individual nodes using 1,000 replicates. Random

216 starting trees were used for each independent ML tree search and all other parameters

217 were set on default values. The ML analyses were conducted under different models for

218 each partition of the matrix as evaluated for the software PartitionFinder (Lanfear *et al.*

219 2012) (Table S4). Bayesian Inference (BI) (Huelsenbeck & Ronquist 2001) was

220 performed evaluating alternative tree topologies through the estimation of posterior

221 probabilities (P) using MrBayes v.3.0 (Ronquist & Huelsenbeck 2003). The Bayesian

222 Inference was conducted under different models for each partition of the matrix as

223 evaluated for the software PartitionFinder (Lanfear *et al.* 2012) (Table S4). The ML tree

224 obtained from ML analysis was used as a starting three for the Marko searches. Eight

225 chains were run simultaneously for 100,000,000 generations and every 1000th

226 generation, a tree was sampled. The above analysis was performed twice. The

227 distribution of log-likelihood scores was examined to determine stationary phase for

228 each search and to decide if extra runs were required to achieve convergence, using the

229 program Tracer 1.5 (Rambaut & Drummond 2007a). All sampled topologies beneath

230 the asymptote (25,000,000 generations) were discarded as part of a burn-in procedure,

231 and the remaining trees were used to construct a 50% majority-rule consensus tree in

232 Paup* (Swofford 2003).

233 Alternative tree topologies were evaluated in the program Treefinder (Jobb *et al.*

234 2004) using the Shimodaira and Hasegawa (SH) test (Shimodaira & Hasegawa 1999),

235 the Approximately Unbiased (AU) test (Shimodaira, 2002), and the Expected

236 Likelihood Weights (ELW) method (Strimmer & Rambaut 2002). All tests were

237 conducted under ML with a 14 partition scheme and with the same model implemented

238 in RAxML analysis (Table S4).

239

240 *Time Calibration and Ancestral Area Reconstruction*

241   Estimates of branch lengths and ancestral biogeographic ranges were conducted

242 in parallel using BEAST v.1.7.5. All clade-age estimates are presented as the mean and

243 95% highest posterior density (HPD) values, which are the upper and lower bounds of

244 the HPD interval. The HPD is a credible set that contains 95% of the sampled values.

245 We included two calibration points to constrain divergence dates for the 154 clades

246 identified in our phylogenetic tree. The first calibration point was implemented as a

247 normally-distributed prior, with an offset of 125 million years ago (Ma), and a standard

248 deviation of 15 million years. These date-estimate parameters were selected to match

249 current knowledge of the timing of siluriform origins. Information from the

250 stratigraphic record and geographic distributions of living taxa indicate an origin for

251 Siluriformes as a whole during the Lower Cretaceous (145 – 100 Ma; Lundberg 1993;

252 Sullivan *et al.* 2006; Lundberg *et al.* 2007).

253   The second calibration point was implemented using a log-normal prior offset to

254 55 Ma with a mean and standard deviation of 1 for the origin of the family

255 Callichthyidae. The oldest known callichthyid fossil, *Corydoras revelatus* Cockerell

256 (1925) was dated by Marshall *et al.* (1997) as Paleocene. This prior assumed 55 Ma as a

257 minimum age. We used a macroevolutionary Birth–Death model for the diversification

258 likelihood values and a starting tree obtained from the RAxML analysis. The analyses

259 were conducted under different models of molecular evolution for each partition of the

260 data matrix as evaluated by the software PartitionFinder (Lanfear *et al.* 2012) (Table

261 S4). The ML tree obtained was used as a starting three for the MCMC searches. The

262 analysis was run for 50 million generations and sampled every 1000th generation.

263 Stationarity and sufficient mixing of parameters (ESS>200) was checked using Tracer

264 v1.5 (Rambaut & Drummond 2007a). A consensus tree was built using TreeAnnotator

265 v1.7.5 (Rambaut & Drummond 2007b).

266   Data on the geographic distributions of species in each of the three subfamilies

267 analysed here (Hypoptopomatinae, Neoplecostominae and Otothyrinae) were taken

268 from the original descriptions and Eschmeyer (2013). Species distribution ranges were

269 located within the following five biogeographic regions: A, Coastal Drainages of

270 Southeastern Brazil; B, Upper Paraná Basin; C, Paraguay, Lower Paraná and Uruguay

271 basins; D, Amazon and Orinoco basins; E, São Francisco basin and Coastal Drainages

272 of Northeastern of Brazil.

273 A maximum-likelihood analysis of biogeographic history was also performed in

274 Lagrange v2.0 (Ree *et al.* 2005; Ree & Smith 2008) using a DEC model of geographic

275 range evolution. The DEC model specifies instantaneous transition rates between

276 discrete distribution areas along the branches of a phylogenetic tree, and uses these rates

277 to assess the likelihoods of ancestral distributions at cladogenetic events (Ree *et al.*

278 2005; Ree & Smith 2008). Four DEC models were tested to estimate distribution ranges

279 inherited by the descending lineages at each node of the tree (see Table S5 for the

280 likelihood values of each model). The model that obtained the highest ML values (M3)

281 constrained dispersal rates between areas separated by one intercalated area at 0.5 the

282 likelihood of dispersal between adjacent areas, and areas separated by two intercalated

283 areas at 0.0001 the likelihood of models between adjacent areas.

284

285 **Results**

286 *Phylogenetic Analysis*

287 Partial sequences of three mitochondrial genes (16S rRNA, COI, Cytb) and one

288 nuclear gene (F-reticulon 4) were obtained from 155 specimens representing 114

289 loricariid species (Table S1). The combined sequence data resulted in a matrix of

290 exactly 4,500 base pairs (bp), of which 1,482 bp (33%) were non-variable (conserved),

291 2,677 bp (59%) were variable and included in the analysis, and 341 bp (8%) were

292 variable indels excluded from the analysis. This matrix was used to perform all

293 phylogenetic and biogeographic analyses and was partitioned by gene and coding

294 positions into 14 sections (Table S4). These data were not saturated considering that the

295 Iss.c value is greater than the Iss, and the R^2 value is greater than 0.70 for transitions

296 and transversions for all the genes (Table S6).

297 Bayesian and ML phylogenetic analyses resulted in very similar topologies

298 (Figs. 1–4). Our results illustrate that the clades Hypoptopomatinae, Neoplecostominae

299 and Otothyrinae are monophyletic with strong statistical support (BS=96, P=0.99 for

300 Hypoptopomatinae; BS=99, P=1.00 for Neoplecostominae; BS=96, P=0.99 with BI for

301 Otothyrinae). Additionally, our results suggest that Neoplecostominae is more closely

302 related to Otothyrinae than to Hypoptopomatinae (BS=98, P=0.99), and that these two

303 clades together form the sister group to Hypoptopomatinae to the exclusion of other

304 Loricariidae (BS=97, P=1.00). Tree topology tests rejected the hypothesis that

Otothyrinae and Hypoptopomatinae are sister groups (as proposed by Schaefer 1991 and 1998) in two (ELW and AU) of the three tests performed (Table S7). The hypothesis that Otothyrinae and Hypoptopomatinae are sister taxa was not supported by the SH test, but this test is considered less reliable than the AU test for the same datasets (Shimodaira 2002).

Within Hypoptopomatinae all examined genera were recovered as monophyletic with strong statistical support values (BS=69, P=1.0 for *Otocinclus*; BS=97, P=1.0 for *Oxyropsis*; BS=100, P=1 for *Acestridium*; BS=100, P=1 for *Hypoptopoma*). *Otocinclus* was recovered as the sister group of *Lampiella gibbosa*, and these taxa together formed the sister group to a clade consisting of *Oxyropsis*, *Acestridium* and *Hypoptopoma*. *Acestridium* and *Hypoptopoma* group together as the sister group to *Oxyropsis*.

Within Neoplecostominae *Kronichthys* and *Isbrueckerichthys* were recovered as monophyletic with high statistical support (BS=100, P=1.0 for *Kronichthys*; BS=69, P=0.99 for *Isbrueckerichthys*), however *Pareiorhaphis*, *Pareiorhina* and *Neoplecostomus* were not recovered as monophyletic. The topology tests rejected the hypothesis of a monophyletic *Neoplecostomus* and *Pareiorhina* (Table S7). *Pareiorhaphis splendens* formed the sister group to species of *Kronichthys,* and this group formed the sister taxon to other species of *Pareiorhaphis*.

Within Otothyrinae *Corumbataia*, *Schizolecis*, *Rhinolekos* and *Epactionotus* were monophyletic with high statistical support (BS=100, P=1.0 for *Corumbataia*; BS=100, P=1.0 for *Schizolecis*; BS=100, P=1.0 with BI for *Rhinolekos*; BS=92, P=1.0 for *Epactionotus*). The genera *Hisonotus*, *Parotocinclus* and *Pseudotothyris* were not monophyletic. There are four lineages within the subfamily Otothyrinae that include species currently assigned to *Hisonotus*. The first lineage includes the species *Hisonotus insperatus*, *H. piracanjuba*, *Hisonotus* sp. 4, *Hisonotus* sp. 5, *Hisonotus* sp. 6, and *Hisonotus* sp. 7 and is supported by high statistical support values (BS=100 with ML and P=1). The second lineage is composed of the species *Hisonotus chromodontus*, *Hisonotus* sp. 1, *Hisonotus* sp. 2, *Hisonotus* sp. 3, *Parotocinclus aripuanensis*, *Parotocinclus* aff. *spilurus*, and *Parotocinclus* sp. 3. The third lineage is composed of *Hisonotus depressicauda*, *H. francirochai* and *H. paulinus,* and is supported by high statistical support values (BS=99, P=1.0). The fourth lineage is composed of the most number of *Hisonotus* species in this analysis, including *Hisonotus aky*, *H. iota*, *H. montanus*, *H. megaloplax*, *H. prata*, *H. carreiro*, *H. ringueleti*, *H. nigricauda*, *H. heterogaster*, *H. notopagos*, *H.* cf. *charrua*, *H. laevior*, *H. charrua*, *H. leucophrys*, *H.*

339 *leucofrenatus*, *H. taimensis*, *H. notatus* and *H. armatus*, and for species *Otothyropsis*

340 *marapoama*, *Eurycheilichthys* sp. 1, *Epactionotus bilineatus*, *E. itaimbezinho* and *E.*

341 *gracilis,* and is supported by high statistical support values (BS=72, P=0.99).

342

343 ***Relaxed Clocks and Historical Biogeography***

344   Our time tree (Figs. 5–7) is the most comprehensive study of its kind to date

345 including 114 loricariid species in the subfamilies Hypoptopomatinae,

346 Neoplecostominae and Otothyrinae. The mean substitution rate for the dataset estimated

347 using BEAST is 0.272% per MY. The Hypoptopomatinae is estimated by BEAST to

348 have originated during the Lower Eocene about 33.6–67.4 Ma 95% HPD (mean 49.9

349 Ma), and is inferred by Lagrange to have originated in the Coastal Drainages region

350 (Fig. 5, Region A). The clade composed of Neoplecostominae + Otothyrinae is

351 estimated by BEAST to also have originated during the Lower Eocene about 31.0–62.2

352 Ma 95% HPD (mean 45.9 Ma), and is also inferred by Lagrange to have originated in

353 the Coastal Drainages region (Fig. 5, Region A).

354   Hypoptopomatinae is distributed across three of the geographic regions in Fig. 5:

355 Atlantic Coastal Drainages (Region A), Paraguay, Lower Paraná and Uruguay Basins

356 (Region C), and Amazon and Orinoco Basins (Region D). The ancestral area

357 reconstructions suggests that the Hypoptopomatinae moved from Coastal Drainages

358 (Region A) to the Amazon and Orinoco Basins (Region D) between 26.7–58.6 Ma 95%

359 HPD (mean 42.5 Ma). The relationships among hypoptopomatine taxa in the Amazon

360 and Paraguay basins also suggest that these two regions were either connected or

361 exchanged headwaters at about 15 Ma (Fig. 6).

362   Neoplecostominae is also distributed across three of the regions in Fig. 5:

363 Coastal Drainages (Region A), Upper Paraná Basin (Region B) and São Francisco Basin

364 and Northeastern Drainages (Region E). The ancestral lineage of *Neoplecostomus*

365 (except *N. ribeirensis*), *Pareiorhina carrancas* is inferred to have reached the Upper

366 Paraná Basin from the Coastal Drainages at c. 14.2–33.4 Ma 95% HPD (mean 22.9

367 Ma). The ancestor of *Pareiorhina carrancas* reached the Upper Paraná Basin from the

368 Coastal Drainages at c. 1.7–8.7 Ma 95% HPD (mean 4.6 Ma). The ancestor of

369 *Neoplecostomus franciscoensis* reached the São Francisco basin from Coastal Drainages

370 at c. 3.9–13.1 Ma 95% HPD (mean 7.5 Ma). The ancestor of *Pseudotocinclus tietensis*

371 reached the Upper Paraná Basin (B) from the Coastal Drainages region (A) about 0.4–

372 5.5 Ma 95% HPD (mean 2.3 Ma). The ancestral lineage of *Pareiorhaphis eurycephalus*,

373   *P. hystrix*, *P. parmula* and *P. vestigipinnis* reached the Uruguay Basins about 2.0–7.5

374   Ma 95% HPD (mean 4.3 Ma) (Fig. 6).

375   The ancestral area reconstructions (Fig. 7) suggest that Otothyrinae originated in

376   the Coastal Drainages (Region A) and then subsequently expanded its range into the

377   other regions by means of biotic dispersal, geodispersal (river capture), or both. The

378   first group to diverge within Otothyrinae is composed for species of the genus

379   *Corumbataia* and six species of the genus *Hisonotus* (*H. insperatus*, *Hisonotus* sp. 4,

380   *Hisonotus* sp. 5, *Hisonotus* sp. 6, *Hisonotus* sp., 7 and *Hisonotus* sp. 8). The ancestral

381   lineage of this group originated in Coastal Drainages region (A) at 29.0–57.1 Ma 95%

382   HPD (mean 42.5 Ma). The second group to diverge is composed of *Schizolecis*

383   *guntheri,* the only known species of *Schizolecis*. Our results suggest that the ancestor of

384   this species originated in the Coastal Drainages region (A) about 28.7–55.8 Ma 95%

385   HPD (mean 41.0 Ma). The third group to diverge within Otothyrinae is composed four

386   species of *Hisonotus* (*Hisonotus* sp. 1, *Hisonotus* sp. 2, *Hisonotus* sp. 3 and *Hisonotus*

387   *chromodontus*) and three species of *Parotocinclus* (*Parotocinclus* sp. 3, *P. aripuanensis*

388   and *P.* aff. *spilurus*). The ancestor of this group dispersed from the Coastal Drainages

389   region (A) to the Amazon and Orinoco Basins (D) about 25.6–51.0 Ma 95% HPD

390   (mean 37.5 Ma). Subsequently, the ancestor of the clade composed of *Hisonotus* sp. 1,

391   *Hisonotus* sp. 2 and *Parotocinclus* aff. *spilurus* reached the São Francisco Basin and

392   Northeastern Basins (E) about 19.3–43.2 Ma 95% HPD (mean 30.7 Ma).

393   The fourth group to diverge within Otothyrinae is composed of species of the

394   genus *Pseudotothyris*, *Otothyris* and the species *Parotocinclus* sp. 2, *P. britiskii* and *P.*

395   *eppleyi*. The ancestor of this group originated in the Coastal Drainages region (A) about

396   23.3–46.1 Ma 95% HPD (mean 33.7 Ma). Subsequently, the ancestor of *Parotocinclus*

397   sp. 2, *P. britiskii* and *P. eppleyi* dispersed from to the Amazon and Orinoco Basins (D)

398   about 19.8–42.6 Ma 95% HPD (mean 31.2 Ma). The ancestor of the group composed of

399   the species *Microlepidogaster dimorpha*, *Rhinolekos* sp. 1, *R. britskii* and *R. garavelloi*

400   dispersed from the Coastal Drainages region (A) to the Upper Paraná Basin (B) about

401   18.3–37.3 Ma 95% HPD (mean 27.3 Ma). The ancestor of the clade composed of

402   *Hisonotus depressicauda*, *H. francirochai* and *H. paulinus* originated in Upper Paraná

403   Basin (B) about 15.7–33.2 95% HPD (mean 23.7 Ma). The ancestor of the clade

404   composed of *Parotocinclus* sp. 1, *Parotocinclus* cf. *bahiensis*, *P. robustus* and *P. prata*,

405   New taxon sp. 1, New taxon sp. 2 and *Hisonotus bocaiuva*, originated in the Coastal

406   Drainages region (A) about 15.7–33.2 Ma 95% HPD (mean 23.7 Ma). The ancestral of

407 the clade composed of the true *Hisonotus* and its closest relatives (*Hisonotus aky*, *H.*

408 *iota*, *H. montanus*, *H. megaloplax*, *H. prata*, *H. carreiro*, *H. ringueleti*, *H. nigricauda*,

409 *H. heterogaster*, *H. notopagos*, *H.* cf. *charrua*, *H. laevior*, *H. charrua*, *H. leucophrys*,

410 *H. leucofrenatus*, *H. taimensis*, *H. notatus* and *H. armatus*), and the species

411 *Otothyropsis marapoama*, *Eurycheilichthys* sp. 1, *Epactionotus bilineatus*, *E.*

412 *itaimbezinho* and *E. gracilis* originated in the Coastal Drainage region about 17.0–35.0

413 Ma 95% HPD (mean 25.6 Ma).

414 Additionally, two dispersal events can be inferred from this area to the Paraguay,

415 Lower Paraná and Uruguay Basins (C). The first is the ancestor of *Hisonotus iota*, *H.*

416 *aky*, *H. montanus*, *H. megaloplax*, *H. prata*, *H. carreiro* and *H. ringueleti* about 10.7–

417 26.3 Ma 95% HPD (mean 17.8 Ma). The second is the ancestor of *Hisonotus cf.*

418 *charrua*, *H. leucophrys*, *H. charrua*, *H. laevior*, *H. taimensis*, *H. leucofrenatus*, *H.*

419 *notatus* and *H. armatus* about 11.4–24.8 95% HPD (mean 17.5 Ma).

420

421 **Discussion**

422 *River capture as a biogeographic process*

423 The time-calibrated molecular phylogeny and ancestral area reconstructions of

424 this study (Figs. 5–7) suggest that river capture has been an important process affecting

425 diversification of taxa in the HNO-clade. These results are largely consistent with those

426 of previous studies of loricariids from Southern and Southeastern Brazil (Reis &

427 Schaefer 1998; Chiachio *et al.* 2008; Roxo *et al.* 2012a). For example, Chiachio *et al.*

428 (2008) recovered a similar division of the HNO-clade into two monophyletic groups,

429 the Hypoptopomatinae and Neoplecostominae + Otothyrinae, inferred the ancestor of

430 Hypoptopomatinae to have inhabited the Amazon basin, and inferred the ancestor of

431 Neoplecostominae + Otothyrinae to have inhabited an area now drained by the Upper

432 Parana and part of the Atlantic coastal drainages.

433 The Atlantic coastal region has a complex and ancient geological history that

434 traces to the final separation of Africa and South America about 100 million years ago

435 (Pitman *et al.* 1993; Cesero & Ponte 1997; Scotese 2004; Blakey 2006; Ribeiro 2006).

436 Roxo *et al.* (2012a) identified the Coastal Drainages of Southeastern Brazil as an

437 important area where many lineages of Loricariidae originated, including the ancestors

438 of Neoplecostominae. Ribeiro (2006) described a series of phylogenetic patterns

439 (termed A, B and C) in which sister group relationships are found between lineages

440 inhabiting the Atlantic coastal drainages and inland drainages such as Amazon and

441   Paraná Basins. Our results suggest a fit to pattern B in Neoplecostominae and

442   Otothyrinae, with sister-group relationships between species endemic to the Brazilian

443   coastal drainages and adjacent portions of the Brazilian shield. Ribeiro (2006) listed

444   *Lignobrycon*, *Rhinelepis*, *Spintherobolus*, and *Triportheus*, the tribes Aspidoradini and

445   Glandulocaudini, and the subfamilies Cheirodontinae and Sarcoglanidinae as examples

446   of pattern B.

447   According to the Lagrange ancestral area reconstructions, the area of the modern

448   Atlantic Coastal Drainages (Region A) is optimized as the ancestral area for three of the

449   deepest nodes of the HNO phylogeny. These nodes include the HNO-clade as a whole

450   (40.8–79.7 Ma 95% HPD, mean 58.4 Ma, Fig. 5), the Hypoptopomatinae (33.6–67.4

451   Ma 95% HPD, mean 49.9 Ma, Fig. 6), and the Neoplecostominae and Otothyrinae

452   (31.0–62.2 Ma 95% HPD, mean 45.9 Ma, Fig. 6–7). The results of the Lagrange

453   analysis are consistent with a river capture event at about 26.7–58.6 Ma 95% HPD

454   (mean 42.5 Ma), allowing range expansion(s) from the Atlantic Coastal Drainages

455   (Region A) to a region comprised of the modern Paraguay/Lower Paraná/Uruguay

456   (Region C) and Amazon/Orinoco Basins (Region D). An important river capture event

457   at this approximate time and place is also consistent with the topology of a General

458   Area Cladogram of fish taxa from tropical South America, as inferred form a Brooks

459   Parsimony (meta)Analysis of all 32 published phylogenies of species-rich fish clades

460   available at that time (fig. 7.1 at Albert & Carvalho 2011).

461   Chiachio *et al.* (2008) explained the division of Hypoptopomatinae, between

462   lineages in the Amazon Basin (Region D) and the Brazilian East Coastal (Region A)

463   and the Upper Paraná (Region B), as the result of limited dispersal of fishes to less

464   favourable areas of the continental margin. Although species of Hypoptopomatinae do

465   inhabit lowland rivers in the Amazon, Orinoco and Guianas regions, most species of

466   Neoplecostominae plus Otothyrinae inhabit rivers and streams in the mountainous

467   Brazilian Shield, where they are adapted to colder and more highly oxygenated waters

468   (Langeani 1990). Additionally, historical paleogeographic connections among the

469   Orinoco, Amazon, and Paraguay basins are hypothesized to have enabled the

470   colonization of Hypoptopomatinae species through these basins (Albert *et al.* 2011).

471   Within Neoplecostominae our time-calibrated phylogeny and Lagrange

472   biogeographic analysis suggest a (geo)dispersal event in the ancestral species of the

473   clade composed of *Neoplecostomus* (except *N. ribeirensis*) and *Pareiorhina carrancas*

474   to move from the Coastal Drainages (Region A) to the Upper Paraná Basin (Region B)

475   at about 14.2–33.4 Ma 95% HPD (mean 22.9 Ma). Roxo *et al.* (2012a) reported an

476   event with a similar date in the range 15.4–38.1 Ma 95% HPD (mean 26.7 Ma), and

477   suggested that this (geo)dispersal event could be a result of a headwater capture. During

478   this time period several headwater capture events have been proposed between the Rio

479   Tietê, Rio Paraíba do Sul, Rio São Francisco, and Rio Ribeira de Iguape basins

480   (Ab'Sáber 1957, 1998; Ribeiro 2006). Headwater capture is likely to have influenced

481   ancestral fish distributions throughout adjacent drainages, allowing the ancestors of this

482   group to reach the Upper Paraná basin.

483   The subfamily Otothyrinae also has a complex biogeographic history among

484   South American basins (Fig. 7). The ancestral area reconstruction with highest ML

485   scores gives us the origin in the Coastal Drainages (Region A). Within one of the two

486   clades of Otothyrinae to diverge (i.e. species of *Corumbataia* and six species of

487   *Hisonotus, H. insperatus*, *H. piracanjuba, Hisonotus* sp. 4, *Hisonotus* sp. 5, *Hisonotus*

488   sp. 6 and *Hisonotus* sp. 7), (geo)dispersal from Coastal Drainage (Region A) to Upper

489   Paraná basin (Region B) is estimated in the time frame 20.7–47.4 Ma 95% HPD (mean

490   33.29 Ma).

491   The results of our Lagrange analysis suggest the influence of river capture in the

492   movement of Otothyrinae from Atlantic Coastal Drainages (Region A) to the Amazon

493   and Orinoco Basins (Region D). Our preferred model (M3) of geographic dispersal

494   among areas posits a connection between regions C (Paraguay, Lower Paraná and

495   Uruguay Basins) and D (Amazon and Orinoco Basins) before 15 Ma. For more than a

496   century authors have suggested historical dispersal routs of fishes between Paraguay

497   and Amazon basins (Eigenmann & Eigenmann 1891; Jordan 1896; Eigenmann 1906;

498   Pearson 1937; Carvalho & Albert 2011; Ribeiro *et al.* 2013). These authors suggested

499   that most of the fish lineages represented in the Paraguay Basin can be explained by

500   dispersal, presumably by means of headwater capture (geodispersal) of Amazon

501   tributaries (Madeira, Tocantins, Xingu) on the Brazilian Shield. However, geodispersal

502   events in the reverse direction, from south to north, must also be considered for taxa

503   with origins in the La Plata and Atlantic coastal drainages, and with derived lineages in

504   the Amazon and Orinoco basins.

505   The Lagrange analysis also infers a river capture event affecting the ancestor of

506   the clade including New taxon sp. 1, New taxon sp. 2, *Hisonotus bocaiuva*,

507   *Parotocinclus* cf. *bahiensis*, *P. robustus* and *P. prata* from the Atlantic Coastal

508   Drainages (Region A) to the São Francisco Basin and Northeastern Drainages (Region

509   E) in the time frame 11.3–26.1 Ma 95% HPD (mean 18.2 Ma). These two regions also

510   share extensive watershed divides with the many separate Atlantic coastal drainages of

511   the eastern margin of the Brazilian Shield. Ribeiro (2006) suggested that the origin of

512   the Taubaté Graben probably resulted in the capture of several other adjacent rivers,

513   such as headwaters of the Tietê, Grande, São Francisco and Doce rivers. A river capture

514   event at this approximate time and place is also consistent with the General Area

515   Cladogram of fish taxa from tropical South America (Albert & Carvalho, 2011; fig.

516   7.1).

517   The results of our Lagrange analysis point to the influence of several river

518   capture events permitting movements of Otothyrinae lineages from the Atlantic Coastal

519   Drainages (Region A) to the Paraguay, Lower Paraná and Uruguay Basins (Region C)

520   (Fig. 7). These events occurred within the group of the true *Hisonotus* species

521   (including the type species *Hisonotus notatus*), and the species of the genera

522   *Eurycheilichthys* and *Epactionotus*. While most of the early-branching clades in this

523   group inhabit the eastern margin of the Brazilian Shield, a few early-branching lineages

524   occur in the Uruguay Basin (Region C). Ribeiro (2006) reported that several species are

525   shared between the isolated coastal drainages and the adjacent upland as: *Cnesterodon*

526   *decemmaculatus* and *Cnesterodon brevirostratus* (Lucinda, 2005), *Bryconamericus*

527   *patriciae* (Silva 2004), *Hypostomus commersoni* and *H. aspilogaster* (Reis *et al.* 1990).

528   The results of our Lagrange analysis also indicate a geodispersal event from the

529   Amazon and Orinoco basins (Region D) to the São Francisco Basin and Northeastern

530   Drainages (Region E) in the ancestor of *Hisonotus* sp. 1, *Hisonotus* sp. 2 and

531   *Parotocinclus* aff. *spilurus* at about 19.3–43.2 Ma 95% HPD (mean 30.7 Ma). Rosa *et*

532   *al.* (2004) previously suggested that some fish species in Northeastern Brazil are

533   widespread in two or more basins, encompassing the São Francisco, Parnaíba and

534   several adjacent coastal rivers basins. This is the case, for example, in *Triportheus*

535   *signatus, Prochilodus brevis*, *Cichlasoma orientale* and *Parauchenipterus galeatus*.

536

537   ***Sea-level changes as a biogeographic process***

538   Periods of alternating sea-level stands can also influence the distributions of

539   lowland freshwater taxa (Lovejoy *et al.* 2006; Albert & Reis 2011; Bloom & Lovejoy

540   2011). Eustatic sea-level changes under global climate controls, and regional subsidence

541   or uplift under tectonic controls, have resulted in multiple marine transgressions and

542   regressions over the course of the Cenozoic, alternately flooding and exposing low lying

543 areas of the continental platforms, and converting lowland and coastal plains from
544 freshwater to shallow marine ecosystems. López-Fernandez & Albert (2011) identify
545 six marine transgressions during the Eocene, one in the Eocene and Oligocene, three in
546 Oligocene and one in the Miocene, the time interval during which most lineages of
547 Hypoptopomatinae diversified (Fig. 6). Ancestral lineages of Hypoptopomatinae were
548 present in the lowland portions of the Amazon and Orinoco basins (Region D) from
549 about 26.7–58.6 Ma 95% HPD (mean 42.5 Ma) to the present, and our results suggests
550 three events of (geo)dispersal to the lowland portions of the La Plata basin from about
551 12.6–33.1 Ma 95% HPD (mean 21.7 Ma), 9.9–26.4 Ma 95% HPD (mean 17.0 Ma) and
552 0.0–10.6 Ma 95% HPD (mean 5.3 Ma) to the present. These populations were therefore
553 presumably influenced by numerous regional marine transgressions and regressions.

554   Marine transgressions can isolate and fragment lowland fish populations,
555 promoting both speciation and extinction by reducing the total amount and connectivity
556 of freshwater habitat patches (Lundberg *et al.* 1998; Lovejoy *et al.* 2006; Ribeiro 2006;
557 Sabaj-Perez *et al.* 2007; López -Fernandes & Albert 2011). Marine transgressions can
558 also result in local population extirpations and/or allopatric speciation in upland refugia
559 (e.g. Albert *et al.* 2006). Marine regressions can expand lowland and coastal freshwater
560 habitats, thereby promoting dispersal and reducing extinction (López-Fernández &
561 Albert 2011; López-Fernández *et al.* 2013).

562   The relatively small areal extent of river basins in the Atlantic coastal drainages,
563 combined with areal expansions and contractions due to Pleistocene shoreline
564 fluctuations, may have acted in concert to elevate speciation and extinction rates in this
565 region (e.g., Beheregaray *et al.* 2002; do Amaral 2012). Indeed many extant fish species
566 in the Atlantic coastal drainages are of high conservation concern (Reis 2013).
567 However, the effect of Pleistocene shoreline fluctuations on fish diversity was
568 presumably restricted to the coastal plain (areas below 100m elevation), whereas most
569 of the fish species of the Atlantic coastal drainages inhabit canyons in the piedmont,
570 especially larger rivers such as the Rio Doce, Ribeiro de Iguape and Rio Maquine (e.g.
571 Malabarba *et al.* 2013).

572

573 *Peripheral location of low-diversity, early-branching lineages*

574   The ancestral-area reconstructions generated by Lagrange (Fig. 5) permit one to
575 infer the geographic origin of the HNO-clade, and of all three HNO subfamilies, in the
576 Atlantic Coastal Drainages (Region A), a relatively narrow strip of rivers basins that

extends along the eastern continental margin. This is a reasonable interpretation given the disproportionately high number of low-diversity, early-branching clades in all three subfamilies restricted to Region A. This interpretation also conforms to widespread expectations about the relative rates of macroevolutionary parameters that affect net rates of diversification (Bloom 2013; Pyron & Burbrink 2013; Rabosky 2013). The Lagrange DEC model of species range evolution assumes a model of biogeographic history dominated by vicariance, in which dispersal and extinction are treated as relatively rare events (Ree & Smith 2008; Ronquist & Sanmartín 2011). The Lagrange model is also entirely neutral (*sensu* Hubbell 2001) with respect to DEC parameter values among clades and regions.

An alternative interpretation of HNO biogeographic history may also be considered, in which the ancestral species range was distributed over a wide portion of southern South America in the early Cenozoic, including much of the modern Atlantic Coastal (Region A), Upper Parana (Region B), and Paraguay/Lower Parana/Uruguay (Region C) areas. Under this alternative interpretation, the accumulation of many low-diversity, early-branching clades in the Atlantic coastal drainages are expected from patterns of diversification on landscapes with low rates of river capture.

In places like the eastern margin of South America, where geographic range evolution is thought to have been dominated by river capture (Ribeiro 2006; Buckup 2011; Lima & Ribeiro 2011; Pereira *et al.* 2012), vicariance and geodispersal events are expected to be coupled (see the Introduction; see also Albert & Campton 2010). Under these conditions, rates of speciation and dispersal should be approximately matched as sources for the introduction of new species (*sensu* Vellend 2010). Further, because dispersal expands species ranges, it tends to reduce extinction rates, and freshwater fish species with larger ranges generally have lower extinction risk (Fagan 2002; O'Grady *et al.* 2004). Therefore, the combination of low speciation and extinction rates in the Atlantic coastal drainages may have contributed to an accumulation of low-diversity clades. By contrast, the relatively higher rates of speciation and extinction in the La Plata basin is predicted to have resulted in a phylogeny with few or no low-diversity early-branching clades (see e.g. Albert *et al.* 2011 fig. 2.15). In other words, regions with high species turnover are less likely to retain low-diversity early-branching clades (i.e. the Effect Hypothesis of Vrba 1984).

This alternative interpretation predicts the presence of many low-diversity, early-branching fish lineages on landscapes with low rates of river capture. This

611   alternative interpretation differs from the Lagrange-generated ancestral-area

612   reconstructions by positing different rates of speciation and extinction in clades

613   inhabiting the Atlantic coastal drainages and La Plata basin. In other words, this

614   alternative interpretation it not neutral with respect to DEC parameter values among

615   regions, positing instead that rates of speciation and extinction are correlated with rates

616   of river capture.

617

618   *Museums and cradles*

619   In evaluating distributional patterns of Neotropical fish distributions in

620   southeastern Brazil, Ribeiro (2006) concluded that the Atlantic coastal drainages

621   (Region A of the present study) served as both a cradle and a museum of diversity for

622   different fish groups. The terms "evolutionary cradle" and "evolutionary museum" are

623   alternative hypotheses for the occurrence of areas with high species richness (Stebbins

624   1974). An "evolutionary cradle" is an area with high rates of speciation, where

625   environmental conditions promote speciation. By contrast, an "evolutionary museum" is

626   an area with low rates of extinction, where low rates of environmental disturbance act to

627   preserve early-branching taxa, and where species richness accumulates through long

628   periods of geological time.

629   Results of this study on the Hypoptopomatinae, Neoplecostominae and

630   Otothyrinae broadly concur with these conclusions of Ribeiro (2006) (Figs. 8–9). All

631   three HNO subfamilies are inferred by Lagrange ancestral-area reconstructions to have

632   originated in the Atlantic coastal drainages, suggesting that this region served as the

633   cradle for early diversification in these clades. In addition, several lineages of

634   Neoplecostominae remain confined to the region of the Atlantic coastal drainages,

635   which therefore also appears to serve as a museum for these clades. These major

636   patterns of diversification in Neoplecostominae in the Atlantic coastal drainages and

637   Brazilian Shield were previously recognized by Roxo *et al.* (2012a). For

638   Hypoptopomatinae, most of the diversification occurred in lowlands of the Amazon,

639   Orinoco and Paraguay basins, and the species *Lampiella gibbosa* appears to be a

640   relictual lineage confined to the Atlantic coastal drainages. Diversification within

641   Otothyrinae exhibits a pattern with monophyletic lineages in each of the several regions

642   and basins of the South American platform (Fig. 9).

643

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653

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Number	Collection No	Fish No	GenBank (Reticulon/16S/COI/CytB)	Species	Location (river, city, state, country)
1	LBP 6037	29054	Not submitted	*Hisonotus armatus*	Rio Maquiné/Osório/RS/Brazil
2	MCP 21375		-/-/EU371011/-	*Hisonotus armatus*	Lagoa dos Patos/São José do Norte/RS/Brazil
3	MCP 37682		Not submitted	*Hisonotus armatus*	Arroio Arambare/Pedro Osório/RS/Brazil
4	LBP 3472	20258	Not submitted	*Hisonotus notatus*	Afluente Rio Aduelas/Macaé/RJ/Brazil
5	MNRJ 37474		Not submitted	*Hisonotus notatus*	-/Casimiro de Abreu/RJ/Brazil
6	LBP 4765	25554	Not submitted	*Hisonotus taimensis*	Rio Guaíba/Barra do Ribeiro/RS/Brazil
7	LBP 13147	51066	Not submitted	*Hisonotus taimensis*	Riacho sem nome/Agudo/RS/Brazil
8	LBP 7407	35655	Not submitted	*Hisonotus leucofrenatus*	Rio Batatau/Itapeúna/SP/Brazil
9	MCP 31819		Not submitted	*Hisonotus leucofrenatus*	Afluente Rio Nhundiaquara/Morretes/PR/Brazil
10	MCP 41351		Not submitted	*Hisonotus leucophrys*	Rio Ariranhas/Xavantina/SC/Brazil
11	MCP 21644		Not submitted	*Hisonotus charrua*	Rio Quaraí Mirim/Quaraí/RS/Brazil
12	MCP 37684		Not submitted	*Hisonotus laevior*	Arroio Arambare/Pedro Osório/ RS/Brazil

				Species	Locality
13	LBP 13187	51070	Not submitted	*Hisonotus laevior*	Riacho sem nome/Camaquã/RS/Brazil
14	LBP 3376	21246	Not submitted	*Hisonotus laevior*	Arroio dos Corrientes/Pelotas/RS/Brazil
15	LBP 3376	21248	Not submitted	*Hisonotus laevior*	Arroio dos Corrientes/Pelotas/RS/Brazil
16	LBP 4720	24941	Not submitted	*Hisonotus cf. charrua*	Arroio Cuaró Grande/Artigas/Uruguay
17	MCP 40762		Not submitted	*Hisonotus notopagos*	Arroio Mantiqueira/Lavras do Sul/RS/Brazil
18	LBP 4723	24951	Not submitted	*Eurycheilichthys sp. 1*	Arroio Jaboticaba/Veranópolis/RS/Brazil
19	LBP 4871	24919	Not submitted	*Epactionotus bilineatus*	Rio Carvalho/São Francisco de Paula/RS/Brazil
20	MCP 23683		-/-/EU371004/-	*Epactionotus itaimbezinho*	Rio Mangue/Morrinho do Sul/RS/Brazil
21	MCP 23606		-/-/EU371005/-	*Epactionotus gracilis*	Rio Morto/Meleiro/SC/Brazil
22	LBP 3335	20465	Not submitted	*Hisonotus heterogaster*	Arroio sem nome/Rio Grande/RS/Brazil
23	LBP 579	8565	Not submitted	*Hisonotus heterogaster*	Afluente Rio Guaíba/Eldorado do Sul/RS/Brazil
24	LBP 4719	25636	Not submitted	*Hisonotus nigricauda*	Arroio do Nariz/Rosário do Sul/RS/Brazil
25	LBP 5528	25343	Not submitted	*Otothyropsis marapoama*	Ribeirão Cubatão/Marapoama/SP/Brazil
26	LBP 4863	24930	Not submitted	*Hisonotus ringueleti*	Arroio Cuaró Grande/Artigas/Uruguay
27	MCP 40943		Not submitted	*Hisonotus carreiro*	Arroio Guabiju/Guabiju/RS/Brazil
28	MCP 40492		Not submitted	*Hisonotus prata*	Rio da Prata/Nova Prata/RS/Brazil

29	MCP 41352		Not submitted	*Hisonotus megaloplax*	Arroio Cragoata/Passo Fundo/RS/Brazil
30	LBP 13055	51035	Not submitted	*Hisonotus montanus*	Rio Canoas/Vargem/SC/Uruguay
31	MCP 41474		Not submitted	*Hisonotus aky*	Rio Forquilha/Paim Filho/RS/Brazil
32	LBP 13072	51046	Not submitted	*Hisonotus iota*	Rio Chapecó/Coronel Freitas/SC/Brazil
33	MCP 40029		Not submitted	*Hisonotus iota*	Rio Chapeco/Coronel Freitas/SC/Brazil
34	LBP 8249	38464	Not submitted	*Parotocinclus prata*	Rio Santo Antônio/Luizlândia/MG/Brazil
35	LBP 7472	32387	Not submitted	*Parotocinclus prata*	Córrego Guarda-Mor/Guarda-Mor/MG/Brazil
36	LBP 8258	38298	Not submitted	*Parotocinclus robustus*	Córrego Cachoeira/Bocaiúva/MG/Brazil
37	LBP 7182	34694	Not submitted	*Parotocinclus cf. bahiensis*	Rio Coité/Lençóis/BA/Brazil
38	LBP 5867	28345	Not submitted	*New taxon 1*	Rio Curral das Éguas/Três Marias/MG/Brazil
39	LBP 17402	67143	Not submitted	*Hisonotus bocaiuva*	Córrego Cachoeira/Bocaiuva/MG/Brazil
40	LBP 7244	33302	Not submitted	*New taxon 2*	Afluente Rio Araguari/Perdizes/MG/Brazil
41	LBP 3457	20293	Not submitted	*Parotocinclus sp. 1*	Rio da Conceição/Guarapari/ES/Brazil
42	LBP 13923	58218	Not submitted	*Hisonotus francirochai*	Córrego sem nome/Capitinga/MG/Brazil
43	LBP 871	8801	Not submitted	*Hisonotus paulinus*	Afluente do rio Grande/Paranapiacaba, SP/Brazil
44	LBP 3932	22661	Not submitted	*Hisonotus paulinus*	Rio Paraitinguinha/Salesópolis/SP/Brazil

45	LBP 17583	69477	Not submitted	*Hisonotus depressicauda*	Riacho sem nome/Sapucaí Mirim/MG/Brazil
46	LBP 17583	69479	Not submitted	*Hisonotus depressicauda*	Riacho sem nome/Sapucaí Mirim/MG/Brazil
47	LBP 8051	37802	Not submitted	*Hisonotus depressicauda*	Ribeirão do Salto/Delfim Moreira/MG/Brazil
48	MCP 22602		Not submitted	*Hisonotus depressicauda*	Arroio Barra Grande/Paulo Frontin/PR/Brazil
49	LBP 2910	18756	Not submitted	*Hisonotus depressicauda*	Ribeirão da Quinta/Botucatu/SP/Brazil
50	LBP 4854	25626	Not submitted	*Microlepidogaster dimorpha*	Riacho Grotão/Araxá/MG/Brazil
51	LBP 7253	33309	Not submitted	*Rhinolekos britskii*	Córrego sem nome/Pires do Rio/GO/Brazil
52	LBP 7245	34405	Not submitted	*Rhinolekos britskii*	Afluente Rio Arapuca/Bela Vista de Goias/GO/Brazil
53	LBP 5848	28365	Not submitted	*Rhinolekos* sp. 1	Riacho sem nome/Águas Fria de Goiás/GO/Brazil
54	LBP 7246	34410	Not submitted	*Rhinolekos garavelloi*	Córrego Fazenda Lageado/Caldas Novas/GO/Brazil
55	LBP 7246	33305	Not submitted	*Rhinolekos garavelloi*	Córrego Fazenda Lageado/Caldas Novas/GO/Brazil
56	LBP 2869	18571	EU817527/-/-/-	*Parotocinclus maculicauda*	Rio Fau/Miracatu/SP/Brazil
57	LBP 4722	24946	EU817525/-/-/-	*Pseudotothyris* sp. 1	Lago Acaraí/São Francisco do Sul/SC/Brazil
58	LBP 2094	13851	Not submitted	*Pseudotothyris obtusa*	Riacho Descoberto/Guaratuba/PR/Brazil
59	LBP 1971	13685	EU817526/-/-/-	*Otothyris travassosi*	Riacho Rosário/Canavieiras/BA/Brazil
60	MHNG 2586.95	BR1200	EU817552/-/-/-	*Pseudotothyris janeirensis*	Rio Taiaçupeba/-/SP/Brazil

#	Voucher	Number	GenBank	Species	Locality
61	LBP 6950	35328	Not submitted	*Parotocinclus* sp. 2	Igarapé Nouba Uba/São Gabriel da Cachoeira/AM/Brazil
62	ANSP 179131		-/-/GQ225426/-	*Parotocinclus britskii*	-/-/-/
63	LBP 4787	25579	Not submitted	*Parotocinclus eppleyi*	Caño Tama Tama/-/-/Venezuela
64	AUM 43947		-/-/EU359455/-	*Parotocinclus eppleyi*	-/-/-/
65	LBP 5624	35327	Not submitted	*Parotocinclus* aff. *spilurus*	Rio Maravilha/Balsas/MA/Brazil
66	LBP 5624	35326	Not submitted	*Parotocinclus* aff. *spilurus*	Rio Maravilha/Balsas/MA/Brazil
67	LBP 334	4276	Not submitted	*Hisonotus* sp. 1	Ribeirão Santo Inácio/Moema/MG/Brazil
68	LBP 8276	38487	Not submitted	*Hisonotus* sp. 2	Rio Verde Grande/Jaíba/MG/Brazil
69	LBP 16277	61771	Not submitted	*Hisonotus* sp. 3	Rio Feio/Querência/MT/Brazil
70	LBP 7948	35750	Not submitted	*Hisonotus chromodontus*	Rio dos Patos/Nova Mutum/MT/Brazil
71	LBP 7948	35749	Not submitted	*Hisonotus chromodontus*	Rio dos Patos/Nova Mutum/MT/Brazil
72	LBP 10981	50459	Not submitted	*Parotocinclus aripuanensis*	Rio Lajeado/Guajará Mirim/RO/Brazil
73	LBP 1572	11537	Not submitted	*Parotocinclus* sp. 3	Ribeirão Ínsula/Barra do Garça/MT/Brazil
74	LBP 2414	16276	Not submitted	*Parotocinclus* sp. 3	Córrego Fundo/Barra do Garça/MT/Brazil
75	LBP 2514	13846	EU817539/-/-/-	*Schizolecis guntheri*	Rio Sagrado/Morretes/PR/Brazil
76	LBP 3238	19471	EU817536/-/-/-	*Schizolecis guntheri*	Rio Garuva/Garuva/SC/Brazil

#	Voucher	Number	GenBank	Species	Locality
77	LBP 2513	15240	EU817535/-/-/-	*Schizolecis guntheri*	Rio Macacu/Itaboraí/RJ/Brazil
78	LBP 2401	15272	EU817538/-/-/-	*Schizolecis guntheri*	Rio São Pedro/-/RJ/Brazil
79	LBP 2988	19646	EU817537/-/-/-	*Schizolecis guntheri*	Rio Indaiá/Ubatuba/SP/Brazil
80	LBP 1653	11477	EU817520/-/-/-	*Corumbataia tocantinensis*	Rio Vermelho/Goias/GO/Brazil
81	LBP 2001	12191	EU817521/-/-/-	*Corumbataia cuestae*	Rio Alambari/Botucatu/SP/Brazil
82	LBP 5529	25381	Not submitted	*Hisonotus insperatus*	Ribeirão Cubatão/ Marapoama/SP/Brazil
83	LBP 1325	11238	Not submitted	*Hisonotus* sp. 4	Ribeirão Keller/Marialva/PR/Brazil
84	LBP 17256	66651	Not submitted	*Hisonotus piracanjuba*	Córrego sem nome/Morrinhos/GO/Brazil
85	LBP 17256	66655	Not submitted	*Hisonotus piracanjuba*	Córrego sem nome/Morrinhos/GO/Brazil
86	MNRJ 29502	490	Not submitted	*Hisonotus* sp. 5	-/Cotriguaçu/MT/Brazil
87	LBP 17532	68706	Not submitted	*Hisonotus* sp. 6	Rio Maracanã/Tangará da Serra/MT/Brazil
88	LBP 5062	26057	Not submitted	*Hisonotus* sp. 7	Afluente Rio Aricá Mirim/Chapada dos Guimarães/MT/Brazil
89	LBP 5062	26056	Not submitted	*Hisonotus* sp. 7	Afluente Rio Aricá Mirim/Chapada dos Guimarães/MT/Brazil
90	LBP 2931	18994	JN689285/JN089823/JN089799/JN089774	*Pseudotocinclus tietensis*	Rio Paraitinga/Salesópolis/SP/Rio Tietê Basin
91	LBP 616	7564	JN689286/FJ625810/FJ625819/FJ965511	*Pseudotocinclus juquiae*	Rio Juquiá/Juquitiba/SP/Rio Ribeira do Iguape Basin
92	LBP 4391	24189	JN689280/ FJ434517/FJ434540/FJ965510	*Pareiorhina rudolphi*	Ribeirão Guaxinduva/Jundiaí/SP/Rio Tietê Basin

93	LBP 7383	34843	JN689310/ FJ965496/FJ965507/FJ965517	*Neoplecostomus selenae*	Ribeirão das Batéias/Riacho Grande/SP/Rio Paranapanema Basin
94	LBP 7525	34832	-/JN089843/JN089818/JN089794	*Neoplecostomus botucatu*	Cachoeira Véu da Noiva/Botucatu/SP/Rio Paranapanema Basin
95	NUP 3560	9701	JN689293/ FJ434506/FJ434529/FJ965516	*Neoplecostomus yapo*	Rio Tibagi/Fortaleza/PR/Rio Paranapanema Basin
96	LBP 2732	17444	JN689303/FJ965495/FJ965505/FJ965515	*Neoplecostomus paranensis*	Córrego Mocoquinha/Cajuru/SP/Rio Grande Basin
97	LBP 5901	27990	JN689304/JN089837/JN089812/JN089788	*Neoplecostomus langeanii*	Córrego São Domingos/Muzambinho/MG/Rio Grande Basin
98	LBP 2861	18616	JN689298/JN089833/JN089808/JN089784	*Neoplecostomus bandeirante*	Rio Paraitinga/Salesópolis/SP/Rio Tietê Basin
99	NUP 2528	9423	JN689295/FJ434520/FJ434543/FJ965513	*Neoplecostomus corumba*	Rio Corumbá/GO/Rio Paranaíba Basin/Upper Rio Paraná Basin
100	LBP 645	7593	JN689311/FJ434508/FJ434531/FJ965518	*Neoplecostomus microps*	Ribeirão Cajarana/Pindamonhangaba/SP/Rio Paraíba do Sul Basin
101	LBP 6537	31681	JN689313/FJ965494/FJ965504/FJ965519	*Neoplecostomus franciscoensis*	Rio das Velhas/Brumadinho/MG/Rio São Francisco Basin
102	LBP 2551	15243	JN689312/FJ434507/FJ434530/ FJ965512	*Neoplecostomus espiritosantensis*	Rio Jucu/Domingos Martins/ES/Oriental Coastal Basin
103	LBP 8380	37559	JN689288/JN089826/JN68927?/JN089777	*Pareiorhina hyptiorhachis*	Rio Pomba/Santa Barbara do Tugúrio/MG/Rio Paraíba do Sul Basin
104	LBP 1087	10256	JN689290/JN089828/JN089803/JN089779	*Pareiorhina carrancas*	Córrego do Sapateiro/Barbacena/MG/Rio Grande Basin
105	LBP 8368	37559	JN689291/JN089829/JN089804/JN089780	*Pareiorhina carrancas*	Córrego Beijinho/Carrancas/MG/Rio Grande Basin
106	LBP7385	34852	JN689324/FJ965491/FJ965502/FJ965526	*Isbrueckerichthys epakmos*	Rio Água Doce/Tapiraí/SP/Rio Ribeira do Iguape Basin
107	LBP 6389	29765	JN689326/FJ965489/FJ965503/FJ965529	*Isbrueckerichthys cf. calvus*	Rio Taquará/California/PR/Rio Paranapanema Basin

#	Voucher	Catalog	GenBank Accession	Species	Locality
108	LBP 7373	34853	JN68297/FJ965490/FJ965506/FJ965525	*Isbrueckerichthys alipionis*	Rio Betari/Iporanga/SP/Rio Ribeira do Iguape Basin
109	LBP 2650	17402	JN689325/FJ625812/FJ625821/FJ965528	*Isbrueckerichthys duseni*	Rio Pulador/Campinhos/PR/Rio Ribeira de Iguape Basin
110	LBP 7384	34837	JN689323/JN089845/JN089820/JN089796	*Neoplecostomus ribeirensis*	Rio Água Doce/Tapiraí/SP/Rio Ribeira do Iguape Basin
111	LBP 515	6334	JN689316/FJ965492/FJ965500/FJ965523	*Kronichthys subteres*	Rio Betari/Iporanga/SP/Rio Ribeira do Iguape Basin
112	LBP 795	8304	JN689315/FJ434503/FJ434526/FJ965522	*Kronichthys lacerta*	Rio Marumbi/Morretes/PR/Oriental Coastal Basin
113	LBP 2122	15096	JN689314/FJ434502/FJ434525/FJ965520	*Kronichthys heylandi*	Rio Parati-Mirim/Parati/RJ/Oriental Coastal Basin
114	LBP 1766	12886	JN689317/FJ965493/FJ965501/FJ965521	*Kronichthys* sp. 1	Rio Sítio do Meio/Mongaguá/SP/Oriental Coastal Basin
115	LBP 748	8257	JN689318/FJ625811/FJ625820/FJ965524	*Pareiorhaphis splendens*	Rio São João/Guaruva/PR/Oriental Coastal Basin
116	MCP 41275		-/-/EU359438/-	*Pareiorhaphis cameroni*	Rio Cubatão/Águas Mornas/SC/Brazil
117	LBP 902	7989	JN689319/FJ434514/FJ434537/FJ965532	*Pareiorhaphis steindachneri*	Rio Itapucu/Jaraguá do Sul/SC/Oriental Coastal Basin
118	MCP 41909		-/-/EU359437/-	*Pareiorhaphis azygolechis*	Rio Araraquara/Guaratuba/PR/Brazil
119	LBP 1161	8935	JN689320/FJ434512/FJ434535/FJ965530	*Pareiorhaphis vestigipinnis*	Rio Caveiras/Painel/SC/Rio Uruguai Basin
120	LBP 701	7363	JN689321/FJ434513/FJ434536/FJ965531	*Pareiorhaphis hystrix*	Rio Tainhas/Tainhas/RS/Oriental Coastal Basin
121	MCP 41747		EU359443/-/-/-	*Pareiorhaphis parmula*	Rio dos Patos/Lapa/PR/Brazil
122	MCP 41458		EU359439/-/-/-	*Pareiorhaphis eurycephalus*	Rio Rufino/Rio Rufino/SC/Brazil
123	LBP 4042	22905	Not submitted	*Hypoptopoma inexspectatum*	Rio Moa/Cruzeiro do Sul/AC/Brazil

124	LBP 693	7084	Not submitted	*Hypoptopoma inexspectatum*	Afluente rio Pirai/ Poconé/MT/Brazil
125	MHNG 2678.015	PR 12	JN689282/FJ965486/FJ965498/FJ965508	*Hypoptopoma inexspectatum*	Rio Paraná/Santa Fé/Argentina
126	LBP 3081	19713	EU817541/-/-/-	*Hypoptopoma gulare*	Rio Orinoco/Caicara del Orinoco/Bolivar/Venezuela
127	MHNG 2709.024	MUS 388	EU817554/-/-/-	*Hypoptopoma* sp. 1	Export Iquitos, Upper Amazon basin, Peru
128	LBP 3165	19315	EU817543/-/-/-	*Acestridium discus*	Rio Preto da Eva/Rio Preto da Eva/AM/Brazil
129	LBP 7204	35332	Not submitted	*Acestridium* sp. 1	Igarapé Ya-Mirim/São Gabriel da Cachoeira/AM/Brazil
130	LBP 6973	35324	Not submitted	*Oxyropsis* sp. 1	Igarapé Demuriari/São Gabriel da Cachoeira/AM/Brazil
131	LBP 4300	23945	Not submitted	*Oxyropsis acutirostra*	Igarapé Zamula/Barcelos/AM/Brazil
132	LBP 2652	17407	Not submitted	*Lampiella gibbosa*	Rio Carombé/Campinhos/PR/Brazil
133	LBP 877	8564	EU817546/-/-/-	*Otocinclus flexilis*	-/Santo Antônio da Patrulha/RS/Brazil
134	MCP 25234		-/-/EU370983/-	*Otocinclus arnoldi*	Rio Inhacunda/São Francisco de Assis/RS/Brazil
135	LBP 5310	26831	Not submitted	*Otocinclus hoppei*	Igarapé Uiratapuru/Laranjal do Jari/AP/Brazil
136	MHNG 2613.057	CA25	EU817556/-/-/-	*Otocinclus hoppei*	Arroio Huangana, Alto Pisqui/Loreto/Ucay/Peru
137	LBP 5132	26233	EU817544/-/-/-	*Otocinclus vittatus*	Lagoa Bairro Caiçara/Cáceres/MT/Brazil

#	Voucher	Catalog	GenBank	Species	Locality
138	MCP 34842		-/-/EU359432/-	*Otocinclus cocama*	Afluente do Cano da Concha Supay/Jenaro Herrera/Loreto/Peru
139	MHNG no number	SU07-350	EU817558/-/-/-	*Otocinclus mariae*	Witoto Ecu creek/Sipaliwini/Suriname
140	MHNG 2601.060	BR98-040	EU817557/-/-/-	*Otocinclus mariae*	Rio Acará/-/PA/Brazil
141	LBP 3510	21309	JN689283/FJ625809/FJ625818/FJ965533	*Hypostomus nigromaculatus*	Córrego Hortelã/Botucatu/SP/Rio Paranapanema Basin
142	NUP 1725	16652	Not submitted	*Hypostomus microstomus*	Rio Paraná/Guaíra/PR/Brazil
143	LBP 2544	10887	Not submitted	*Hypostomus ancistroides*	Rio Corumbataí/Corumbataí/SP/Brazil
144	LBP 1557	11505	Not submitted	*Rineloricaria lanceolata*	Ribeirão Ínsula/Barra do Garça/MT/Brazil
145	LBP 1557	11506	Not submitted	*Spatuloricaria* sp. 1	Ribeirão Ínsula/Barra do Garça/MT/Brazil
146	MCP 31467		-/AY307290/-/-	*Delturus parahybae*	-/Laranjal/MG/Brazil
147	LBP 2368	15363	JN689278/FJ434499/FJ434524/FJ965535	*Hemipsilichthys gobio*	Rio Macaquinho/Bairro dos Macacos/SP/Rio Paraíba do Sul Basin
148	LBP 4956	10241	JN689279/FJ625808/FJ625817/FJ965534	*Hemipsilichthys papillatus*	Ribeirão da Jacutinga/Bom Jardim de Minas/MG/Rio Paraíba do Sul Basin
149	LBP 1352	11454	Not submitted	*Astroblepus* sp. 1	Rio Jequetepeque/Magdalena/Cajamarca/Peru
150	LBP 3284	20010	Not submitted	*Astroblepus* sp. 2	Rio Chorobamba/Huancabamba/Pasco/Peru
151	LBP 485	6040	Not submitted	*Callichthys callichthys*	Córrego do Pombo/Marília/SP/Brazil

152	LBP 210	*Hoplosternum littorale*	Igarapé São Francisco/Rio Branco/AC/Brazil	4134	-/GU210868/-/-
153	LBP 2809	*Corydoras oiapoquensis*	Guyana coastal rivers/-/-/Guyana		GU210997/-/-/-
154	LBP 6862	*Corydoras imitator*	Igarapé Puranga/São Gabriel da Cachoeira/AM/Brazil	32502	-/GU210613/-/-
155	LBP 449	*Diplomystes mesembrinus*	Rio Chubut/Los Altares/Chubut/Argentina	5815	Not submitted

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1029 **Table 2.** Summary of taxonomic information for species of Hypoptopomatinae, Neoplecostominae and Otothyrinae included in the analysis.
1030 Total number of valid names from Eschmeyer & Fong (2013).

Subfamily	Genus	Species	Ecoregions
Otothyrinae	*Corumbataia*	*cuestae*	B
Otothyrinae	*Corumbataia*	*tocantinensis*	D
Otothyrinae	*Eurycheilichthys*	sp. 1	A
Otothyrinae	*Epactionotus*	*bilineatus*	A
Otothyrinae	*Epactionotus*	*gracilis*	A
Otothyrinae	*Epactionotus*	*itaimbezinho*	A
Otothyrinae	*Hisonotus*	*aky*	C
Otothyrinae	*Hisonotus*	*armatus*	A/C
Otothyrinae	*Hisonotus*	*armatus*	A/C
Otothyrinae	*Hisonotus*	*armatus*	A/C
Otothyrinae	*Hisonotus*	*bocaiuva*	E
Otothyrinae	*Hisonotus*	*carreiro*	A
Otothyrinae	*Hisonotus*	*charrua*	A/C
Otothyrinae	*Hisonotus*	cf. *charrua*	A/C
Otothyrinae	*Hisonotus*	*chromodontus*	D
Otothyrinae	*Hisonotus*	*chromodontus*	D
Otothyrinae	*Hisonotus*	*depressicauda*	B
Otothyrinae	*Hisonotus*	*depressicauda*	B
Otothyrinae	*Hisonotus*	*depressicauda*	B

Otothyrinae	*Hisonotus*	*depressicauda*	B
Otothyrinae	*Hisonotus*	*depressicauda*	B
Otothyrinae	*Hisonotus*	*francirochai*	B
Otothyrinae	*Hisonotus*	*heterogaster*	A
Otothyrinae	*Hisonotus*	*heterogaster*	A
Otothyrinae	*Hisonotus*	*insperatus*	B
Otothyrinae	*Hisonotus*	*iota*	C
Otothyrinae	*Hisonotus*	*iota*	C
Otothyrinae	*Hisonotus*	*laevior*	A/C
Otothyrinae	*Hisonotus*	*laevior*	A/C
Otothyrinae	*Hisonotus*	*laevior*	A/C
Otothyrinae	*Hisonotus*	*leucofrenatus*	A
Otothyrinae	*Hisonotus*	*leucofrenatus*	A
Otothyrinae	*Hisonotus*	*leucophrys*	C
Otothyrinae	*Hisonotus*	*megaloplax*	C
Otothyrinae	*Hisonotus*	*montanus*	C
Otothyrinae	*Hisonotus*	*nigricauda*	A/C
Otothyrinae	*Hisonotus*	*notatus*	A
Otothyrinae	*Hisonotus*	*notatus*	A
Otothyrinae	*Hisonotus*	*notopagos*	A
Otothyrinae	*Hisonotus*	*paulinus*	B
Otothyrinae	*Hisonotus*	*paulinus*	B

Otothyrinae	*Hisonotus*	*piracanjuba*	B
Otothyrinae	*Hisonotus*	*piracanjuba*	B
Otothyrinae	*Hisonotus*	*prata*	A
Otothyrinae	*Hisonotus*	*ringueleti*	C
Otothyrinae	*Hisonotus*	*taimensis*	A
Otothyrinae	*Hisonotus*	*taimensis*	A
Otothyrinae	*Hisonotus*	sp. 1	E
Otothyrinae	*Hisonotus*	sp. 2	E
Otothyrinae	*Hisonotus*	sp. 3	D
Otothyrinae	*Hisonotus*	sp. 4	C
Otothyrinae	*Hisonotus*	sp. 5	A
Otothyrinae	*Hisonotus*	sp. 6	C
Otothyrinae	*Hisonotus*	sp. 7	C
Otothyrinae	*Hisonotus*	sp. 7	C
Otothyrinae	*Microlepidogaster*	*dimorpha*	B
Otothyrinae	*New taxon*	sp. 1	E
Otothyrinae	*New taxon*	sp. 2	B
Otothyrinae	*Otothyris*	*travassosi*	A
Otothyrinae	*Otothyropsis*	*marapoama*	B
Otothyrinae	*Parotocinclus*	*aripuanensis*	D
Otothyrinae	*Parotocinclus*	*cf. bahiensis*	A
Otothyrinae	*Parotocinclus*	*britskii*	D

Otothyrinae	*Parotocinclus*	*eppleyi*	D
Otothyrinae	*Parotocinclus*	*eppleyi*	D
Otothyrinae	*Parotocinclus*	*maculicauda*	A
Otothyrinae	*Parotocinclus*	*prata*	E
Otothyrinae	*Parotocinclus*	*prata*	E
Otothyrinae	*Parotocinclus*	*robustus*	E
Otothyrinae	*Parotocinclus*	aff. *spilurus*	E
Otothyrinae	*Parotocinclus*	aff. *spilurus*	E
Otothyrinae	*Parotocinclus*	sp. 1	D
Otothyrinae	*Parotocinclus*	sp. 2	D
Otothyrinae	*Parotocinclus*	sp. 3	D
Otothyrinae	*Parotocinclus*	sp. 3	D
Otothyrinae	*Pseudotothyris*	*obtusa*	A
Otothyrinae	*Pseudotothyris*	*janeirensis*	A
Otothyrinae	*Pseudotothyris*	sp. 1	A
Otothyrinae	*Rhinolekos*	*britskii*	B
Otothyrinae	*Rhinolekos*	*britskii*	B
Otothyrinae	*Rhinolekos*	*garavelloi*	B
Otothyrinae	*Rhinolekos*	*garavelloi*	B
Otothyrinae	*Rhinolekos*	sp. 1	B
Otothyrinae	*Schizolecis*	*guntheri*	A
Otothyrinae	*Schizolecis*	*guntheri*	A

Otothyrinae	*Schizolecis*	*guntheri*	A
Otothyrinae	*Schizolecis*	*guntheri*	A
Otothyrinae	*Schizolecis*	*guntheri*	A
Neoplecostominae	*Isbrueckerichthys*	*alipionis*	A
Neoplecostominae	*Isbrueckerichthys*	*cf. calvus*	A
Neoplecostominae	*Isbrueckerichthys*	*epakmos*	A
Neoplecostominae	*Isbrueckerichthys*	*duseni*	A
Neoplecostominae	*Kronichthys*	*heylandi*	A
Neoplecostominae	*Kronichthys*	*lacerta*	A
Neoplecostominae	*Kronichthys*	*subteres*	A
Neoplecostominae	*Kronichthys*	sp. 1	A
Neoplecostominae	*Neoplecostomus*	*bandeirante*	B
Neoplecostominae	*Neoplecostomus*	*botucatu*	B
Neoplecostominae	*Neoplecostomus*	*corumba*	B
Neoplecostominae	*Neoplecostomus*	*espiritosantensis*	A
Neoplecostominae	*Neoplecostomus*	*franciscoensis*	E
Neoplecostominae	*Neoplecostomus*	*langeanii*	B
Neoplecostominae	*Neoplecostomus*	*microps*	A
Neoplecostominae	*Neoplecostomus*	*paranensis*	B
Neoplecostominae	*Neoplecostomus*	*ribeirensis*	A
Neoplecostominae	*Neoplecostomus*	*selenae*	B
Neoplecostominae	*Neoplecostomus*	*yapo*	B

Neoplecostominae	*Pareiorhaphis*	*azygolechis*	A
Neoplecostominae	*Pareiorhaphis*	*cameroni*	A
Neoplecostominae	*Pareiorhaphis*	*eurycephalus*	C
Neoplecostominae	*Pareiorhaphis*	*hystrix*	A
Neoplecostominae	*Pareiorhaphis*	*parmula*	C
Neoplecostominae	*Pareiorhaphis*	*steindachneri*	A
Neoplecostominae	*Pareiorhaphis*	*vestigipinnis*	C
Neoplecostominae	*Pareiorhina*	*carrancas*	B
Neoplecostominae	*Pareiorhina*	*carrancas*	B
Neoplecostominae	*Pareiorhina*	*hyptiorhachis*	A
Neoplecostominae	*Pareiorhina*	*rudolphi*	A
Neoplecostominae	*Pseudotocinclus*	*juquiae*	A
Neoplecostominae	*Pseudotocinclus*	*tietensis*	B
Hypoptopomatinae	*Acestridium*	*discus*	D
Hypoptopomatinae	*Acestridium*	sp. 1	D
Hypoptopomatinae	*Hypoptopoma*	*gulare*	D
Hypoptopomatinae	*Hypoptopoma*	*inexspectatum*	C/D
Hypoptopomatinae	*Hypoptopoma*	*inexspectatum*	C/D
Hypoptopomatinae	*Hypoptopoma*	*inexspectatum*	C/D
Hypoptopomatinae	*Hypoptopoma*	sp. 1	D
Hypoptopomatinae	*Lampiella*	*gibbosa*	A
Hypoptopomatinae	*Otocinclus*	*arnoldi*	C

Hypoptopomatinae	*Otocinclus*	*cocama*	D
Hypoptopomatinae	*Otocinclus*	*flexilis*	A/C
Hypoptopomatinae	*Otocinclus*	*hoppei*	D
Hypoptopomatinae	*Otocinclus*	*hoppei*	D
Hypoptopomatinae	*Otocinclus*	*mariae*	D
Hypoptopomatinae	*Otocinclus*	*mariae*	D
Hypoptopomatinae	*Otocinclus*	*vittatus*	C/D
Hypoptopomatinae	*Oxyropsis*	*acutirostra*	D
Hypoptopomatinae	*Oxyropsis*	sp. 1	D

Total valid names for Hypoptopomatinae, Neoplecostominae and Otothyrinae	**182**
Total number of ingroup valid names	**86**
Total number of possible new species in the present work	**19**
Percent of valid species names included in the present work	**48 %**

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1038 **Supplementary Table 3.** Primers used in the present study to amplify partial sequences of F-reticulon 4, 16S rRNA, cytochrome oxidase subunit
1039 I (COI) and cytochrome B (CytB).

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'
COI	FishF1	Ward et al. (2005)	5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3'
	FishR1		5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3'
CytB	L14841	Oliveira et al. (2011)	5'-CCA TCC AAC ATC TCA GCA TGA TGA AA3'
	H15915b		5'-AAC CTC CGA TCT TCG GAT TAC AAG AC3'

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Extra References Table

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1050 sampling. BMC Evolutionay Biology, 11, 275.

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1052 of the Royal Society B, 360, 1847–1857.

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Supplementary Table 4. Nucleotide substitution models for each partition evaluated in the software PartitionFinder (Lanfear et al., 2012) and
used in the phylogenetic analyses. *These partitions were analyzed in one partition.

Gene	Maximum Likelihood RAxML Analysis	Bayesian Inference with Mrbayes v.3.0	Molecular Clock Analysis with Beast v.1.6.2	Bases
COI first base of codon	GTR+I+G	GTR+I+G	GTR+I+G	1 – 534 \3
COI second base of codon	GTR+I+G	SYM+I+G	TrNef+I+G	2 – 534 \3
COI third base of codon	GTR+I	GTR+I	GTR+I	3 – 534 \3
CytB first base of codon	GTR+I+G	SYM+I+G	SYM+I+G	535 – 1325 \3
CytB second base of codon	GTR+I+G	GTR+I+G	GTR+I+G	536 – 1325 \3
CytB third base of codon	GTR+G	GTR+G	GTR+G	537 – 1325 \3
16S	GTR+I+G	GTR+I+G	GTR+I+G	1326 – 1849
F-reticulon intron 1	GTR+G	GTR+G	GTR+G	1850 – 3274
F-retex2 first base of codon	GTR+G	SYM+G	SYM+G	3275 – 3536 \3
*F-retex2 second base of codon	GTR+G	SYM+G	SYM+G	3276 – 3536 \3
*F-retex2 third base of codon	GTR+G	GTR+G	GTR+G	3277 – 3536 \3
F-reticulon intron 2	GTR+G	GTR+G	GTR+G	3538 – 3881
F-retex3 first base of codon	GTR+I+G	SYM+I+G	SYM+I+G	3882 – 4500 \3
F-retex3 second base of codon	GTR+I+G	GTR+I+G	GTR+I+G	3883 – 4500 \3
F-retex3 third base of codon	GTR+I+G	GTR+I+G	GTR+I+G	3884 – 4500 \3

1064 **Supplementary Table 5.** DEC models tested to estimate distribution ranges inherited by the descending lineages at each node of the tree. *
1065 Represent the model used in the analysis.

	Prediction	Dispersal rates between adjacent areas	Dispersal rates between no adjacent areas	Likelihood
M1	Dispersal between no adjacent areas not permitted	1.0	–	lnL = - 255.9
M2	Dispersal between no adjacent areas permitted	1.0	1.0	lnL = - 254.8
*M3	Dispersal between no adjacent areas permitted	0.5	0.0001	lnL = - 252.5
M4	Dispersal between no adjacent areas permitted	0.1	0.0001	lnL = - 263.0

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1073 **Supplementary Table 6.** Substitution Saturation estimated for each gene using the index of substitution saturation (Iss) (Xia et al., 2003 and Xia

1074 and Lemey 2009) and the rate of transitions/transversions evaluated in software DAMBE 5.2.31 (Xia and Xie, 2001).

	Model	Transition	Transversion	Index of Substitution Saturation (Iss)
COI	TN93	0.769	0.754	Iss<Iss.c
CytB	GTR	0.783	0.850	Iss<Iss.c
16S	GTR	0.914	0.844	Iss<Iss.c
Reticulon	GTR	0.937	0.950	Iss<Iss.c

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1083 **Supplementary Table 7.** Likelihood-based tests for alternative topologies. SH and AU are probability values obtained for the Shimodaira-

1084 Hasegawa and the Approximately Unbiased tests (Shimodaira, 2002). Asterisks denote significant values (P<0.05 for SH and P<0.01 for AU and

1085 ELW), that imply the topology is rejected.

Test	Topology	-Ln L	Δ - Ln L	ELW	SH	AU
	ML	76971.76				
1	Hypoptopomatinae sister group of Otothyrinae[a]	77001.46	29.7	0.0021*	0.4540	<0.001*
2	Relationship of Otothyrinae genus consistent with Clade B of Schaefer (1991), Fig. 13[a]	77523.21	551.45	<0.001*	<0.001*	<0.001*
3	*Neoplecostomus* genus monophyletic[a]	77120.75	148.99	<0.001*	0.0027*	<0.001*
4	*Pareiorhina* genus monophyletic[a]	77102.71	130.95	<0.001*	0.0025*	<0.001*
5	*Epactionotus* and *Eurycheilichthys* sister group of *Hisonotus* from South of Brazil[a]	77010.47	38.71	0.0089*	0.3299	0.0136

[a] The alternative topology was defined as the ML tree forcing the desired relationship.

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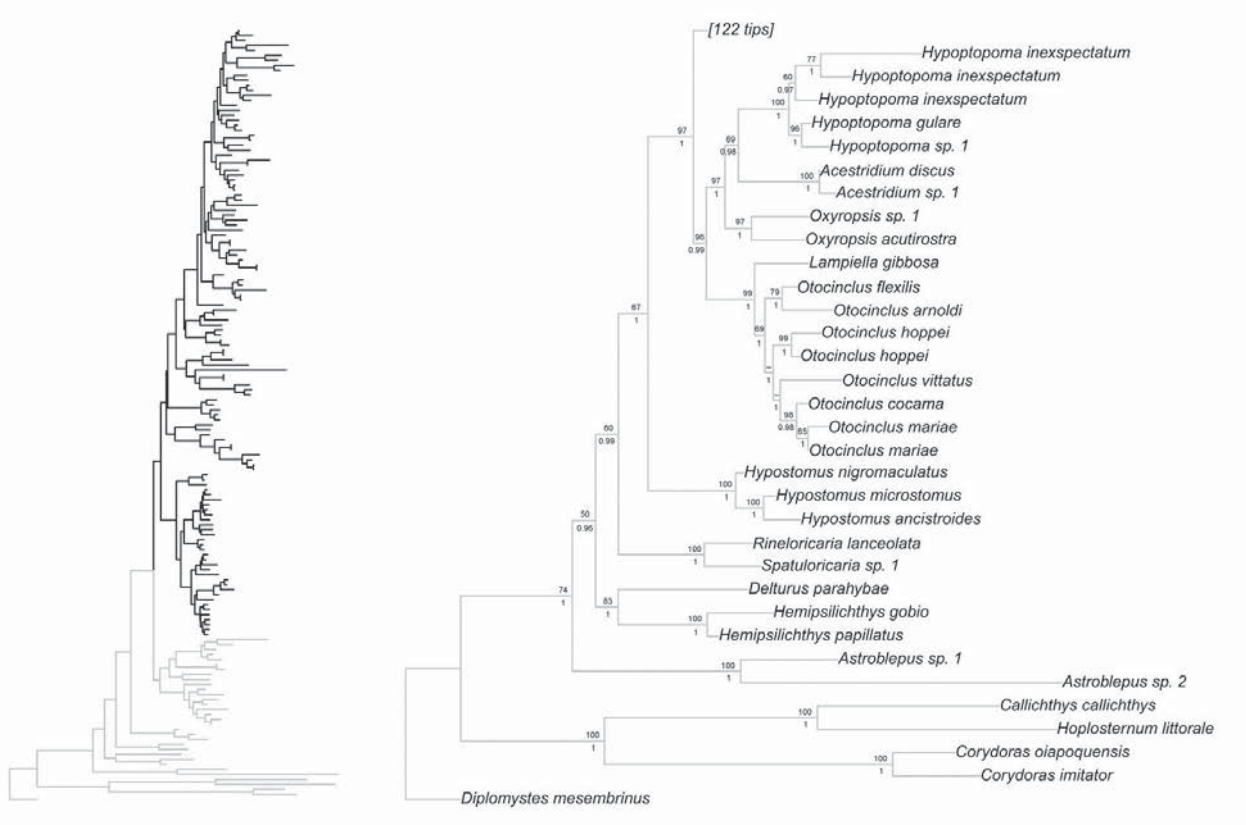
Fig. 1 - Partial ML tree showing outgroups and interrelationship among species of the subfamily Hypoptopomatinae. Numbers above branches are bootstrap values from 1000 bootstrap pseudoreplicates obtained from ML analysis. Bootstrap values below 50% (−) are not shown. Numbers below branches are posterior probabilities obtained in the BI analysis. Posterior probabilities values below 0.95 (−) or when the nodes were not obtained by B analyses are not shown.
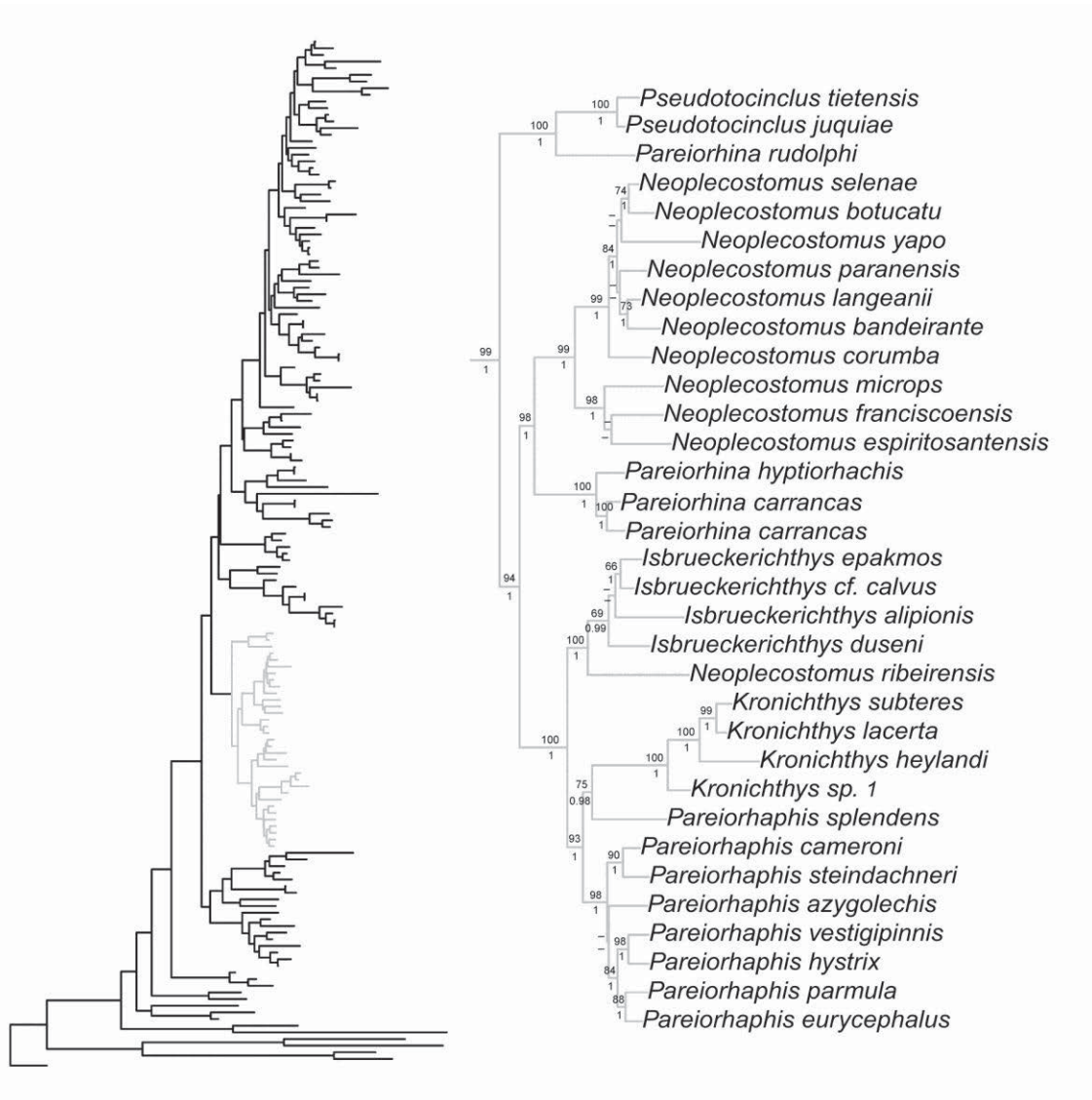
Fig. 2 - Partial ML tree showing interrelationship among species of the subfamily Neoplecostominae. Numbers above branches are bootstrap values from 1000 bootstrap pseudoreplicates obtained from ML analysis. Bootstrap values below 50% (−) are not shown. Numbers below branches are posterior probabilities obtained in the BI analysis. Posterior probabilities values below 0.95 (−) or when the nodes were not obtained by B analyses are not shown.

Fig. 3 - Partial ML tree showing interrelationship among species of the subfamily Otothyrinae. Numbers above branches are bootstrap values from 1000 bootstrap pseudoreplicates obtained from ML analysis. Bootstrap values below 50% (−) are not shown. Numbers below branches are posterior probabilities obtained in the BI analysis. Posterior probabilities values below 0.95 (−) or when the nodes were not obtained by B analyses are not shown.

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Fig. 4 - Partial ML tree showing the interrelationship among species of the subfamily
Otothyrinae. Numbers above branches are bootstrap values from 1000 bootstrap
pseudoreplicates obtained from ML analysis. Bootstrap values below 50% (−) are not
shown. Numbers below branches are posterior probabilities obtained in the BI analysis.
Posterior probabilities values below 0.95 (−) or when the nodes were not obtained by B
analyses are not shown.
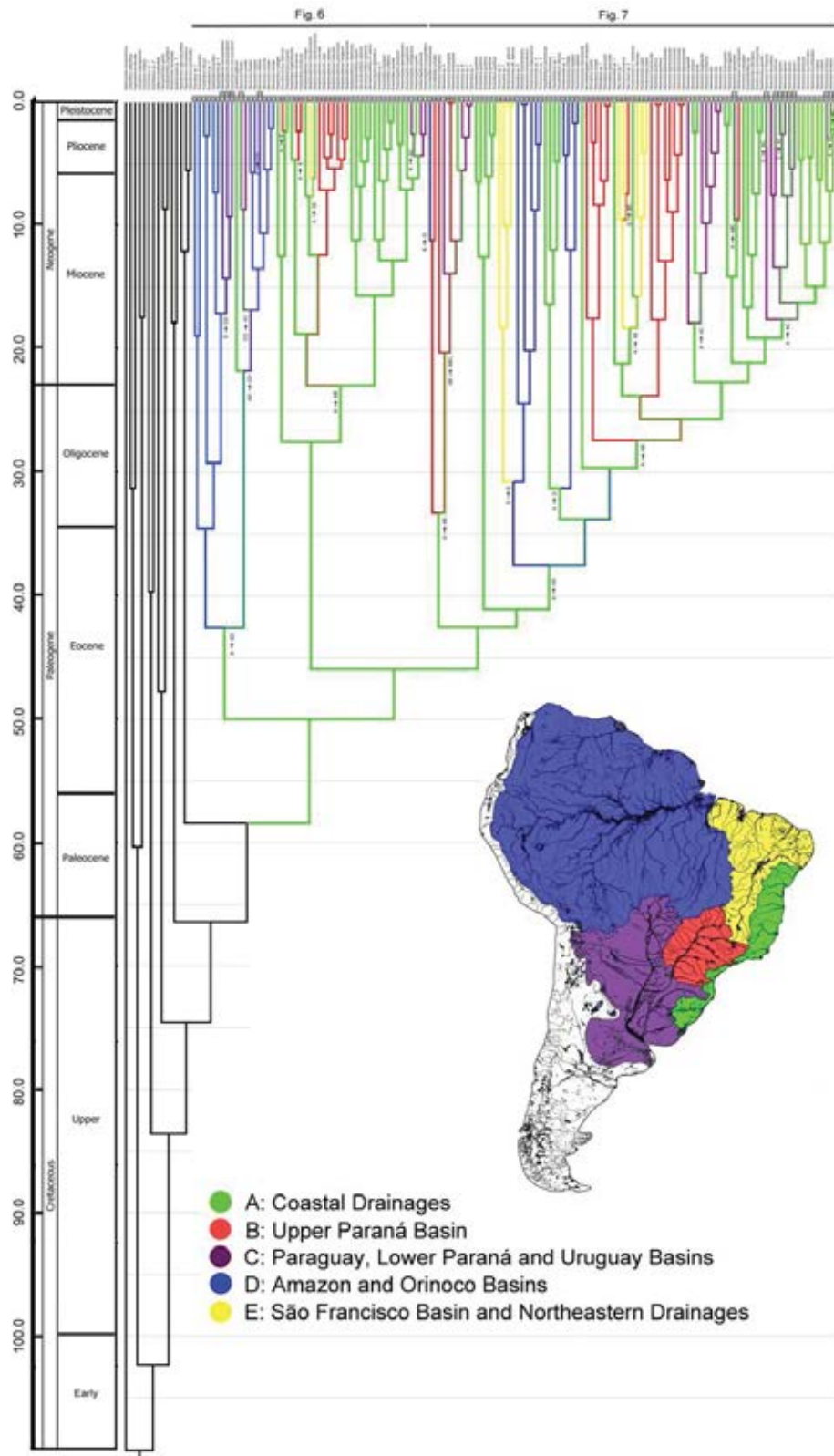
1121
1122 Fig. 5 - Time-calibrated phylogeny for Hypoptopomatinae, Neoplecostominae and
1123 Otothyrinae. Tree topology from BEAST analysis of 155 specimens representing 114
1124 loricariid species. Divergence ages calibrated by origins of Siluriformes (120 Ma) and
1125 Callichthyidae (55 Ma). Regions: A, Atlantic Coastal Drainages (Green); B, Upper
1126 Paraná Basin (Red); C, Paraguay, Lower Paraná and Uruguay Basins (Purple); D,
1127 Amazon and Orinoco Basins (Blue); E, São Francisco Basin and Northeastern
1128 Drainages (Yellow).

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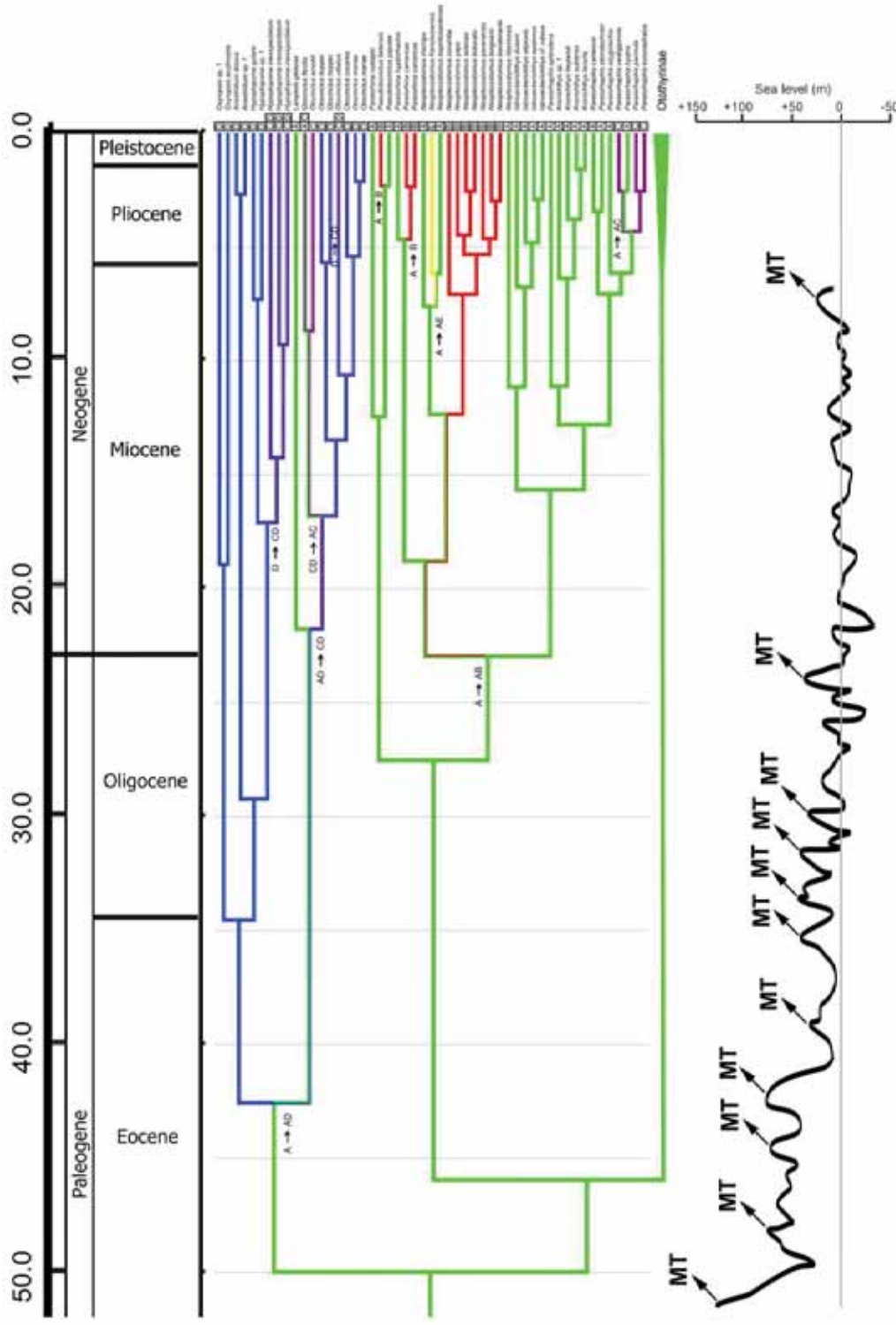
Fig. 6 - Partial time-calibrated tree from BEAST analysis, showing divergence ages for taxa in Hypoptopomatinae and Neoplecostominae. The curve below the phylogeny represented sea levels with the marine transgressions (MT), modified from Zachos *et al.* (2001) and Miller *et al.* (2005).
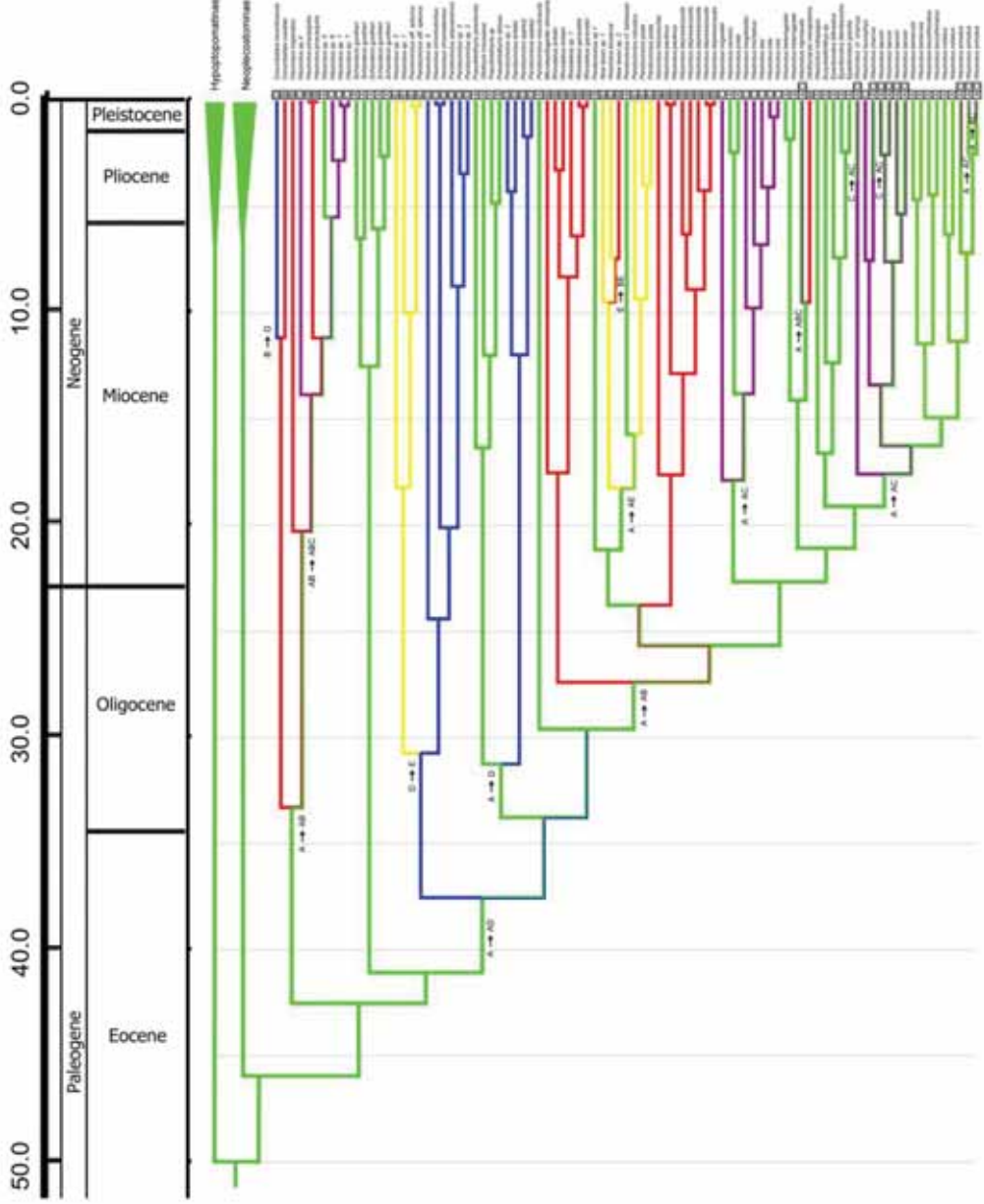
Fig. 7. Partial time-calibrated tree from BEAST analysis, showing divergence ages for taxa in Otothyrinae.
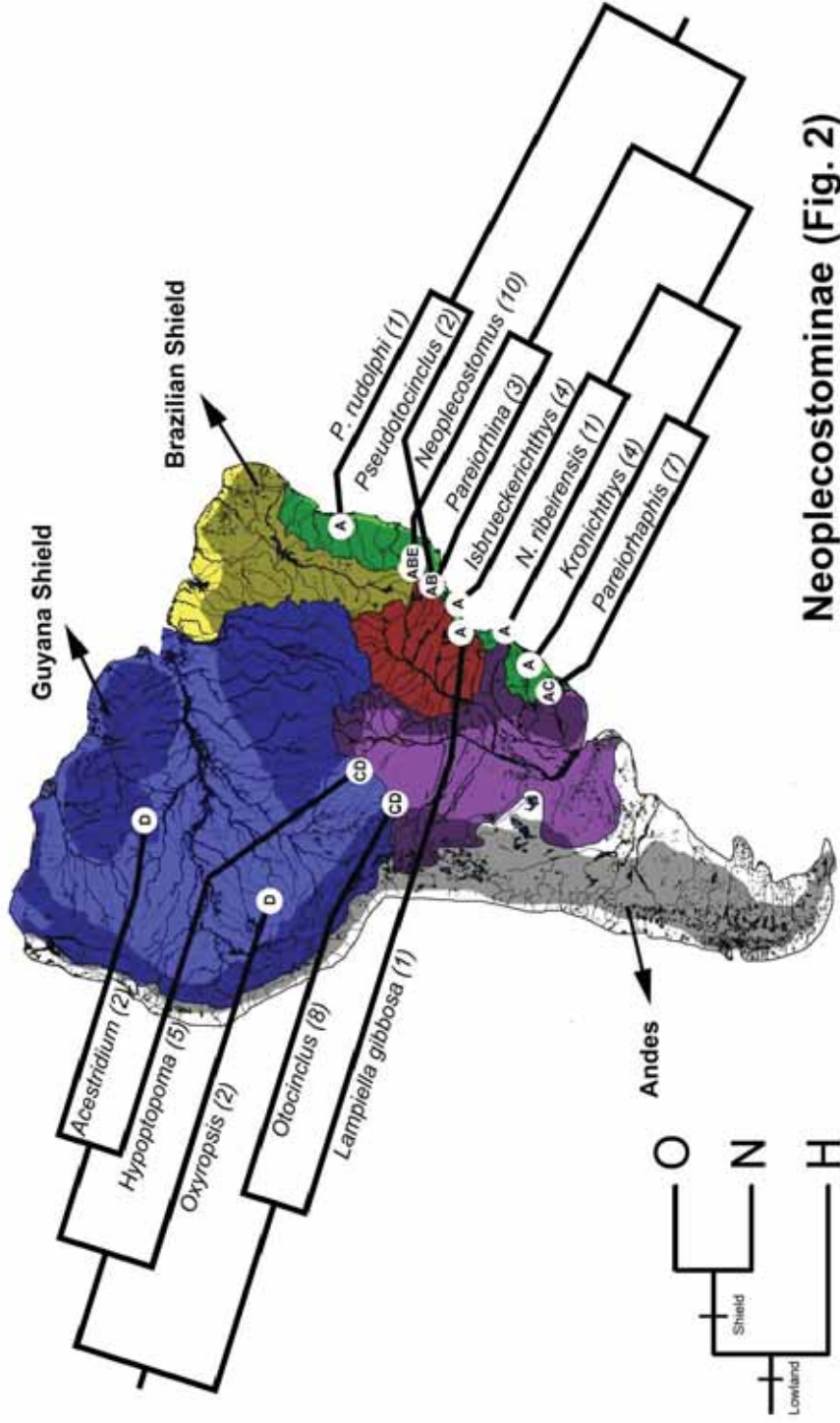
Fig. 8. Geographic distribution and phylogeny of major clades of Hypoptopomatinae and Neoplecostominae in tropical South America. Terminal taxa may represent species or monophyletic higher taxa (see figs. 1–2 for all taxa). Numbers in parentheses after each taxon name are number of taxa sampled for each lineage. Regions: A, Coastal Drainages (Green); B, Upper Paraná Basin (Red); C, Paraguay, Lower Paraná and Uruguay Basins (Purple); D, Amazon and Orinoco Basins (Blue); E, São Francisco Basin and Northeastern Drainages (Yellow).
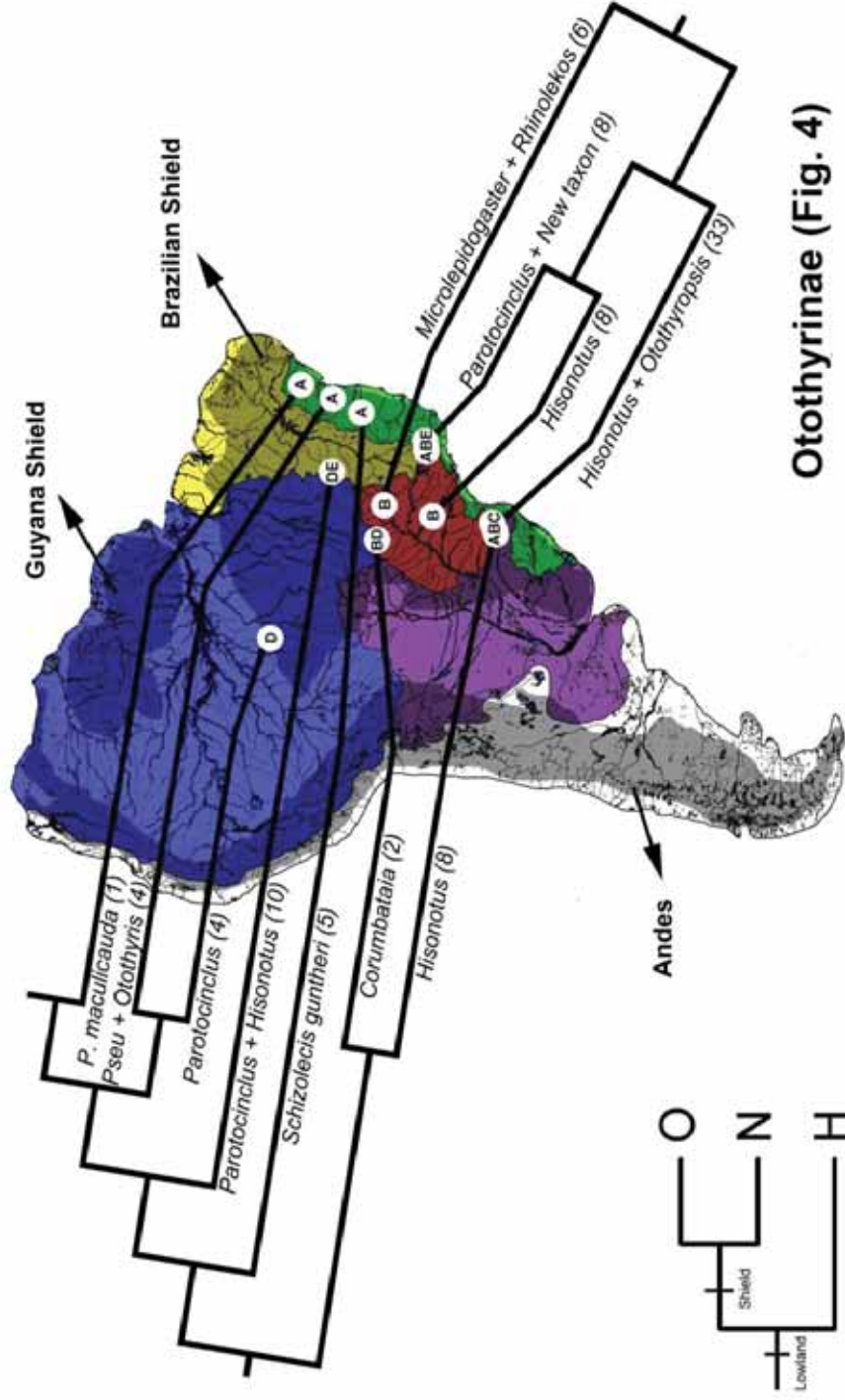
Fig. 9. Geographic distribution and phylogeny of major clades of Otothyrinae in tropical South America. Terminal taxa may represent species or monophyletic higher taxa (see figs. 1–2 for all taxa). Numbers in parentheses after each taxon name are number of taxa sampled for each lineage. Regions: A, Coastal Drainages (Green); B, Upper Paraná Basin (Red); C, Paraguay, Lower Paraná and Uruguay Basins (Purple); D, Amazon and Orinoco Basins (Blue); E, São Francisco Basin and Northeastern Drainages (Yellow).

Chapter 3

1 **[Manuscript formatted to Evolution]**

2

3 **Diversity and evolution of body size in armoured catfishes (Siluriformes:**
4 **Loricariidae)**

5

6 **Abstract**

7 Considering that size is one of the more important quantitative traits on which evolution
8 acts, the objective of the present paper is investigate and describe the major patterns of size
9 evolution in three Neotropical fish subfamilies Hypoptopomatinae, Neoplecostominae and
10 Otothyrinae. First we evaluate different methods of ancestral reconstruction to continuous
11 characters to tracking size evolution and used a maximum likelihood approach to estimate
12 ancestral sizes. Second considering that the size distribution patterns within fish lineages at
13 low taxonomic levels as subfamilies remains understudied we used a measuring of rates of
14 evolution in *darwins (d)* in a phylogenetic context, to evaluate if lineages tend to increase in
15 BS over evolutionary time (e.g. Cope's rule). Our results suggested that the most similar
16 models in the distribution of *darwins (d)* are PIC vs. GLS, seeing that, the distributions of *d*
17 values fits more a line compared with the other models and the most different models are
18 ML vs. PIC, seeing that, dots are more widespread through the graphic. Additionally, our
19 results suggested that the range of size-change values *(d)* along branches phylogeny
20 increased rapidly during the Later Miocene. The distribution of size-changes *(d)* is slightly
21 right-skewed (consistent with Cope` rule) considering all together subfamilies, is slight left-
22 skewed (reverse of Cope`s Rule) within Hypoptopomatinae, is slight right-skewed within
23 Neoplecostominae, however, if we exclude the three lower *(d)* values the distribution stays
24 quite right-skewed (consistent with Cope` rule), and is quite left-skewed within Otothyrinae
25 (reverse of Cope`s rule). These results document the ways in which macroevolutionary
26 processes may produce the size diversity within armoured "cascudinhos".

27

28 **Introduction**

29   Size is one of the more important aspects of an animal functional design and one of
30 the principal quantitative traits on which evolution acts (Schmidt-Nielsen 1984, 1997;

121

31 Blanckenhorn 2000). The evolution of body size (BS) has attracted particular attention,

32 because it is related to many physiological, ecological and biogeographic variables (Peters

33 1983; Calder 1984; Reiss 1989; Roff 1992; Stearns 1992), and has profound consequences

34 for structure, function, and survival (Schmidt-Nielsen 1984, 1997). Therefore, disparity in

35 BS among organisms raises several important questions: What are the biological

36 consequences of changes in BS? What are the main genetic, ecological and physiological

37 processes responsible for these changes? How do differences in size affect net rates of

38 diversification among different lineages? (Schmidt-Nielsen 1984; Blanckenhorn 2000).

39 Disparity in BS has been the focus of a significant amount of research, much of it

40 relying on a comparative macroevolutionary framework (Van Valen 1974; Stanley 1979;

41 Schmidt-Nielsen 1984; Stanley 1998; Blanckenhorn 2000; Jablonski 2007, 2008, Albert

42 and Johnson 2011). Many of these studies ultimately aim to shed light on the diversification

43 of phylogenetic lineages as a function of change in organismal traits, often using BS as a

44 proxy for physiological and ecological attributes (Newell 1949; Stanley 1973; Kochmer

45 and Wagner 1988; Maurer et al. 1992; Brown et al. 1993; Fenchel 1993; Gaston and

46 Blackburn 1995; Poulin and Morand 1997; Munday and Jones 1998; Kingsolver and

47 Pfennig 2004; Webster et al. 2004; Brown and Sibly 2006; Clauset and Erwin 2008; Purvis

48 et al. 2003; Albert and Johnson 2011). A widely cited trend in the literature known as

49 Cope's rule (also known as the Cope-Depéret rule, Depéret 1907), predicts a tendency for

50 BS to increase within lineages over time (Cope 1877, 1887, 1896; Newell 1949; Stanley

51 1973). The most extensive macroecological analyses of size-frequency distributions have

52 focused on extant mammals and extant birds (Clauset and Erwin 2008; Clauset et al. 2009;

53 Monroe and Bokma 2009; Olson et al. 2009; Capellini et al. 2010) and in fishes as a whole

54 (Albert and Johnson 2011), however, BS evolution in other animal groups remains unclear.

55 Explanations for this trend include the ability of individuals to tolerate short-term variation

56 in the physical environment, their capacity to extract energy and nutrients from a wider

57 variety of poorer-quality food, and their ability to avoid predators (Stanley 1973; Brown

58 and Maurer 1986, 1987). Furthermore, studies of natural and artificial selection have shown

59 that microevolutionary processes operating through the differential survival and

60 reproduction of individual organisms can indeed cause substantial directional evolution in

61 BS (Wolda 1963; Bantock and Bayley 1973; Knights 1979; Boag and Grant 1981; Price

62 1984; Price and Grant 1984; Price et al. 1984). However, Gould (1988) questioned this

63 microevolutionary interpretation, suggesting that macroevolutionary processes (speciation

64 and extinction) are more important drivers of size differences among lineages.

65   Early research supported the hypothesis of right-skewed distributions of BSs among

66 species, with a tendency to be higher right-skewed in high taxonomic levels as classes and

67 diverse skewed at lower taxonomic levels as order and families (Kozlowski and Gawelczyk

68 2002; Stanley 1973). More recent work have being supported the pattern of right-skewed

69 distribution among species, even when log transformed (Brown and Maurer 1986; Gaston

70 and Blackburn 2000; Maurer et al. 2004; Albert and Johnson 2011). Different patterns of

71 size distribution among clades could be explained as a result of different evolutionary

72 pressures and histories acting at different scales and rates. Within large-scale taxonomic

73 pattern Newell (1949) and Kingsolver and Pfennig (2004) predicted models of size

74 evolution resulting in larger sizes within lineages, otherwise Brown and Sibly (2006)

75 predicted models of size evolution resulting in smaller sizes within lineages. Some studies

76 interpreted the right-skewed size distributions as evidence for the selective advantage of

77 small size (Damuth 1993; Blanckenhorn 2000; Maurer et al. 2004). Additionally, right-

78 skewed size distributions could be interpreted as an increase rate of diversification

79 (Jablonski 1997; Maurer 1998; Gardezi and da Silva 1999; Knouft and Page 2003), or

80 extinction risks that threaten large bodied species (Maurer et al. 1992; Purvis et al. 2003;

81 Clauset and Erwin 2008; Clauset et al. 2009). Despite the plethora of hypotheses, size

82 distribution patterns within fish lineages as a whole, mainly at low taxonomic levels as

83 family and subfamilies, remains understudied (Albert & Johnson 2011).

84   The advent of gene sequencing techniques starting in the 1970s (Maxan and Gilbert

85 1977; Sanger et al. 1977; Gilbert 1981), and the development of molecular phylogenic

86 statistical methodologies starting in the 1980s with the works of Joseph Felsenstein

87 (Felsenstein 1981; Felsenstein 1985a, b; Felsenstein 1988) has led to a rapid proliferation

88 of phylogenetic studies over the past decade (Nei and Kumar 2000). This field of molecular

89 phylogenetics has revolutionized our ability to pose hypothesis within a comparative

90 evolutionary framework (Donoghue & Smith 2003; Benton & Emerson 2007; Wiley and

91 Lieberman 2011). This methodology afforded us to reconstruct the evolutionary

92 relationships of specific lineages, and allowing us to measure the rates of change in key

93   traits (Paradis 2012; Harmon et al. 2008). At past decades a series of papers has appeared

94   regarding the measurement of the long-term rates of morphological evolution (Haldane

95   1949; Lerman 1965; Cherry et al. 1978, 1982; Atchley 1980; Kunkel et al. 1980; Gingerich

96   1983, 1984a, b; Wyles et al. 1983; Charlesworth 1984; Gould 1984; Lomolino 1985; Lynch

97   1990; Jablonski 1997; Alroy 1998; Boback 2003; Boback and Guyer 2003; Campbell and

98   Echternacht 2003; Clauset and Erwin 2008; Clauset et al. 2009; Monroe and Bokma 2009;

99   Olson et al. 2009; Capellini et al. 2010; Albert and Johnson 2011). In terms of BS

100   evolution, Haldane (1949) proposed a method for measuring the rate of BS evolution

101   expressed in *darwin (d)* unit defined to be the change of a morphological trait over one

102   million years. Albert and Johnson (2011) introduce a new use of a method for measuring

103   rates of evolution in *darwins* (Haldane 1949) of continuous traits in an explicitly

104   phylogenetic context to evaluate how a non-directional model of trait evolution can produce

105   skewed size-frequency distributions and trends in BS evolution in agreement with Cope's

106   prediction.

107   Chiachio et al. (2008) hypothesized that small BS may explain the limited dispersal

108   abilities observed within certain armoured catfish subfamilies Hypoptopomatinae,

109   Neoplecostominae and Otothyrinae (HNO-clade) throughout Brazilian hydrographic basins.

110   These three monophyletic subfamilies are part of the family Loricariidae, the most diverse

111   and widespread members of the Neotropical freshwater fish fauna (Reis et al. 2003). It has

112   been shown that dispersal distance increases with BS due to a smaller energetic migratory

113   cost per unit distance in large fishes (Bernatchez and Dodson 1987). Chiachio et al. (2008)

114   explored a hypothesis where the comparatively small BS of the most recent common

115   ancestor of the HNO-clade limited the dispersal across the Northern and Southern River

116   Systems of Brazil. However, their work did not evaluate the pattern of size distribution and

117   rate of BS evolution in a specific biological context or rule (i.e. Cope´s rule).

118   Here we investigate and describe the major patterns of size evolution in three

119   Neotropical fish subfamilies Hypoptopomatinae, Neoplecostominae and Otothyrinae. First

120   we evaluate different methods of ancestral reconstruction to continuous characters to

121   tracking size evolution and used a maximum likelihood approach to estimate ancestral

122   sizes. Second considering that the size distribution patterns within fish lineages at low

123   taxonomic levels as subfamilies remains understudied we used a measuring of rates of

124   evolution in *darwins (d)* (Haldane 1949; Albert and Johnson 2011), in a phylogenetic

125   context, to evaluate if lineages tend to increase in BS over evolutionary time (e.g. Cope's

126   rule).

127

128   **Material and Methods**

129   *Time calibrated tree and ancestral characters estimation*

130   Size data are available for includes 114 loricariid (86 described and 19 undescribed

131   species) from the HNO-clade, including 8 outgroup species (unpublished paper). The

132   taxonomic summary, the GenBank accessing number and the species BS for this study is

133   provided in Supplementary Table 1. Size was assessed as maximum recorded standard

134   length (SL) in cm as measured from the tip of the snout to the posterior end of the last

135   vertebra or to the posterior end of the midlateral portion of the hypural plate, excluding the

136   length of the caudal fin. Standard length was measured directly from specimens deposited

137   at biological collection of LBP (Laboratório de Biologia e Genética de Peixes,

138   Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, São Paulo), specimens

139   in original species description papers, or published lengths in Reis et al. (2003). All

140   measurements were taken point to point to the nearest 0.1 mm with digital calipers.

141   The uncorrelated relaxed molecular clock (lognormal) was calibrated using BEAST

142   (Bayesian evolutionary analysis sampling trees) v1.6.2, (Drummond and Rambaut 2007).

143   The models utilized for each partition are shown in Table S3. We included two calibration

144   points to constrain divergence dates for the 154 clades identified in our phylogenetic tree.

145   The first calibration point was implemented as a normally-distributed prior, with an offset

146   of 125 million years ago (Ma), and a standard deviation of 15 million years. These date-

147   estimate parameters were selected to match current knowledge of the timing of siluriform

148   origins. Information from the stratigraphic record and geographic distributions of living

149   taxa indicate an origin for Siluriformes as a whole during the Lower Cretaceous (145 – 100

150   Ma; Lundberg 1993; Sullivan et al. 2006; Lundberg et al. 2007). The second calibration

151   point was implemented using a log-normal prior offset to 55 Ma with a mean and standard

152   deviation of 1 for the origin of the family Callichthyidae. The oldest known callichthyid

153   fossil, *Corydoras revelatus* Cockerell (1925) was dated by Marshall et al. (1997) as

154 Paleocene. This prior assumed 55 Ma as a minimum age. We used a Birth–Death model for

155 speciation likelihood and a starting tree obtained from ML analysis. The analysis was run

156 for 100 million generations and sampled every 1000th generation. Stationarity and

157 sufficient mixing of parameters (ESS>200) was checked using Tracer v1.5 (Rambaut and

158 Drummond 2007a). A consensus tree was built using TreeAnnotator v1.6.2 (Rambaut and

159 Drummond 2007b).

160   Different methods for ancestral state reconstruction (ASR) of continuous characters

161 could result in different estimates of ancestral species size thereby having a differential

162 effect on resulting *darwins (d)* values (Haldane 1949). In order to avoid such discrepancies,

163 we employed three different ASR strategies using continuous variables to see how and if *d*

164 values ware affected. The methods used were: Maximum Likelihood (ML), Phylogenetic

165 Independent Contrasts (PIC) and Generalized Least Squares (GLS). These were employed

166 using package "ape" (Paradis 2012) in program R version 3.0.0 (R Core Team 2013). For

167 the analysis of ancestral species size we used a Maximum Likelihood (ML) method

168 (Schluter et al. 1997), since this model can reconstruct the ancestral character history with

169 significant accuracy (Schluter et al. 1997). The water river volume and water river velocity

170 was treated as continuous characters, and with altitude the ancestral estimations were

171 employed using package "ape" under a Maximum Likelihood (ML) method and Brownian

172 motion model.

173   During model selection it is necessary to determine whether a stable model of

174 continuous character evolution fits the data better than a Brownian motion model. Schluter

175 et al. (1997) predicted that the accuracy of the estimation of ancestral character

176 reconstruction under likelihood methods depends on how a model of character evolution

177 fits a specific dataset. We evaluated macroevolutionary models using the function

178 fit.continuous of package "geiger" (Harmon et al. 2008) in the program R version 3.0.0 (R

179 Core Team 2013) for ancestral estimation of BS. The best fitting model was determined

180 using the Akaike's Information Criterion (AICc) (Sugihara 1978). The models tested are:

181 Brownian Motion (BM) (Maddison 1991) that predicts a random walk with a central

182 tendency proportional to the parameter alpha; Ornstein-Uhlenbeck (OU) (Hansen 1997;

183 Butler and King 2004) that can be seen as a generalization of the Brownian motion process

184 and also can fit a random walk model with a central tendency proportional to the parameter

185 alpha; and Early Burst model (EB) (Blomberg et al. 2003) where the rate of evolution can

186 increases or decrease through time.

187 The phylogenetic correlation among of the log transformed maximum BS with

188 water river volume, water river velocity and altitude were performed in program R (R Core

189 Team 2013), as well as the "abline", "confidence" and "prediction" bands.

190

191 *Rate of Body Size Evolution*

192 We examined rates of evolutionary change of log transformed BS expressed in

193 *darwins (d)* (Haldane 1949) for a test for Cope's rule. This unit represents the difference in

194 size per unit time along internal branches of a phylogenetic tree. A right skewed size

195 distribution of *darwins (d)* was interpreted as concordant with Cope's rule (evolution

196 toward large size); a left skewed size distribution was interpreted as reverse Cope's rule

197 (evolution toward small size); and a symmetric size distribution was interpreted as no

198 change of size from ancestors to extant species.

199

200 $d = \ln S_2 - \ln S_1 / t_2 - t_t$

201

202 where S_n is the difference in size between nodes (*n*), and t_n is the difference in geological

203 age between nodes (*n*) expressed in Ma.

204

205 Therefore, a *darwin (d)* is a standardized change in the value of a trait per million years

206 (Albert and Johnson 2011).

207

208 *Normality Test and Diversification Analysis*

209 We used Kuiper's test to evaluate if the log sample sizes and the distribution of

210 *darwins (d)* follow a normal distribution. This test compares a null distribution with the

211 empirical distribution of the observed data (Kuiper 1960) and is similar to the Kolmogorov-

212 Smirnov test. This was implemented in package "truncgof" in R version 3.0.0 (R Core

213 Team 2013) according to the tabulation given in Stephens (1970) and under a parameter of

214 mean = 1, sd = 1 and H = -1 (see Table S1 to R commands). We also performed a QQ-Plot

215 (Quantile-Quantile Plots) for Hypoptopomatinae, Neoplecostominae, Otothyrinae

216 subfamilies separated and the three subfamilies together referred here as HNO-clade to

217 evaluate the distribution of the log sizes and *darwins (d)* compared to a normal distribution.

218 This analysis was performed in the package 'stats' in R (R Core Team 2013).

219 The lineages-through-time plots the number of lineages, on a logarithmic scale,

220 observed on a tree with respect to time. If diversification has been constant through time,

221 and the numbers of lineages then a straight line is expected. If diversification rates

222 decreased through time, then the observed plot is expected to lay above the straight line,

223 whereas the opposite result is expected if diversification rates increase through time. This

224 analysis was performed according to ideas of Nee et al. (1992) and Harvey et al. (1994) and

225 was implemented using the package "ape" in program R version 3.0.0 (R Core Team 2013).

226

227 **Results**

228 *Time Calibrated Tree and Ancestral Characters Estimation*

229 The size of specimens used for our analysis, including 114 loricariid (86 valid

230 species and 19 undescribed species of the HNO-Clade), ranged from 2.1 cm (*Parotocinclus*

231 sp. 3 and *P. aripuanensis*) to 17 cm (*Pareiorhaphis cameroni*). Species of

232 Neoplecostominae reach the greatest mean size (mean of 9.0 cm), species of Otothyrinae

233 the lowest mean size (mean 4.0 cm) and species of Hypoptopomatinae a intermediated size

234 pattern (mean of 6.1 cm).

235 The results of BEAST (Figs. 1–2) showed that the mean substitution rate for the

236 dataset was 0.272% per MY. The Hypoptopomatinae is estimated to have originated during

237 the Lower Eocene about 33.6–67.4 Ma 95% HPD (mean 49.9 Ma) and the clade composed

238 of Neoplecostominae + Otothyrinae is estimated to have originated during the Lower

239 Eocene about 31.0–62.2 Ma 95% HPD (mean 45.9 Ma).

240 In Neoplecostominae the species sizes were increasing from the ancestral to the

241 present and most of the modifications occurred in the Miocene (Fig. 1), achieving

242 approximately the modern dimensions. Within Hypoptopomatinae we can observe an

243 increase of ancestral sizes in the lineage of *Hypoptopoma* and a decreasing ancestral size of

244 *Otocinclus* lineages occurring in Miocene (Fig. 1). Within Otothyrinae the changes of the

245 species size was most constant during the times evolution of Otothyrinae lineages.

246 However, changes to a small sizes can be observed in our results and these changes

247 apparently occurred in a more recent time in the end of Miocene to Pleistocene (Fig. 2).

248 Furthermore, the genus *Hisonotus* has the largest (*Hisonotus taimensis* 6.6 cm and *H.*

249 *leucofrenatus* 6.0 cm) and the smallest species (*Hisonotus bocaiuva* and *H. piracanjuba* 2.5

250 cm) of Otothyrinae, and these results can be explained be the polyphyletic aspect of this

251 genus.

252   Considering that different methods of ancestral character reconstruction can result

253 in different BS estimations and consequently in different pattern of distribution of *darwins*

254 *(d),* we compared three models for estimation of ancestral states of continuous variables

255 under a Brownian motion using Maximum Likelihood (ML), Phylogenetic Independent

256 Contrasts (PIC) and Generalized Least Squares (GLS). We found that the most similar

257 models in the distribution of *darwins (d)* are PIC vs. GLS, seeing that, the distributions of *d*

258 values fits more a line compared with the other models ($R^2 = 0.66$) and the most different

259 models are ML vs. PIC, seeing that, dots are more widespread through the graphic ($R^2 =$

260 0.36) (see models comparison in Fig. 3).

261   We observe positive correlation of BS with water river volume (p-value < 0.001)

262 and water river velocity (p-value < 0.001), however, no correlation of BS with altitude (p-

263 value = 0.75) was observed (Fig. 4).

264

265 *Rate of body size evolution*

266   The pattern of extant size distribution in a macroecological perspective exhibits a

267 broad range of skewness values among Hypoptopomatinae (n = 18; skewness = 0.55; P<

268 0.01, Kuiper test), Neoplecostominae (n = 33; skewness = -0.71; P< 0.01, Kuiper test),

269 Otothyrinae (n = 89; skewness = -0.34; P< 0.01, Kuiper test) and HNO-clade (n = 140;

270 skewness = 0.80; P< 0.01, Kuiper test) (Fig. 5). Within Neoplecostominae we can observe a

271 left skewed size distribution of extant species suggesting a predominant large size species.

272 Our ancestral character reconstruction plotted in a time calibrated tree (Fig. 1) suggested

273 that evolution to a large size within lineages of Neoplecostominae occurred in Miocene.

274   Patterns of size evolution (as measured in *darwins, d*) in a macroevolutionary

275 perspective (i.e. Cope´s rule) within HNO-clade fishes through Paleogene to present are

276 similar to those of absolute size itself. The range of size-change values *(d)* along branches

129

277   phylogeny increased rapidly during the Later Miocene (10 Ma to the present). Additionally,

278   our results suggested that the distribution of size-changes *(d)* is slightly right-skewed

279   considering all together subfamilies (HNO-Clade; Fig. 6a; skewness = 0.40, P < 0.01,

280   Kuiper test; *d* ranging -0.14 – 0.20). Within Hypoptopomatinae our results suggested that

281   the distribution of size-changes *(d)* is slight left-skewed (Fig. 6b; skewness = -0.03, P <

282   0.01, Kuiper test; *d* ranging -0.05 – 0.05) and has an intermediate range compared with

283   Neoplecostominae and Otothyrinae as showed our box plot graphic (Figs. 7; mean = -

284   0.003).

285   Within Neoplecostominae we can observe that the pattern of distribution of size-

286   changes in *darwins (d)* is slight right-skewed (Fig. 6c; skewness = 0.01, P < 0.01, Kuiper

287   test; range -0.14 – 0.20). However, if we exclude the three lower *(d)* values the distribution

288   stays quite right-skewed (excluding *(d)* values -0.14, -0.13 and -0.12, skewness = 1.34, P <

289   0.01, Kuiper test). The boundary values of the distribution of size can great influence in the

290   calculation of the skewness and low values in left boundaries of Neoplecostominae

291   tendency the results been symmetric. The range distribution of *(d)* is the greatest compared

292   with the other subfamilies (Fig. 7, mean = 0.008). Within Otothyrinae we can observe that

293   the distribution of size-changes *(d)* is quite left-skewed (Fig. 6d; skewness = -1.22; range -

294   0.10 – 0.05; P < 0.01, Kuiper test) and has the lowest range compared with the others

295   subfamilies (Figs. 7, mean = -0.006). Although, the mean BSs of ancestral species of HNO-

296   Clade do not change substantially from Paleogene to the present (Fig. 8).

297

Normality test and diversification analysis

299   The Kuiper's test suggested that the log sample sizes and the distribution of *darwins*

300 *(d)* do not follow a normal distribution, since the p-value is always smaller than 0.01 for all

301 normal distribution tests (Hypoptopomatinae, Neoplecostominae, Otothyrinae and the three

302 clades together; see figs. 5 and 6 for all values). We also evaluated the log sample sizes and

303 the distribution of *darwins (d)* using a graphical tool for assessing normality, a quantile-

304 quantile plot (QQ plot). The graphical analysis also implied that our data do not follow a

305 normal distribution (Figs. S1 and S2).

306   The results of the lineages-through-time plots implies a curve following the straight

307 line from 40 Ma to 10 Ma and slight above the straight line from the last 10 Ma, indicating

308 an increase rate of diversification near the present within Neoplecostominae. Within

309 Hypoptopomatinae the curve following the straight line indicating a constant rate of

310 diversification and within Otothyrinae the LTT plot curve is above the straight line,

311 strongly implying a rate of diversification increasing through time (more speciation and less

312 extinction).

313

314 **Discussion**

315

316 *Time calibrated tree and size-dispersal hypothesis*

317   Our time calibrated tree and ancestral size estimation analysis suggested that no

318 abrupt size changes were observed within Hypoptopomatinae ancestral to extant species

319 (Fig. 1). However, species of the genus *Hypoptopoma* possess the larger maximum

320 standard length (SL) across analyses species of Hypoptopomatinae (ranging from 6 to 10.5

321 cm) and *Otocinclus* the smallest (3.3 to 5.5 cm). Our results suggested that despite the

322 origin of this genus is from Oligocene the evolution to a large size in *Hypoptopoma*

323 occurred in Miocene, as well as the evolution to a small size in species of *Otocinclus* (Fig.

324 1). Chiachio et al. (2008) suggested that the differences in size should influenced dispersal

325 of Hypoptopomatinae through Brazilian hydrographic basins. Fishes of large sizes tend to

326 moves over great distances and has more ability to disperse compared with fishes of small

327 sizes (Bernatchez and Dodson, 1987). Chiachio et al. (2008) predicted that *H.*

328   *inexspectatum* crossed the border of what they call Northern River Systems and Southern

329   River Systems of Brazil. Our calibrated tree suggested that the ancestral of *H.*

330   *inexspectatum* crosses this barrier from Northern River Systems to Southern River Systems

331   in Miocene (unpublished paper) and the ancestral of *H. inexspectatum* had around 8.0 cm

332   (Fig. 1). A size higher than the mean of size of the extant species of Hypoptopomatinae of

333   6.1 cm and a higher that the maximum size found for the species today of 7.1 cm,

334   suggesting that size was not a factor that should impeded the dispersal of ancestral of *H.*

335   *inexspectatum* or of other species of *Hypoptopoma* as a whole. Our results also suggested

336   that the ancestral this genus had around 5.8 cm and that the size of this lineage was getting

337   higher from Oligocene, until reaches 8.0 cm during the Miocene.

338   Chiachio et al. (2008) suggested that the ancestral lineages of Hypoptopomatinae

339   have crossed the Northern River Systems and Southern River Systems several times in the

340   evolution of the group what are corroborated by our results. Within the large *Otocinclus*

341   lineage, the ancestral of *Lampiella* and *Otocinclus* have crossed this barrier at the early

342   Miocene and the size of the ancestral of this group reached at 5.3 cm. Our results suggested

343   that both genera display large maximum SL (ranging from 5 cm to *Lampiella* and 5.5 cm to

344   *Otocinclus*). Our results of unpublished paper also suggested that most of the dispersal of

345   Hypoptopomatinae among hydrographic basins occurred in a period of the middle Miocene

346   or before in time and that our analysis suggested that species of the genus *Otocinclus*

347   reaches small size from Later Miocene to the present, a period with few dispersal events

348   within Hypoptopomatinae as shown in Chapter 1.

349   Within Neoplecostominae species reaches the greatest sizes compared with species

350   of the other subfamilies (Hypoptopomatinae and Otothyrinae). Our results suggested that

351   the size of the extant species evaluated from a small size to a large size occurred in the

352   Miocene. This result suggested that this period of time was very important to changes in

353   size among species of Hypoptopomatinae and Neoplecostominae. Miocene was period of

354   great oscillation in the climate and a period where several marine transgression in South

355   America low lands. The Miocene is characterized by a series of climatic events that

356   affected the climate of the subsequent periods and the dawn of modern biota. After the

357   Miocene Climatic Optimum (around 18 to 16 Ma) a wave of extinctions of terrestrial and

358   aquatic life forms occurred (around 15 to 14 Ma) (Shackleton and Kennett 1975; Savin et

359 al. 1975; Miller et al. 1987; Hornibrook 1992). These climate oscillation associated with

360 available ecological niches could be resulted in different pressures in different fishes

361 lineages to reaches large or smaller sizes.

362   The *Hisonotus* group that includes the type species *Hisonotus notatus* and species of

363 the Lagoa dos Patos and Uruguay basin (Fig. 2) represent the large species of this genus.

364 Most of the species of this group lives in large rivers and is widespread through South and

365 Southeaster of Brazil. Living in large river could be selective pressure to reach a large size

366 and as we discussed previously, Chiachio et al. (2008) hypothesized that large species has

367 more ability to dispersal through great distance compared with small species (Bernatchez

368 and Dodson 1987).

369

370 *Macroecological pattern*

371   Despite of importance of study of size in a macroevolutionary perspective, several

372 authors reinforced the aspect of in a macroecological perspective have recognized the role

373 of environmental conditions and ecological processes in differential speciation and

374 extinction rates (Stanley 1973, 1979, 1998; McKinney 1990). In this perspective the

375 macroecological field has rapid advanced towards answering many of the questions that

376 originally occupied macroecologists, such as variations in BS among species (Beck et al.

377 2012).

378   In our results the extant species distributions within subfamilies lineages exhibit a

379 broad range of skewness values, even when log transformed: 0.55 for Hypoptopomatinae, -

380 0.71 for Neoplecostominae and -0.34 for Otothyrinae (Fig. 5). The pattern of skewness of

381 extant species can vary depending of the taxonomy order, and can be interpreted as, more

382 frequent small bodied organisms or evolution toward large size when skewed to the right;

383 and more frequent large organisms or evolution toward small sizes when skewed to the left

384 (Kozlowski and Gawelczyk 2002; Albert and Johnson 2011). According to Kozlowski and

385 Gawelczyk (2002) if we consider narrower systematic groups, orders instead of classes for

386 example, the skewness becomes more variable. However, as a general pattern, the

387 distribution of large groups as fishes the BS distribution is right skewed (Albert and

388 Johnson 2011).

389   The symmetric, right-skewed or left-skewed species shape of BS distributions can

390   be explained by different evolutionary process (Kozlowski and Gawelczyk 2002; Albert

391   and Johnson 2011). The first well-documented feature is that most clades originate at small

392   size (McKinney 1990); this is possible because a clade is most likely to originate after mass

393   extinctions, and small animals are less prone to extinction during a catastrophe. After that,

394   the clade diversification can go in both directions (toward smaller and larger size),

395   considering that diversification is a relation among speciation and extinction.

396   In a macroecological pattern this study revealed that BS of species of HNO-clade

397   were correlated with increase water river volume and velocity, however were not correlated

398   with altitude were species lives. The studies of body fish sizes and the river size, as well as

399   with water temperature are really scarce in Neotropical fishes (Rypel 2014). However,

400   apparently it is logical to think that species of larger sizes lives in large rivers as we can

401   observe in species of HNO-clade.

402

403   *Macroevolution perspective*

404   In macroevolutionary perspective BS diversity is strong related with phylogeny

405   (Ramirez et al. 2008). For that we used the method of measuring rates of evolution in

406   *darwins* (Haldane 1949; Albert and Johnson 2011) of continuous traits in an explicitly

407   phylogenetic context. Therefore, our results suggested that the distribution of size-changes

408   *(d)* is slightly right-skewed considering the HNO-Clade (Fig. 6a; skewness = 0.40, P <

409   0.01, Kuiper test; *d* ranging -0.14 – 0.20) predicting a rate of evolution toward a large size

410   consistent with Cope`s rule. However, considering each subfamily clade separated the

411   *darwins (d)* distribution is slight left-skewed within Hypoptopomatinae reverse of Cope`s

412   rule (Fig. 6b; skewness = -0.03, suggesting a rate of evolution near zero, but slight tending

413   toward a small size); right-skewed within Neoplecostominae consistent with Cope`s rule

414   (Fig. 6c; skewness = 0.01, rate of evolution near zero slighting tending toward a large size).

415   However excluding the three lower *(d)* values (-0.14, -0.13 and -0.12) the distribution stays

416   quite right-skewed (skewness = 1.34, P < 0.01, Kuiper test); and quite left-skewed within

417   Otothyrinae reverse of Cope`s rule (Fig. 6d; skewness = -1.22, rate of evolution tending

418   toward a small size).

419   Albert and Johnson (2011) predicted that species richness and average BS do not

420   predict skewness values in extant fish, and most species-rich families have skewness values

421   near zero. However, right-skewed size frequency distributions have been interpreted as

422   evidence for the selective advantage of small size (Damuth 1993; Blanckenhorn 2000;

423   Maurer et al. 2004). Additionally, this distribution pattern has been interpreted as increase

424   rate of diversification, more speciation and less extinction, at small size (Jablonski 1997;

425   Maurer 1998; Gardezi and da Silva 1999; Knouft and Page 2003), or severe existential risks

426   that threat large species sizes (Maurer et al. 1992; Purvis et al. 2003; Clauset and Erwin

427   2008; Clauset et al. 2009). Our results of LTT (Fig. 9) suggested a curve following the

428   straight line at rate of constant evolution in Hypoptopomatinae, indicating a constant rate of

429   diversification in evolution of this group. However, within Otothyrinae the LTT plot curve

430   is above the straight line (Fig. 9), strongly implying a rate of diversification increasing

431   through time (more speciation and less extinction) may suggesting a selective pressure to

432   small size with in this group.

433   Morse et al. (1985) extend the idea of Hutchinson and MacArthur's (1959) that the

434   world is vaster for small animal. The consequence of this idea is that because there is more

435   usable space for small animals, small-bodied species should be over represented in nature.

436   However, according to Kozlowski and Gawelezyk (2002) this hypothesis alone cannot

437   explain the frequency size distributions among different animal groups.

438

439   *Evolutionary Toward Small and Large Sizes*

440   Our results within Hypoptopomatinae predicted a quite right-skewed size

441   distribution of extant species (0.55, Fig. 5), exhibit a large number of small BS, however

442   value near zero for *darwins (d)* distribution (-0.03, Fig. 6b) suggests a symmetric rate of

443   evolution within this lineage or balanced evolution among Hypoptopomatinae lineages. In

444   Figure 1 we can observe that size evolution vary among Hypoptopomatinae genera. Species

445   of *Hypoptopoma* are known to be the large species since species of *Otocinclus* the smallest,

446   resulting in a symmetric skewness values for Hypoptopomatinae clade as a whole. For both

447   genera the evolution to extant species occurred from Miocene and Pliocene for today from

448   an intermediate size. However, the right-skewed distribution of extant species can be a

449   result of more number of small species within Hypoptopomatinae.

450   Within Otothyrinae the distribution of size of extant species is left skewed (-0.34).

451   This pattern can be interpreted as evolution to a large size in this group (Fig. 5). However,

452   this pattern is not consistent with size distribution in fishes as a whole (Albert and Johnson

453   2011), or with mammals (Maurer et al. 1992; Gardezi and da Silva 1999), birds (Gaston

454   and Blackburn 1995) and also with most terrestrial species (Hutchinson and MacArthur

455   1959; Bonner 1988; May 1988). Additionally, our results of *darwins (d)* analysis suggested

456   a quite left skewed distribution of *darwins* (*d*, -1.22) with most frequency negative values,

457   strongly suggesting a great rate of evolution toward a small sizes within lineages of

458   Otothyrinae (Fig. 6d). We hypothesized that the discontinuity among extant species size

459   distribution and evolution in *darwins (d)* occurred because two factors: first because the

460   study of size distribution should be interpreted in a phylogenetic context (*darwins, d*);

461   second the boundary values of the distribution of size can great influence in the calculation

462   of the skewness and low values in left boundaries of Otothyrinae.

463   Evolutionary change is often thought of as gradual, the asymmetric distribution of

464   size among internal phylogenetic lineages may result from a variety of real biological

465   processes, including punctuated evolution (Pagel et al. 2006; Monroe and Bokma 2009) or

466   extinction of taxa with intermediate trait values (Butler and Losos 1997). Olden et al.

467   (2007) predicted that large BS is associated with elevated extinction risk in many living fish

468   taxa. Additionally, demographic factors, as small effective population sizes and long

469   generation times, can predict elevated extinction risk and consequently resulting in

470   evolution to large size in different lineages (Knouft 2003; Knouft and Page 2003; Hardman

471   and Hardman 2008). Although the relative roles of speciation, extinction and adaptive

472   evolution have been parsed in an explicitly phylogenetic context in some fish taxa (Knouft

473   2003; Near et al. 2005; Hardman and Hardman 2008), the generality of these processes

474   among fishes as a whole remain poorly understood (Smith 1981; Smith et al. 2010).

475   The LTT analysis of diversification within Neoplecostominae (Fig. 9), suggested a

476   curve following the straight line from 40 Ma to 10 Ma and slight above the straight line

477   from the last 10 Ma, indicating an increase rate of diversification near the present.

478   Therefore, increase difference of speciation and extinction rate (possible a low rate of

479   extinction) could result in an evolution to a large size within Neoplecostominae lineages in

480   middle Miocene to the present, since most size change occurs in speciation events (Stanley

481 1973; McKinney 1990). Additionally, a higher rate of extinction within Neoplecostominae

482 from 40 Ma to 10 Ma, as we can observe a low rate of diversification in this period of time

483 associated with a friendly habitat of associated with rocks of bottom of streams, could

484 result in evolution to large size species.

485   Several hypotheses were proposed to explain the asymmetric pattern leading to

486 large sizes among animal lineages as: (1) biased rates of anagenesis to larger sizes (Cope's

487 rule), (2) higher extinction risk at larger sizes, and (3) taxon-specific ecophysiological

488 limits at lower sizes (Stanley 1973, 1998; Schmidt-Nielsen 1984; McShea 1994; Cope

489 1877; Newell 1949; Damuth 1993; Knouft 2003; Knouft and Page 2003; Hardman and

490 Hardman 2008). However, studies analyzing the evolution toward small or large BS in

491 specific groups remain scant (Blanckenhorn 2000; Albert and Johnson 2011). Additionally,

492 Stanley (1973) related that an evolutionary expansion into an ecologically or

493 physiologically limited size space from a small ancestral size could lead towards larger

494 average size within lineages (McShea 1994).

495   The changes in size in Hypoptopomatinae, Neoplecostominae and Otothyrinae

496 clades occurred in the Pliocene and Pleistocene a period know to have great earth climate

497 oscillation (Suguio et al. 1985; Santos et al. 2008; Zachos et al. 2008; Albert and Reis

498 2011). Change in climate is known to be linked with lineages diversification (Kohn and

499 Fremd 2008; Finarelli and Badgley 2010; Renema et al. 2008) and consequently more

500 speciation and changes in size.

501

502 *Sexual-dimorphism size hypothesis*

503   Loricariidae species are known to have sexual dimorphisms and these

504 characteristics may vary greatly among lineages. The selective processes producing sexual

505 dimorphism result in dimorphism for overall BS (sexual size dimorphism). Across species

506 within a lineage, size dimorphism will increase with increasing BS when the male is the

507 larger sex, and decrease with increasing average BS when the female is the larger sex. This

508 process is known as Rensch's rule (Rensch 1959; Fairbairn 1997).

509   Generally all males of the family Loricariidae bear a papilla in the urogenital

510 opening. However, different Loricariidae groups have may have different sexual

511   dimorphism, and sometimes these differences can be variation in size among males and

512   females. Species of *Neoplecostomus* is an example of males normally reaching a large size

513   than females (Langeani 1990; Zawadzki et al. 2008; Roxo et al. 2012). However, within

514   species of Otothyrinae we can observe the opposite with males normally reaching a small

515   size than females. Martins and Langeani (2011) suggested that the small number of teeth in

516   males of *Microlepidogaster dimorpha* could be associated with the fact that males of this

517   species reach a small size than females. According, to Blanckenhorn (2000) the fecundity

518   selection tends to select for increased BS in females, and sexual selection for increased BS

519   in male and these two selective pressures plus the viability selection are responsible to

520   particular species sex dimorphisms. Additionally, in Cope's rule (Bonner 1988; McLain

521   1993; Jablonski 1997) taxa are believed to evolve to larger BSs over evolutionary time, and

522   sexual size dimorphism tend to increases when males are the larger sex. However, in

523   Rensch's rule (Rensch 1959; Fairbairn 1997) sexual size dimorphism tends to decrease

524   when females are the larger sex. Considering that both rules should be influenced the

525   evolution of members of subfamilies Hypoptopomatinae, Neoplecostominae and

526   Otothyrinae.

527

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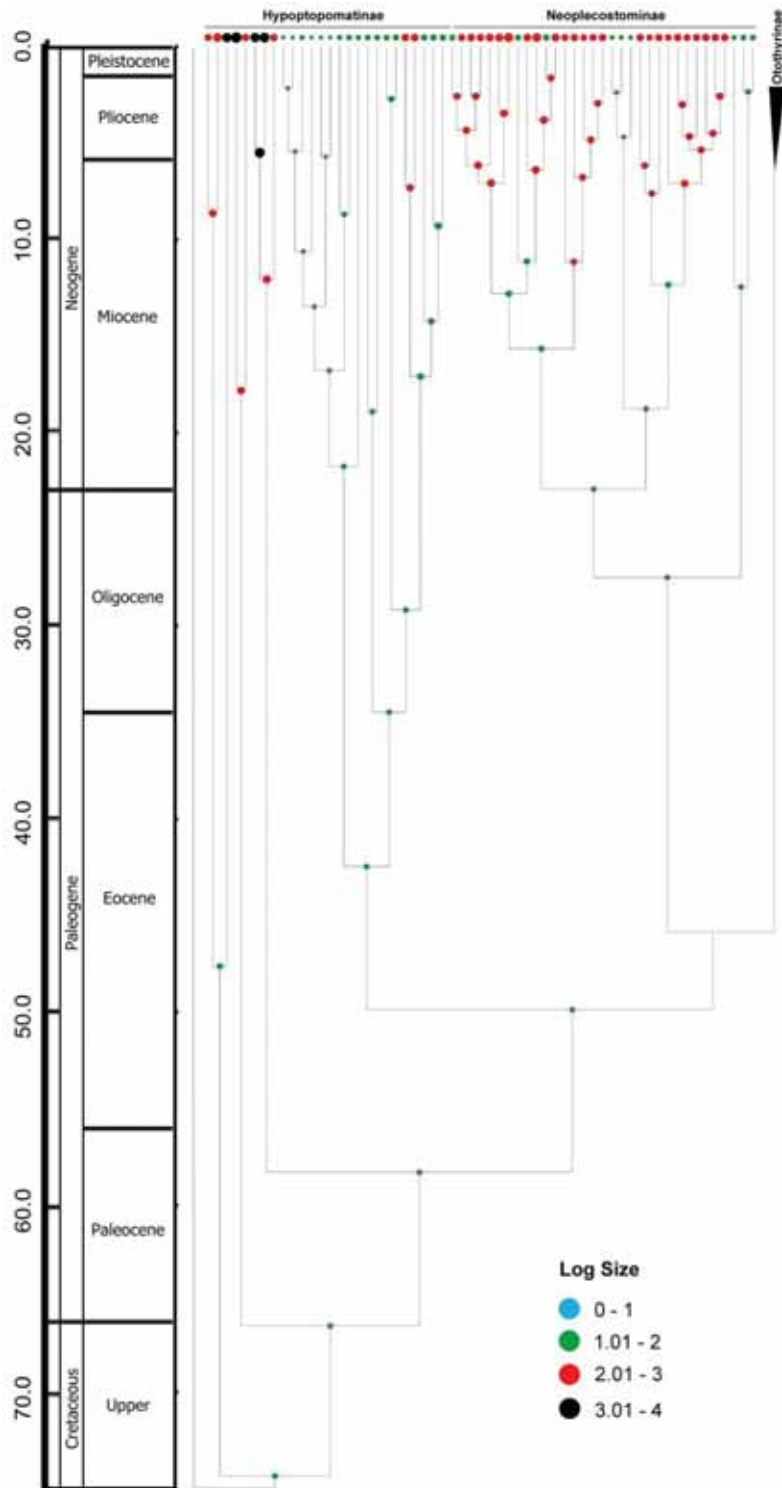
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Figure 1. Phylogeny of Hypoptopomatinae and Neoplecostominae species with ancestral
character reconstruction of size. Calibrated tree topology of unpublished phylogenetic
paper. Circle size and colors in phylogeny branches and tips are proportional to ln(cm) BS.
Branch lengths in Ma.
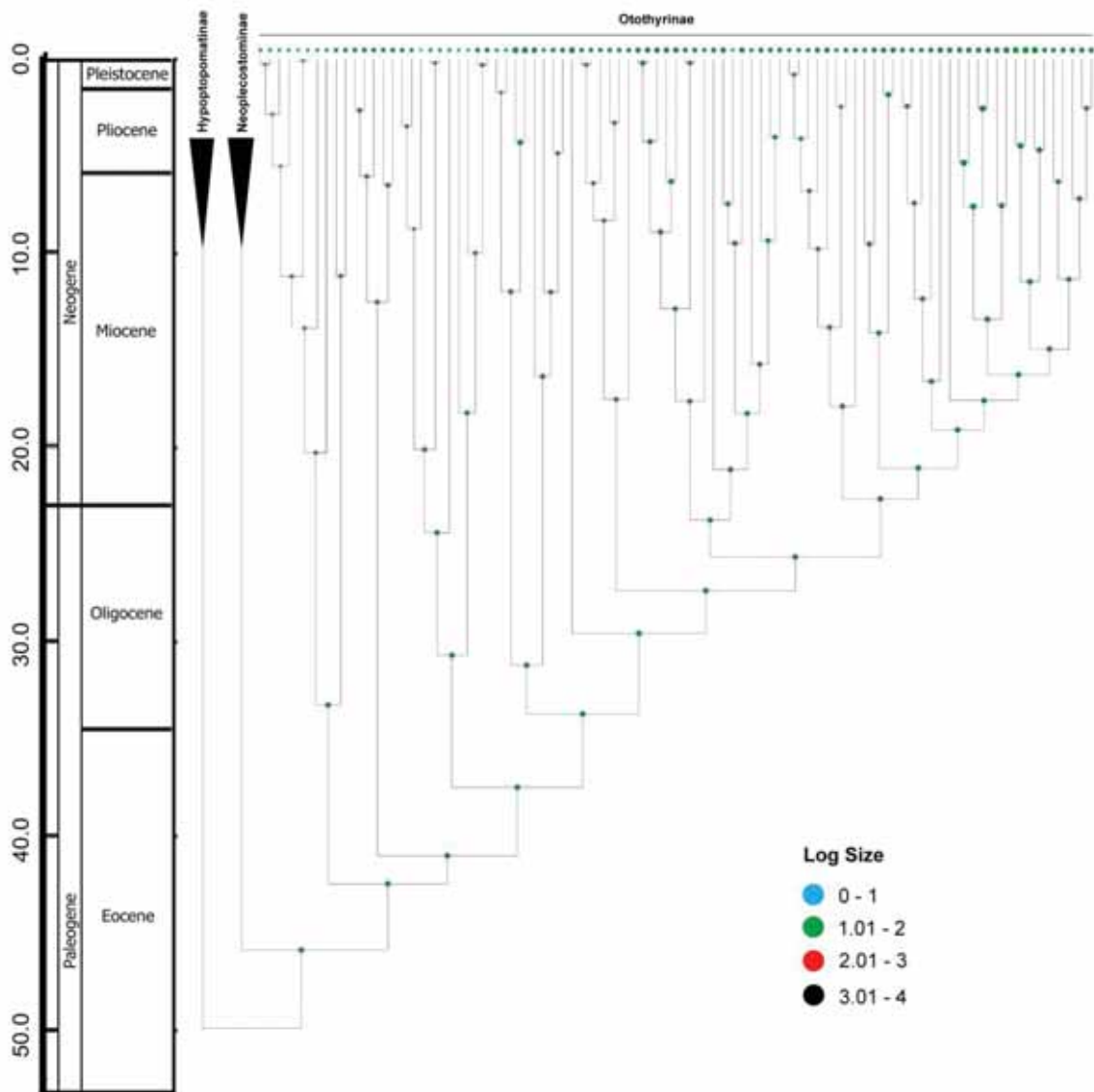
846
847 **Figure 2.** Phylogeny of Otothyrinae species with ancestral character reconstruction of size.
848 Calibrated tree topology of unpublished phylogenetic paper. Circle size and colors in
849 phylogeny branches and tips are proportional to ln(cm) BS. Branch lengths in Ma
850 estimated. See taxa names in Supplementary Table 1.

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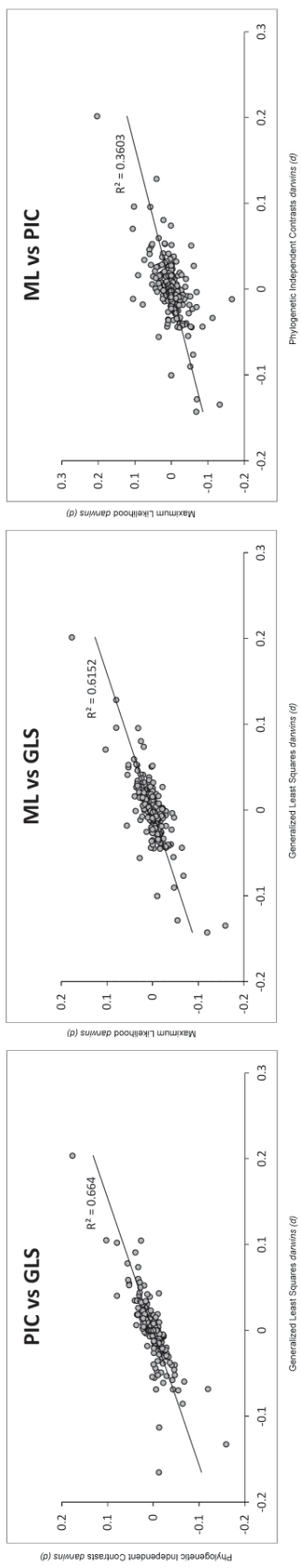
Figure 3. Comparison among *darwins* (*d*) obtained from different methods of ancestral characters reconstructions. We can observe that the most similar models in the distribution of *darwins* (*d*) are PIC vs. GLS ($R^2 = 0.664$), and the models with more differences are ML vs. PIC ($R^2 = 0.3603$).
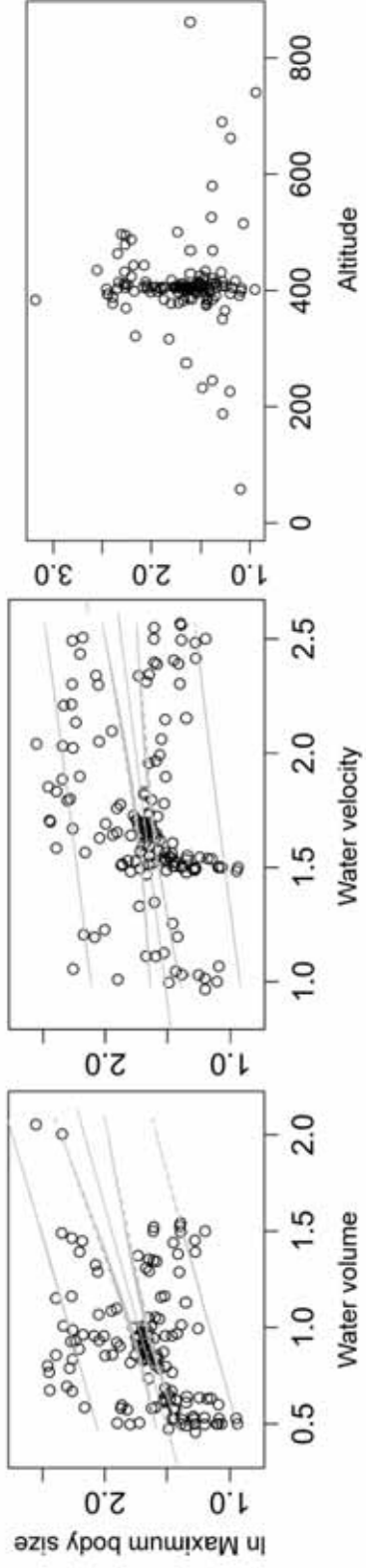
Figure 4. Correlations for log BS with water volume (P < 0.001), water velocity (P < 0.001) and altitude (P = 0.75). The bands are median line, prediction and confidence bands.

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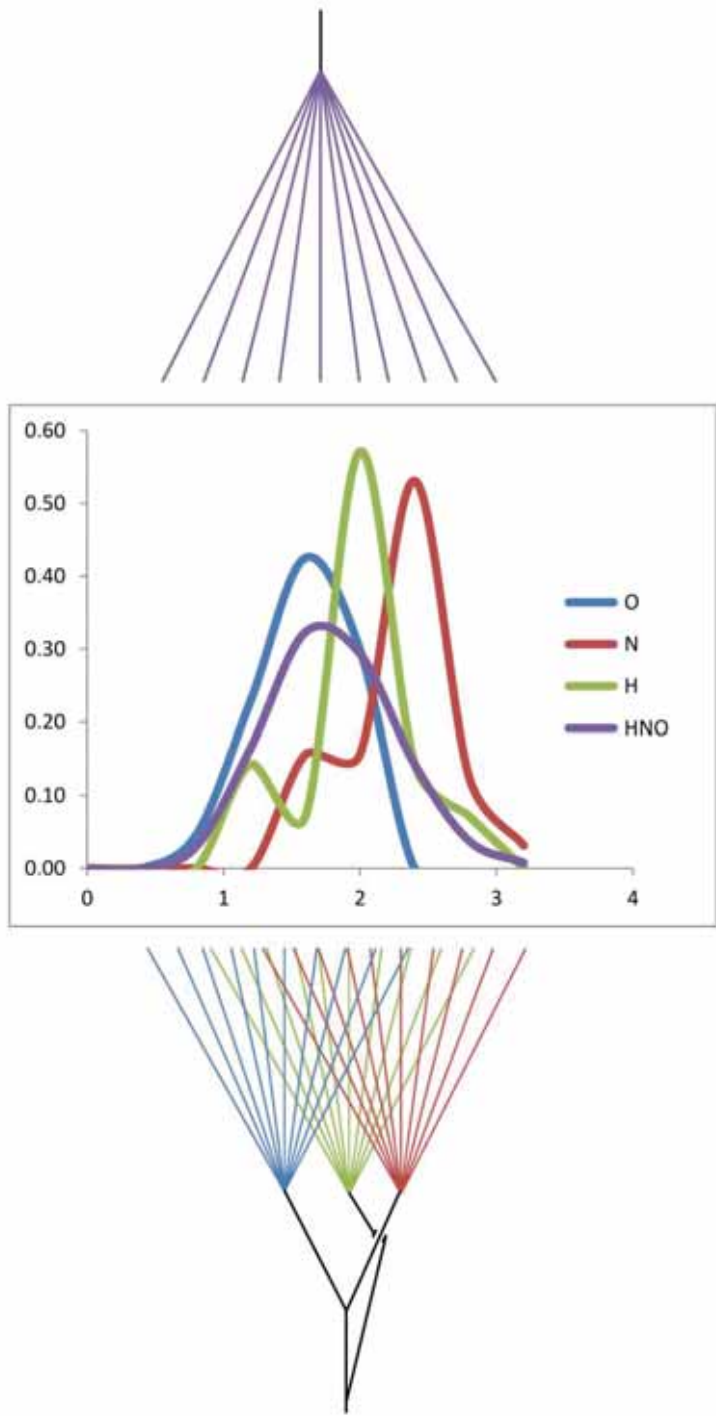
Figure 5. Size-frequency distributions of Hypoptopomatinae (H; n = 18; skewness = 0.55; P< 0.01, Kuiper test), Neoplecostominae (N; n = 33; skewness = -0.71; P< 0.01, Kuiper test), Otothyrinae (O; n = 89; skewness = -0.34; P< 0.01, Kuiper test) and HNO-Clade (HNO; n = 140; skewness = 0.80; P< 0.01, Kuiper test). Size Ranging from 2.1 cm to 17 cm.
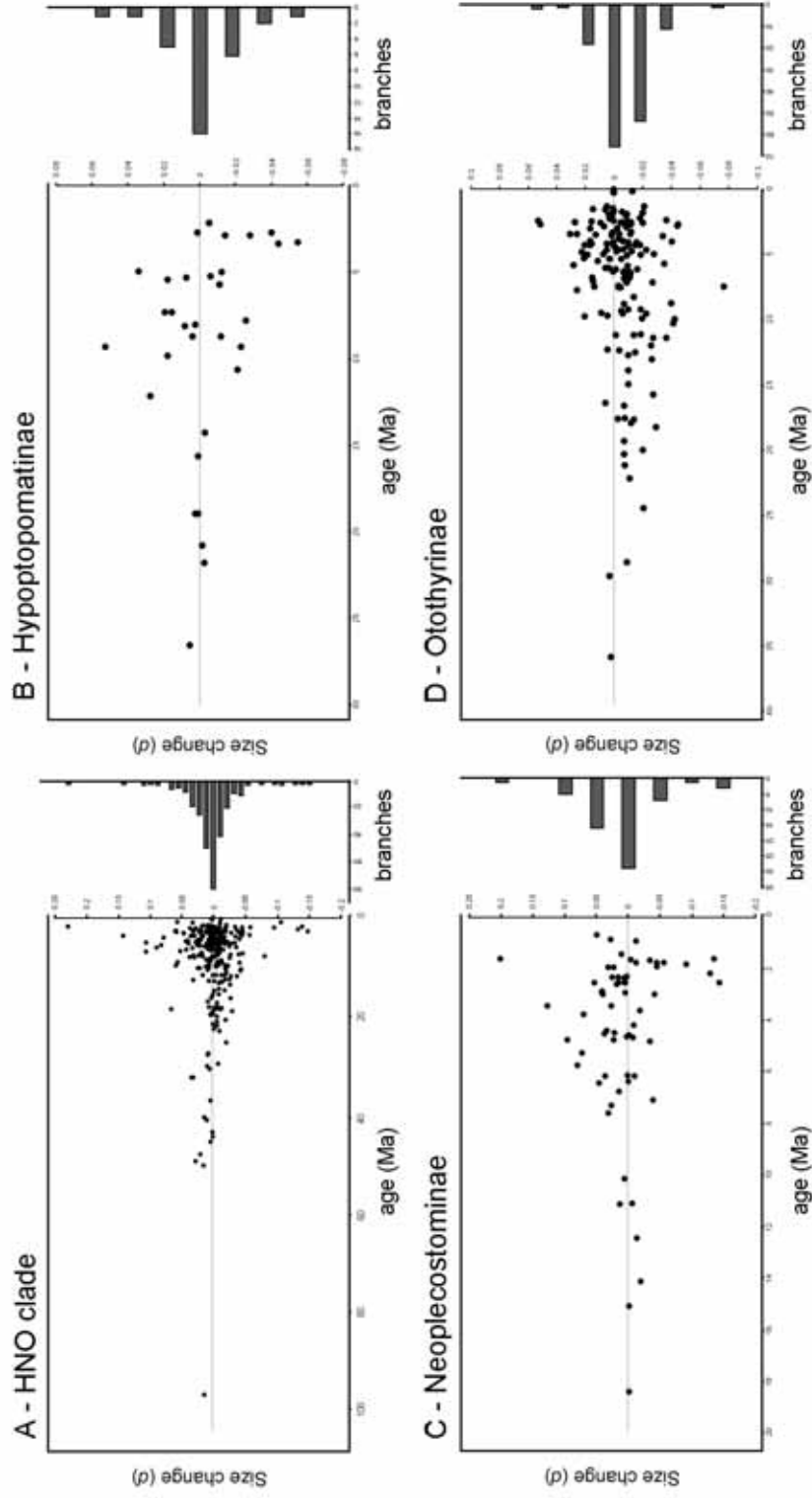
Figure 6. Rates of size-change in *darwins* (*d*) through time estimated along branches with frequency distributions. (a) Values estimated for the three subfamilies together (HNO-clade branches on the phylogeny of Figs. 1 and 2) and frequency distributions of size-change events (60 – 0 Ma; n = 276; skewness = 0.42; P < 0.01, Kuiper test). We can observe that most size diversification occurred in the last 10 Ma; (b) Hypoptopomatinae (30 – 0 Ma; n = 34; skewness = -0.03; P < 0.01, Kuiper test); (c) Neoplecostominae (20 – 0 Ma; n = 65; skewness = 0.01; P < 0.01, Kuiper test); (d) Otothyrinae (40 – 0 Ma; n = 177; skewness = -1.22; P < 0.01, Kuiper test).

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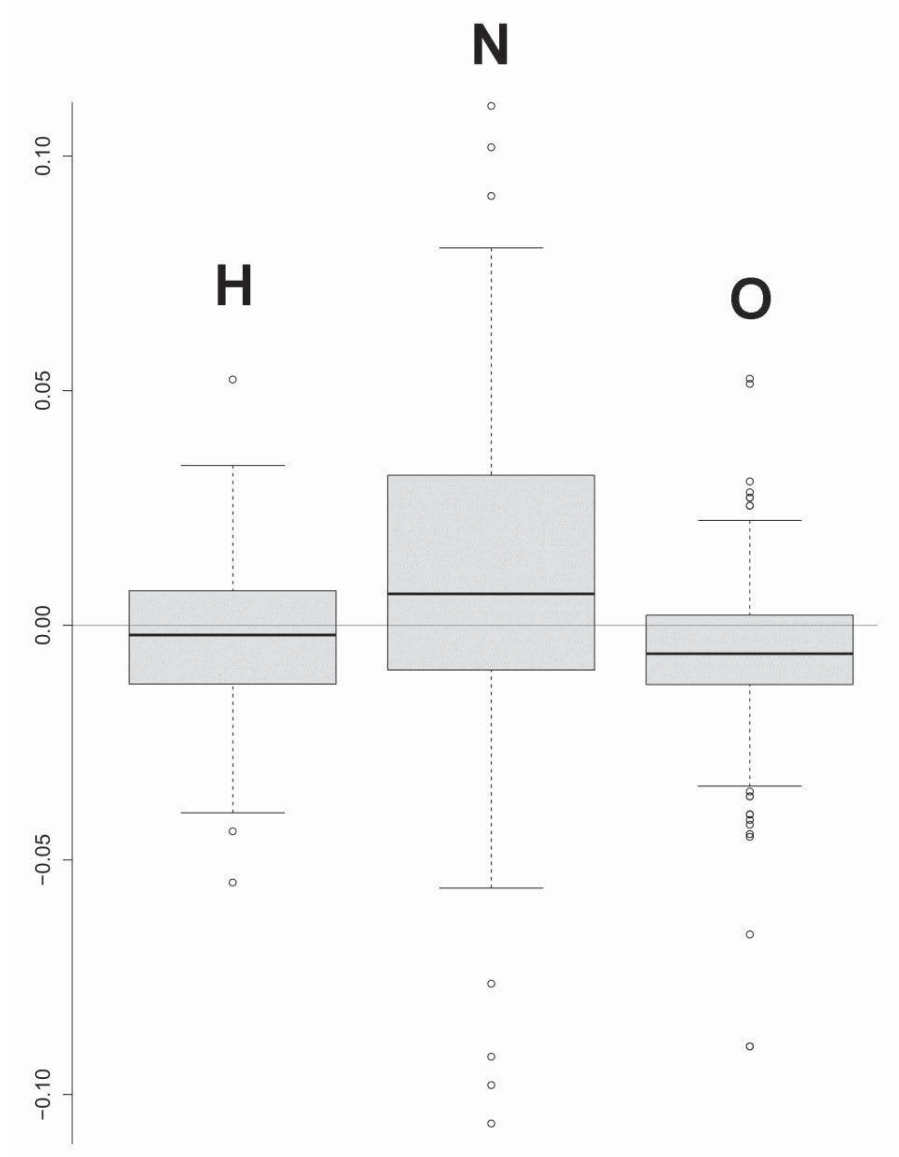
Figure 7. Box Plot comparing the dispersal of *darwins (d)* for ancestors of (H) Hypoptopomatinae (mean = -0.003), (N) Neoplecostominae (mean = 0.008), and (O) Otothyrinae (mean = -0.006).

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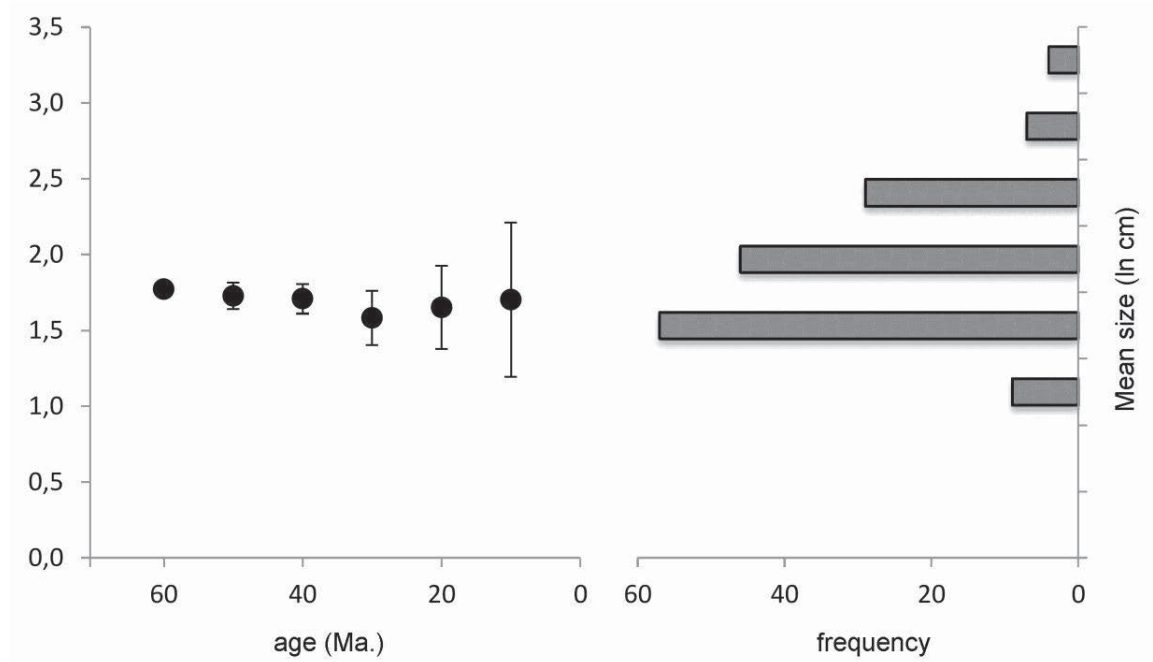
Figure 8. Average BS of all subfamilies together (HNO-Clade) through Paleogene to the
present. Maximum Likelihood ancestral estimation for all branches on the phylogeny of
Figs. 1 and 2. Average size of fossils for each stratigraphic interval (epoch). Error bars ±
one standard deviation of the average of branches or fossils per epoch.
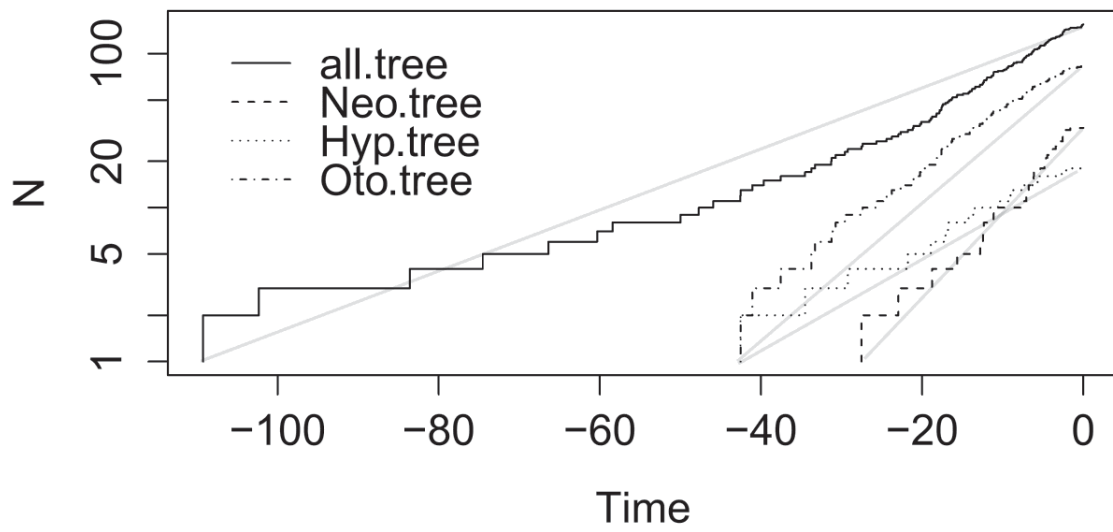
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898 **Figure 9.** Lineage through time plot comparing the diversification pattern among lineages
899 of subfamilies Hypoptopomatinae, Neoplecostominae, Otothyrinae and HNO-clade. The
900 "y" axis is log transformed.

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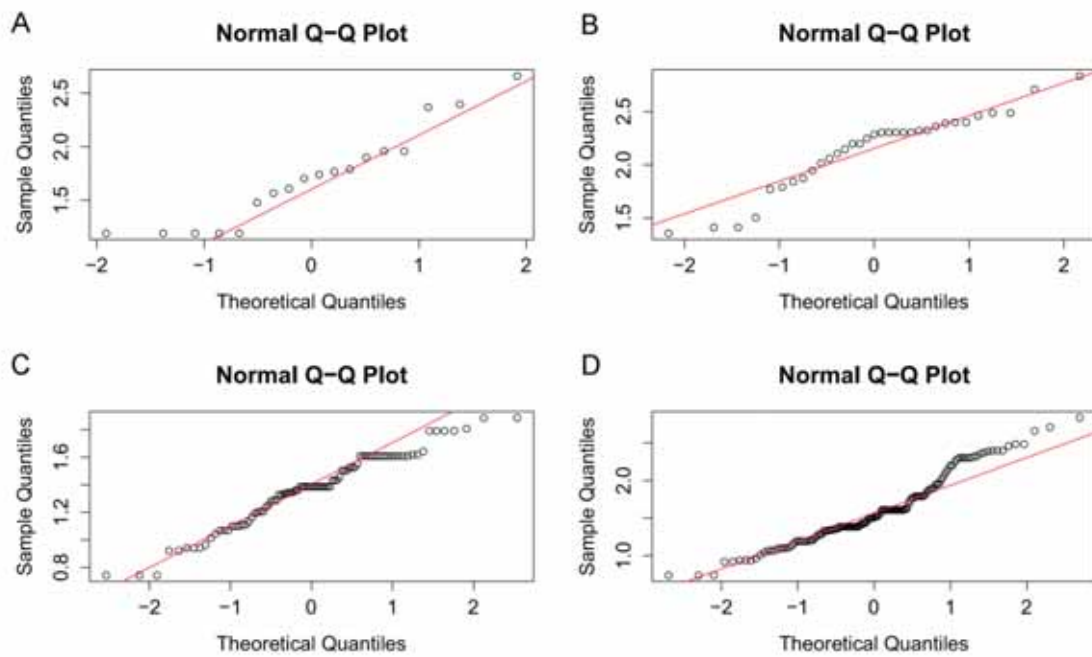
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899 **Figure S1.** Q-Q plot comparing the log size distribution values with a theoretical normal
900 distribution. (a) Hypoptopomatinae; (b) Neoplecostominae; (c) Otothyrinae; (d) HNO-
901 clade.

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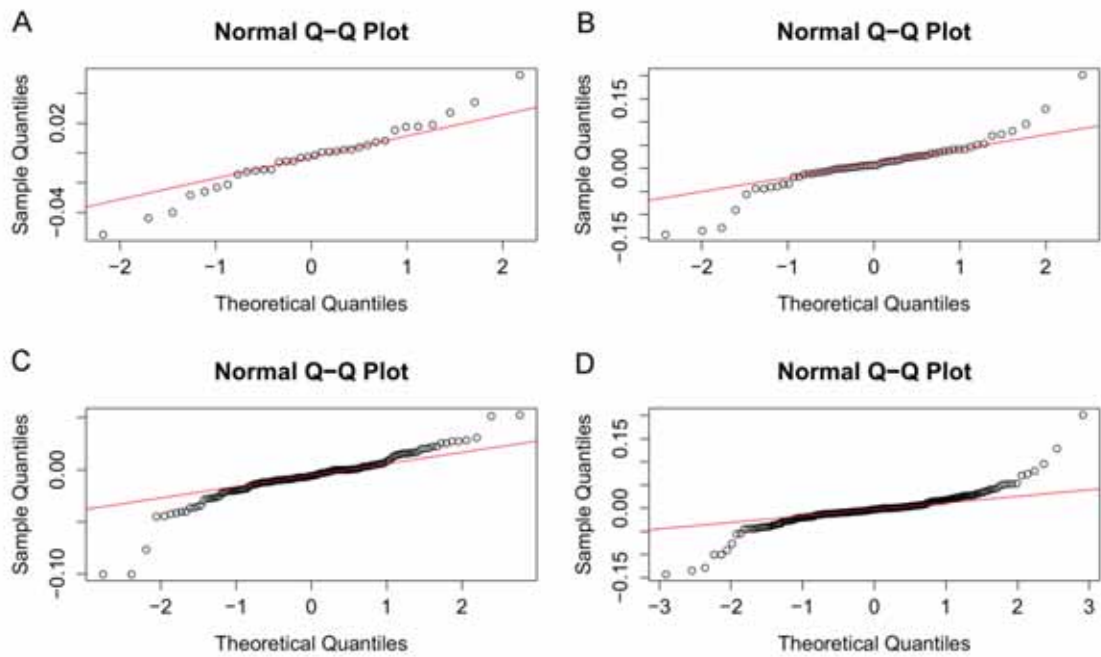
Figure S2. Q-Q plot comparing the *darwins (d)* distribution values with a theoretical
normal distribution. (a) Hypoptopomatinae; (b) Neoplecostominae; (c) Otothyrinae; (d)
HNO-clade.

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917 **Table 1.** Results of model fitting tests combining likelihoods across all clades. Lower AIC values indicate better model support; the best-supported model is in bold.

Clades	EB			OU			BM		
	lnL	AICc	ΔAICc	lnL	AICc	ΔAICc	lnL	AICc	ΔAICc
HNO-Clade	-17.85	**-29.52**	**0.00**	-17.85	**-29.52**	**0.00**	-17.15	-30.22	0.70
Hypoptopomatinae	-1.41	-10.67	2.91	-1.41	-10.67	2.91	-1.45	**-7.76**	**0.00**
Neoplecostominae	-5.58	-18.03	0.49	-5.58	-18.03	0.49	-6.56	**-17.54**	**0.00**
Otothyrinae	-47.06	**-87.85**	**0.00**	-47.06	**-87.85**	**0.00**	-47.06	-89.99	2.94

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928 **Supplementary Table 1.** Species included in the present study. ANSP = Academy of Natural Sciences of Drexel University, Philadelphia; AUM =

929 Auburn University Natural History Museum; LBP = Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista; MCP = Museu de

930 Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul; MNRJ = Museu Nacional da Universidade Federal do Rio de Janeiro;

931 NUP = Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá; MHNG = Museum of Natural History of the

932 City of Geneva.

Number	Collection No	Fish No	GenBank (Reticulon/16S/COI/CytB)	Species	Maximum BS	ln BS
1	LBP 6037	29054	Not submitted	*Hisonotus arnatus*	4.60	1.53
2	MCP 21375		-/-/EU371011/-	*Hisonotus arnatus*	4.60	1.53
3	MCP 37682		Not submitted	*Hisonotus arnatus*	4.60	1.53
4	LBP 3472	20258	Not submitted	*Hisonotus notatus*	4.00	1.39
5	MNRJ 37474		Not submitted	*Hisonotus notatus*	4.00	1.39
6	LBP 4765	25554	Not submitted	*Hisonotus taimensis*	6.60	1.89
7	LBP 13147	51066	Not submitted	*Hisonotus taimensis*	6.60	1.89
8	LBP 7407	35655	Not submitted	*Hisonotus leucofrenatus*	6.00	1.79
9	MCP 31819		Not submitted	*Hisonotus leucofrenatus*	6.00	1.79
10	MCP 41351		Not submitted	*Hisonotus leucophrys*	5.00	1.61
11	MCP 21644		Not submitted	*Hisonotus charrua*	5.05	1.62
12	MCP 37684		Not submitted	*Hisonotus laevior*	5.00	1.61
13	LBP 13187	51070	Not submitted	*Hisonotus laevior*	5.00	1.61
14	LBP 3376	21246	Not submitted	*Hisonotus laevior*	5.00	1.61
15	LBP 3376	21248	Not submitted	*Hisonotus laevior*	5.00	1.61
16	LBP 4720	24941	Not submitted	*Hisonotus cf. charrua*	5.05	1.62
17	MCP 40762		Not submitted	*Hisonotus notopagos*	5.17	1.64
18	LBP 4723	24951	Not submitted	*Eurycheilichthys* sp. 1	4.50	1.50
19	LBP 4871	24919	Not submitted	*Epactionotus bilineatus*	4.00	1.39
20	MCP 23683		-/-/EU371004/-	*Epactionotus itaimbezinho*	3.80	1.34

#	Voucher	Tissue	GenBank	Species		
21	MCP 23606		-/-/EU371005/-	*Epactionotus gracilis*	3.90	1.36
22	LBP 3335	20465	Not submitted	*Hisonotus heterogaster*	5.00	1.61
23	LBP 579	8565	Not submitted	*Hisonotus heterogaster*	5.00	1.61
24	LBP 4719	25636	Not submitted	*Hisonotus nigricauda*	5.00	1.61
25	LBP 5528	25343	Not submitted	*Otothyropsis marapoama*	3.85	1.35
26	LBP 4863	24930	Not submitted	*Hisonotus ringueleti*	3.90	1.36
27	MCP 40943		Not submitted	*Hisonotus carreiro*	3.77	1.33
28	MCP 40492		Not submitted	*Hisonotus prata*	3.32	1.20
29	MCP 41352		Not submitted	*Hisonotus megaloplax*	4.71	1.55
30	LBP 13055	51035	Not submitted	*Hisonotus montanus*	4.50	1.50
31	MCP 41474		Not submitted	*Hisonotus aky*	3.45	1.24
32	LBP 13072	51046	Not submitted	*Hisonotus iota*	3.34	1.21
33	MCP 40029		Not submitted	*Hisonotus iota*	3.34	1.21
34	LBP 8249	38464	Not submitted	*Parotocinclus prata*	4.19	1.43
35	LBP 7472	32387	Not submitted	*Parotocinclus prata*	4.19	1.43
36	LBP 8258	38298	Not submitted	*Parotocinclus robustus*	4.20	1.44
37	LBP 7182	34694	Not submitted	*Parotocinclus cf. bahiensis*	3.10	1.13
38	LBP 5867	28345	Not submitted	*New taxon 1*	5.00	1.61
39	LBP 17402	67143	Not submitted	*Hisonotus bocaiuva*	2.56	0.94
40	LBP 7244	33302	Not submitted	*New taxon 2*	5.00	1.61
41	LBP 3457	20293	Not submitted	*Parotocinclus sp. 1*	4.30	1.46
42	LBP 13923	58218	Not submitted	*Hisonotus francirochai*	3.60	1.28
43	LBP 871	8801	Not submitted	*Hisonotus paulinus*	4.00	1.39
44	LBP 3932	22661	Not submitted	*Hisonotus paulinus*	4.00	1.39
45	LBP 17583	69477	Not submitted	*Hisonotus depressicauda*	5.00	1.61
46	LBP 17583	69479	Not submitted	*Hisonotus depressicauda*	5.00	1.61
47	LBP 8051	37802	Not submitted	*Hisonotus depressicauda*	5.00	1.61
48	MCP 22602		Not submitted	*Hisonotus depressicauda*	5.00	1.61

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49	LBP 2910	18756	Not submitted	*Hisonotus depressicauda*	5.00	1.61
50	LBP 4854	25626	Not submitted	*Microlepidogaster dimorpha*	3.77	1.33
51	LBP 7253	33309	Not submitted	*Rhinolekos britskii*	3.83	1.34
52	LBP 7245	34405	Not submitted	*Rhinolekos britskii*	3.83	1.34
53	LBP 5848	28365	Not submitted	*Rhinolekos* sp. 1	3.50	1.25
54	LBP 7246	34410	Not submitted	*Rhinolekos garavelloi*	3.62	1.29
55	LBP 7246	33305	Not submitted	*Rhinolekos garavelloi*	3.62	1.29
56	LBP 2869	18571	EU817527/-/-/-	*Parotocinclus maculicauda*	6.00	1.79
57	LBP 4722	24946	EU817525/-/-/-	*Pseudotothyris* sp. 1	4.00	1.39
58	LBP 2094	13851	Not submitted	*Pseudotothyris obtusa*	4.00	1.39
59	LBP 1971	13685	EU817526/-/-/-	*Otothyris travassosi*	3.20	1.16
60	MHNG 2586.95	BR1200	EU817552/-/-/-	*Pseudotothyris janeirensis*	5.00	1.61
61	LBP 6950	35328	Not submitted	*Parotocinclus* sp. 2	6.10	1.81
62	ANSP 179131		-/-/GQ225426/-	*Parotocinclus britskii*	6.00	1.79
63	LBP 4787	25579	Not submitted	*Parotocinclus eppleyi*	3.00	1.10
64	AUM 43947		-/-/EU359455/-	*Parotocinclus eppleyi*	3.00	1.10
65	LBP 5624	35327	Not submitted	*Parotocinclus* aff. *spilurus*	4.00	1.39
66	LBP 5624	35326	Not submitted	*Parotocinclus* aff. *spilurus*	4.00	1.39
67	LBP 334	4276	Not submitted	*Hisonotus* sp. 1	2.51	0.92
68	LBP 8276	38487	Not submitted	*Hisonotus* sp. 2	2.54	0.93
69	LBP 16277	61771	Not submitted	*Hisonotus* sp. 3	2.91	1.07
70	LBP 7948	35750	Not submitted	*Hisonotus chromodontus*	3.03	1.11
71	LBP 7948	35749	Not submitted	*Hisonotus chromodontus*	3.03	1.11
72	LBP 10981	50459	Not submitted	*Parotocinclus aripuanensis*	2.10	0.74
73	LBP 1572	11537	Not submitted	*Parotocinclus* sp. 3	2.15	0.75
74	LBP 2414	16276	Not submitted	*Parotocinclus* sp. 3	2.15	0.75
75	LBP 2514	13846	EU817539/-/-/-	*Schizolecis guntheri*	4.00	1.39
76	LBP 3238	19471	EU817536/-/-/-	*Schizolecis guntheri*	4.00	1.39

77	LBP 2513	15240	EU817535/-/-/-	*Schizolecis guntheri*	4.00	1.39
78	LBP 2401	15272	EU817538/-/-/-	*Schizolecis guntheri*	4.00	1.39
79	LBP 2988	19646	EU817537/-/-/-	*Schizolecis guntheri*	4.00	1.39
80	LBP 1653	11477	EU817520/-/-/-	*Corumbataia tocantinensis*	3.80	1.34
81	LBP 2001	12191	EU817521/-/-/-	*Corumbataia cuestae*	3.30	1.19
82	LBP 5529	25381	Not submitted	*Hisonotus insperatus*	3.00	1.10
83	LBP 1325	11238	Not submitted	*Hisonotus* sp. 4	2.84	1.04
84	LBP 17256	66651	Not submitted	*Hisonotus piracanjuba*	2.56	0.94
85	LBP 17256	66655	Not submitted	*Hisonotus piracanjuba*	2.56	0.94
86	MNRJ 29502	490	Not submitted	*Hisonotus* sp. 5	2.75	1.01
87	LBP 17532	68706	Not submitted	*Hisonotus* sp. 6	2.62	0.96
88	LBP 5062	26057	Not submitted	*Hisonotus* sp. 7	2.91	1.07
89	LBP 5062	26056	Not submitted	*Hisonotus* sp. 7	2.91	1.07
90	LBP 2931	18994	JN689285/JN089823/JN089799/JN089774	*Pseudotocinclus tietensis*	6.00	1.79
91	LBP 616	7564	JN689286/FJ625810/FJ625819/FJ625511	*Pseudotocinclus juquiae*	5.87	1.77
92	LBP 4391	24189	JN689280/ FJ434517/FJ434540/FJ965510	*Pareiorhina rudolphi*	4.50	1.50
93	LBP 7383	34843	JN689310/ FJ965496/FJ965507/FJ965517	*Neoplecostomus selenae*	10.17	2.32
94	LBP 7525	34832	-/JN089843/JN089818/JN089794	*Neoplecostomus botucatu*	10.20	2.32
95	NUP 3560	9701	JN689293/ FJ434506/FJ434529/FJ965516	*Neoplecostomus yapo*	10.60	2.36
96	LBP 2732	17444	JN689303/FJ965495/FJ965505/FJ965515	*Neoplecostomus paranensis*	9.82	2.28
97	LBP 5901	27990	JN689304/JN089837/JN089812/JN089788	*Neoplecostomus langeanii*	8.55	2.15
98	LBP 2861	18616	JN689298/JN089833/JN089808/JN089784	*Neoplecostomus bandeirante*	10.90	2.39
99	NUP 2528	9423	JN689295/FJ434520/FJ434543/FJ965513	*Neoplecostomus corumba*	7.83	2.06
100	LBP 645	7593	JN689311/FJ434508/FJ434531/FJ965518	*Neoplecostomus microps*	10.00	2.30
101	LBP 6537	31681	JN689313/FJ965494/FJ965504/FJ965519	*Neoplecostomus franciscoensis*	7.50	2.01
102	LBP 2551	15243	JN689312/FJ434507/FJ434530/ FJ965512	*Neoplecostomus espiritosantensis*	10.00	2.30
103	LBP 8380	37559	JN689288/JN089826/JN689277/JN089777	*Pareiorhina hyptiorhachis*	3.88	1.36
104	LBP 1087	10256	JN689290/JN089828/JN089803/JN089779	*Pareiorhina carrancas*	4.10	1.41

105	LBP 8368	JN689291/JN089829/JN089804/JN089780	*Pareiorhina carrancas*	4.10	1.41
106	LBP7385	JN689324/FJ965491/FJ965502/FJ965526	*Isbrueckerichthys epakmos*	10.00	2.30
107	LBP 6389	JN689326/FJ965489/FJ965503/FJ965529	*Isbrueckerichthys cf. calvus*	9.00	2.20
108	LBP 7373	JN689297/FJ965490/FJ965506/FJ965525	*Isbrueckerichthys alipionis*	8.20	2.10
109	LBP 2650	JN689325/FJ625812/FJ625821/FJ965528	*Isbrueckerichthys duseni*	10.00	2.30
110	LBP 7384	JN689323/JN089845/JN089820/JN089796	*Neoplecostomus ribeirensis*	9.00	2.20
111	LBP 515	JN689316/FJ965492/FJ965500/FJ965523	*Kronichthys subteres*	12.00	2.48
112	LBP 795	JN689315/FJ434503/FJ434526/FJ965522	*Kronichthys lacerta*	7.00	1.95
113	LBP 2122	JN689314/FJ434502/FJ434525/FJ965520	*Kronichthys heylandi*	15.00	2.71
114	LBP 1766	JN689317/FJ965493/FJ965501/FJ965521	*Kronichthys sp. 1*	11.00	2.40
115	LBP 748	JN689318/FJ625811/FJ625820/FJ965524	*Pareiorhaphis splendens*	6.50	1.87
116	MCP 41275	-/-/EU359438/-	*Pareiorhaphis cameroni*	17.00	2.83
117	LBP 902	JN689319/FJ434514/FJ434537/FJ965532	*Pareiorhaphis steindachneri*	12.00	2.48
118	MCP 41909	-/-/EU359437/-	*Pareiorhaphis azygolechis*	11.70	2.46
119	LBP 1161	JN689320/FJ434512/FJ434535/FJ965530	*Pareiorhaphis vestigipinnis*	10.00	2.30
120	LBP 701	JN689321/FJ434513/FJ434536/FJ965531	*Pareiorhaphis hystrix*	11.00	2.40
121	MCP 41747	EU359443/-/-/-	*Pareiorhaphis parmula*	9.45	2.25
122	MCP 41458	EU359439/-/-/-	*Pareiorhaphis eurycephalus*	6.30	1.84
123	LBP 4042	Not submitted	*Hypoptopoma inexspectatum*	7.10	1.96
124	LBP 693	Not submitted	*Hypoptopoma inexspectatum*	7.10	1.96
125	MHNG 2678.015	JN689282/FJ965486/FJ965498/FJ965508	*Hypoptopoma inexspectatum*	7.10	1.96
126	LBP 3081	EU817541/-/-/-	*Hypoptopoma gulare*	11.00	2.40
127	MHNG 2709.024	EU817554/-/-/-	*Hypoptopoma sp. 1*	10.70	2.37
128	LBP 3165	EU817543/-/-/-	*Acestridium discus*	6.70	1.90
129	LBP 7204	Not submitted	*Acestridium sp. 1*	6.00	1.79
130	LBP 6973	Not submitted	*Oxyropsis sp. 1*	5.87	1.77
131	LBP 4300	Not submitted	*Oxyropsis acutirostra*	5.70	1.74
132	LBP 2652	Not submitted	*Lampiella gibbosa*	5.00	1.61

133	LBP 877	8564	EU817546/-/-	*Otocinclus flexilis*	5.50	1.70
134	MCP 25234		-/-/EU370983/-	*Otocinclus arnoldi*	4.80	1.57
135	LBP 5310	26831	Not submitted	*Otocinclus hoppei*	3.30	1.19
136	MHNG 2613.057	CA25	EU817556/-/-	*Otocinclus hoppei*	3.30	1.19
137	LBP 5132	26233	EU817544/-/-	*Otocinclus vittatus*	3.30	1.19
138	MCP 34842		-/-/EU359432/-	*Otocinclus cocama*	4.40	1.48
139	MHNG no number	SU07-350	EU817558/-/-	*Otocinclus mariae*	3.30	1.19
140	MHNG 2601.060	BR98-040	EU817557/-/-	*Otocinclus mariae*	3.30	1.19
141	LBP 3510	21309	JN689283/FJ625809/FJ625818/FJ965533	*Hypostomus nigromaculatus*	10.20	2.32
142	NUP 1725	16652	Not submitted	*Hypostomus microstomus*	24.00	3.18
143	LBP 2544	10887	Not submitted	*Hypostomus ancistroides*	21.00	3.04
144	LBP 1557	11505	Not submitted	*Rineloricaria lanceolata*	9.50	2.25
145	LBP 1557	11506	Not submitted	*Spatuloricaria* sp. 1	30.00	3.40
146	MCP 31467		-/AY307290/-/-	*Delturus parahybae*	21.00	3.04
147	LBP 2368	15363	JN689278/FJ434499/FJ434524/FJ965535	*Hemipsilichthys gobio*	16.00	2.77
148	LBP 4956	10241	JN689279/FJ625808/FJ625817/FJ965534	*Hemipsilichthys papillatus*	9.20	2.22
149	LBP 1352	11454	Not submitted	*Astroblepus* sp. 1	-	-
150	LBP 3284	20010	Not submitted	*Astroblepus* sp. 2	-	-
151	LBP 485	6040	Not submitted	*Callichthys callichthys*	-	-
152	LBP 210	4134	-/GU210868/-/-	*Hoplosternum littorale*	-	-
153	LBP 2809	32502	GU210997/-/-/-	*Corydoras oiapoquensis*	-	-
154	LBP 6862		-/GU210613/-/-	*Corydoras imitator*	-	-
155	LBP 449	5815	Not submitted	*Diplomystes mesembrinus*	-	-

Appendix 1

Article

urn:lsid:zoobank.org:pub:8E9334E7-8D73-4D4C-9FBF-EB8411525F3F

Description of a new species of *Pareiorhina* (Siluriformes: Neoplecostominae) from Rio São Francisco basin

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Abstract

A new species of *Pareiorhina* (Neoplecostominae) from the Rio São Francisco basin, Minas Gerais State, Brazil, is described. The new species is distinguished from its congeners (*Pareiorhina brachyrhyncha*, *P. carrancas,* and *P. rudolphi*) by traits related to small plates covered with odontodes randomly distributed on the abdomen, minute lateral cusps on the teeth, pointed odontodes covering pectoral, pelvic, and anal-fin first rays, caudal-fin being completely dark with one hyaline bar, absence of a ridge on the postdorsal surface of trunk, shape of premaxillae and dentaries, and by the absence of odontodes at the ventral tip of the snout.

Keywords: cascudinhos, catfish, freshwater, Loricariidae, Neotropical region, South America

Resumo

Uma nova espécie de *Pareiorhina* (Neoplecostominae) é descrita da bacia do Rio São Francisco, no Estado de Minas Gerais, Brasil. A nova espécie é distinguida dos seus congêneres (*Pareiorhina brachyrhyncha*, *P. carrancas* e *P. rudolphi*) por caracteres relacionados à presença de pequenas placas coberta por odontódeos distribuídas aleatoriamente pelo abdômen, pequena cúspide lateral nos dentes, odontódeos pontiagudos cobrindo os primeiros raios das nadadeiras peitorais, pélvicas e anal, nadadeira caudal completamente escura com uma listra hialina, ausência de uma quilha na superfície pósdorsal do tronco, formato das pré-maxilas e dentário e pela ausência de odontódeos na extremidade ventral do focinho.

Palavras-chave: cascudinhos, catfish, água doce, Loricariidae, Região Neotropical, América do Sul

Introduction

In a revision of the family Loricariidae, Gosline (1947) proposed the genus *Pareiorhina* to include *Rhinelepis rudolphi* Miranda-Ribeiro, 1911. Also, among the main changes proposed by Gosline was the recognition of Neoplecostominae as a larger group, including *Corymbophanes*, *Delturus*, *Hemipsilichthys*, *Kronichthys*, *Neoplecostomus*, *Pareiorhaphis*, *Pareiorhina*, *Pogonopoma*, *Pogonopomoides*, *Canthopomus* (=*Pseudorhinelepis)*, *Rhinelepis*, and *Upsilodus*. Armbruster (2004), in a phylogenetic analysis, supported the hypotheses that *Pareiorhina* is a Neoplecostominae and moved it from Hypostominae.

Recently, Chiachio *et al.* (2008) suggested that *Pareiorhina rudolphi* and *Pareiorhina* sp. formed a sister-group to the genus *Pseudotocinclus*. Cramer *et al.* (2011) also identified *Pareiorhina* sp. as a sister group to the genus *Pseudotocinclus*, but that *Pareiorhina brachyrhyncha* and *P. carrancas* formed a polytomy with some *Neoplecostomus* species. Gosline (1947), in the description of the genus *Pareiorhina,* did not propose any synapomorphies to define the group, but a combination of autopomorphic, plesiomorphic, and derived characters. Bockmann and Ribeiro (2003), in the description of *Pareiorhina carrancas,* proposed a combination of

synapomorphic characters to separate the genus from other Loricariidae genera: the lateral borders of the head without developed bristles; teeth simple; abdomen naked; dorsal plates meeting along the mid-dorsal line between the dorsal and caudal-fins; adipose fin absent; ventral plates covering mid-ventral line; and dorsal portion of body behind dorsal fin flattened. However, no exclusive diagnostic characters to define the genus have been proposed thus far.

Currently, *Pareiorhina* comprises three described species (*P. brachyrhyncha*, *P. carrancas* and *P. rudolphi*), but Garavello and Santana (1998) have reported the occurrence of possible new species in tributaries of the Rio Grande, Rio Tietê, Rio Paraíba do Sul, and Rio São Francisco basins. In the present paper, we present a formal description of the first species of *Pareiorhina* from the Rio São Francisco basin.

Material and methods

Measurements and counts were taken from left side. All measurements were taken from point to point to the nearest 0.1 mm with digital calipers. After collection the animals were anaesthetized using 1% Benzocaine in water and fixed in 10% formalin for at least two weeks, then transferred to 70% ethanol for permanent storage for morphological studies. Body plate nomenclature followed Schaefer (1997) and Bockmann and Ribeiro (2003). Osteological examination was made on cleared and double-stained specimens (c&s) according to the procedures of Taylor and Van Dyke (1985). Vertebrae counts included five from the Weberian Apparatus. All materials examined are deposited at following institutions: LBP (Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista Julio de Mesquita Filho, Botucatu, São Paulo); LIRP (Laboratório de Ictiologia de Ribeirão Preto, Ribeirão Preto, São Paulo); MCP (Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul); MZUSP (Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo); NUP (Coleção Ictiológica do Nupélia, Universidade Estadual de Maringá, Maringá, Paraná).

The bone plate abbreviations are parieto-supraoccipital (soc), compound pterotic (cpt), opercle (op), preopercle (pop), sphenotic (sp), infraorbitals (io1–io5), frontal (f), prefrontal (pf), nasal (na), prenasal (pn1–pn3); internasal plates (pni), rostral plates (r), postrostral plates (pr1–pr4), subocular cheek plates (cp1–cp2) and suprarostral plates (sprs).

Results

Pareiorhina cepta, new species
Fig. 1; Table 1

Holotype: MZUSP 111095, 41.5 mm SL, Brazil, Minas Gerais State, municipality of São Roque de Minas, district of São João Batista, Córrego do Lavapés, affluent Rio Santo Antônio, affluent Rio Samburá, Rio São Francisco basin, 20°08'36"S 46°38'21"W, 18 May 2011, Mehanna, M. and Senhorini, J.

Paratypes: All from Brazil, Minas Gerais State, Rio São Francisco basin. LBP 10261, 1, 30.2 mm SL, municipality of São Roque de Minas, Córrego Maria da Prata, 20°15'51"S 46°20'57"W, 9 Apr 2010, Mehanna, M. and Senhorini, J. LBP 10287, 13, 21.5–43.6 mm SL, municipality of São Roque de Minas, district of São João Batista, Córrego do Lavapés, affluent Rio Santo Antônio, affluent Rio Samburá, Rio São Francisco basin, 20°08'36"S, 46°38'21"W, 12 Abr 2010, Mehanna, M. and Senhorini, J. LBP 11835, 1 c&s, 33.4 mm SL, 19, 25.1–44.0 mm SL. Same data as the holotype. LIRP 8950, 2, 41.9–42.7 mm SL. MCP 46896, 2, 32.5–42.7 mm SL. NUP 13578, 2, 38.8–40.6 mm SL.

Diagnosis: The new species *Pareiorhina cepta* differs from all its congeners by the presence of small plates covered with odontodes randomly distributed on the abdomen, Fig. 2a (*vs.* abdomen completely naked) and by color pattern of caudal-fin completely dark with one hyaline bar (*vs.* variegated blotches corresponding to approximately three to four hyaline bars in *P. brachyrhyncha* and *P. rudolphi* and hyaline random spots in *P. carrancas,* Fig. 3). Moreover, the new species also differs from all congeners, except for *P. brachyrhyncha*, by presence of a minute lateral cusp in teeth (*vs.* unicuspid teeth in remaining species); differs from all congeners, except for *P. carrancas*, by having ventral surfaces of first pectoral, pelvic and anal-fin rays covered by pointed

odontodes (*vs.* conspicuously spatulate odontodes). Additionally, *P. cepta* differs from *P. carrancas* by lacking a ridge on the postdorsal surface of trunk (*vs.* postdorsal surface of trunk with a low, elongate ridge formed by 10–13 raised median unpaired plates), anterior ends of the premaxillae and dentaries gently sloped inwards, Bockmann and Ribeiro (2003) (*vs.* strongly sloped); from *P. brachyrhyncha* by lacking odontodes at the ventral tip of the snout (*vs.* tip of snout completely covered by odontodes).

FIGURE 1. *Pareiorhina cepta*, MZUSP 111095, 41.5 mm SL, holotype from Córrego do Lavapés, Rio São Francisco basin, municipality of São Roque de Minas, district of São João Batista, Minas Gerais State, Brazil.

TABLE 1. Morphometric data and counts of *Pareiorhina cepta*, from São Francisco basin. IO = interorbital, OD = orbital diameter, PDS = predorsal, CP = caudal peduncle.

	Pareiorhina cepta n=30			
	Holotype	Range	Mean/Mode	SD
Standard length (SL)	41.5	30.2–44.3	38.5	3.78
Percents of Standard length (SL)				
Predorsal length	43.4	42.1–44.8	43.5	0.74
Preanal length	61.6	57.4–62.0	59.6	1.37
Head length	31.7	31.1–33.7	32.1	0.61
Cleithral width	28.3	27.0–30.5	28.7	0.86
Dorsal-fin spine length	24.6	21.9–26.0	23.7	0.84
Base of dorsal-fin length	16.1	13.2–16.4	15.0	0.81
Thorax length	17.8	14.1–18.3	16.7	0.97
Pectoral-fin spine length	22.1	20.8–25.0	23.1	0.97
Abdomen length	24.8	22.3–26.9	24.7	0.89
Pelvic-fin spine length	20.4	19.3–23.4	21.3	1.01
Anal-fin spine length	17.1	16.2–18.3	17.2	0.58
Lower caudal spine	25.5	24.4–29.7	26.7	1.34
Caudal peduncle depth	8.1	7.7–8.8	8.1	0.23
Caudal peduncle length	32.4	32.2–36.5	34.7	1.20
Anal width	13.4	12.2–15.8	13.8	0.87
Snout-opercle length	24.1	18.4–25.4	23.9	1.20
Percents of Head Length				
Head width	86.4	83.4–90.5	86.7	2.11
Head depth	47.8	46.9–53.4	49.3	1.76
Snout length	56.0	53.3–58.9	56.2	1.45
Interorbital width	38.0	36.1–42.8	39.1	1.44
Orbital diameter	12.3	10.7–14.8	12.4	0.92
Suborbital depth	30.5	28.8–33.7	30.8	1.15
Mandibular ramus	13.6	12.4–16.2	14.5	0.95
Lip length until cleithral	23.0	20.0–25.1	22.6	1.37
Other percents				
Anal width/cleithral width	47.5	40.0–56.0	48.3	3.72
IO/OD	32.5	26.0–36.8	31.8	2.47
IO/Mandibulary ramus	35.9	31.8–41.1	37.2	2.76
PDS length/first dorsal ray length	56.8	51.1–59.0	54.6	1.92
CP length/CP depth	25.1	21.7–25.4	23.6	1.02
Pelvic-fin length/CP depth	39.9	35.5–42.1	38.4	1.74
Lower caudal spine/CP depth	31.9	28.8–33.5	30.7	1.37
Meristics				
Dorsal plates	27	24–27	25	-
Mid-dorsal plates	20	17–21	19	-
Median plates	25	24–27	26	-
Mid-ventral plates	20	18–21	20	-
Ventral plates	23	20–23	21	-
Predorsal plates	6	5–6	5	-
Dorsal plates below dorsal-fin base	6	4–6	5	-
Postdorsal plates	16	15–17	17	-
Ventral plates above anal-fin base	3	2–3	3	-
Ventral plates between end of anal base and caudal-fin membrane	13	11–14	13	-
Premaxillary teeth	42	32–46	36	-
Dentary teeth	39	32–46	38	-

Description: Counts and measurements are summarized in Table 1. Dorsal profile of body elongated and depressed, straight from dorsal-fin to caudal-fin base. Ventral profile of body slightly convex from snout tip to caudal-fin base. Greatest body depth at dorsal-fin origin and smallest on caudal peduncle. Greatest body width at opercular region; lateral profile convex between tip snout and end of dorsal-fin; straight to caudal-fin. Body progressively narrowing from cleithrum to caudal-fin. Caudal peduncle rectangular in cross section. Dorsal profile of caudal peduncle with longitudinal depression and covered with paired plates (postdorsal plates between of 15–17). Ventral profile of caudal peduncle flat.

Head wide, rounded dorsally. Tip of snout partly naked and slightly concave towards nares. Interorbital region straight to slightly concave in frontal view. Small eyes (10.7–14.8% of head length), dorsolaterally placed. Iris with small dorsal flap covering pupil. Nares near and almost with same diameter that eyes. Lips well developed and rounded. Lower lip far from reaching pectoral girdle and covered with papillae, decreasing in size towards posterior margin, wider anteriorly. Maxillary barbel short. Upper lip folded over itself. Teeth long and bicuspid (small lateral cusp located laterally in main crow). Rami of dentaries and premaxillae strongly curved inwards. Teeth organized in single series. Dorsal-fin I,7; spinelet presented and ovoid shaped; locking mechanism not functional. Dorsal-fin base located between vertebrae 10 and 14. Pectoral-fin I,6. Pelvic-fin I,5. Pectoral and pelvic-fins without locking mechanism. Pectoral-fin unbranched ray covered with conspicuously pointed and developed odontodes, largest at ventral portion. Tip of pectoral-fin almost reaching middle of pelvic-fins. Second and third branched pectoral-fins rays surpassing tip of unbranched pectoral-fin ray. Pelvic-fin unbranched ray slightly curved. First unbranched fin ray covered with conspicuously pointed and developed odontodes, largest at ventral portion. Tip of pelvic-fin slightly surpassing insertion of anal fin first ray. Anal-fin I,5; it base located between vertebrae 16 and 19. Adipose fin absent. Dorsal portion of caudal peduncle covered with paired plates. Caudal-fin truncated with lower unbranched ray longer than upper. Caudal-fin with I,7–7,I branched rays.

Upper hypural plate (composed of epural, uroneural, and hypurals 3–5) with I+7 rays; lower hypural plate (composed of hypurals 1, 2, and parahypural) with 7+I rays. Body entirely covered by bony plates, except for ventral surface of head and most part of abdomen. Small platelets randomly distributed near urogenital opening and all abdominal regions, leaving abundant naked areas surrounding them. Dorsal plates with 24 to 27, mid-dorsal 17 to 21, median plates 24 to 27, mid-ventral 18 to 21, and ventral 20 to 23. Head with naked area beside compound pterotic. Head without crests. Head and body plates covered with developed odontodes with uniform distribution. Ventral surfaces of first pectoral, pelvic and anal-fin rays covered by pointed odontodes.
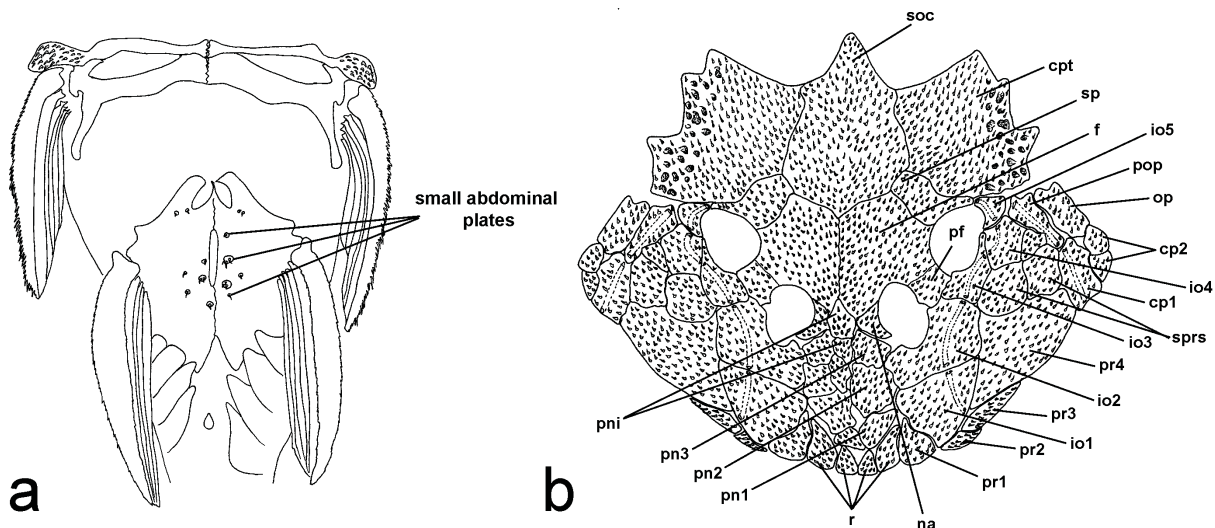
FIGURE 2. (a) Ventral view; small plates covered with odontodes randomly distributed to abdomen. (b) Dorsal view; osteology of the head. Paratype, LBP 11835, c&s, 33.4 mm SL.

Seven pairs of ribs associated with vertebral central 8–15. First pair of ribs large and laminar; remaining ribs slender and poorly ossified. Total number of vertebrae 30. Supraorbital sensory canal with four pores s1, s3, s6+s6 and s8; s1 located on terminal portion of nasal plates; pore s3 located on anterior portion of nasal; pore s6+s6 located between frontal, on horizontal line through anteriormost limits of eyes; pore s8 on division between frontal, sphenotic and parieto-supraoccipital, just above eyes. Infraorbital sensory canals with six pores; pore io1 located
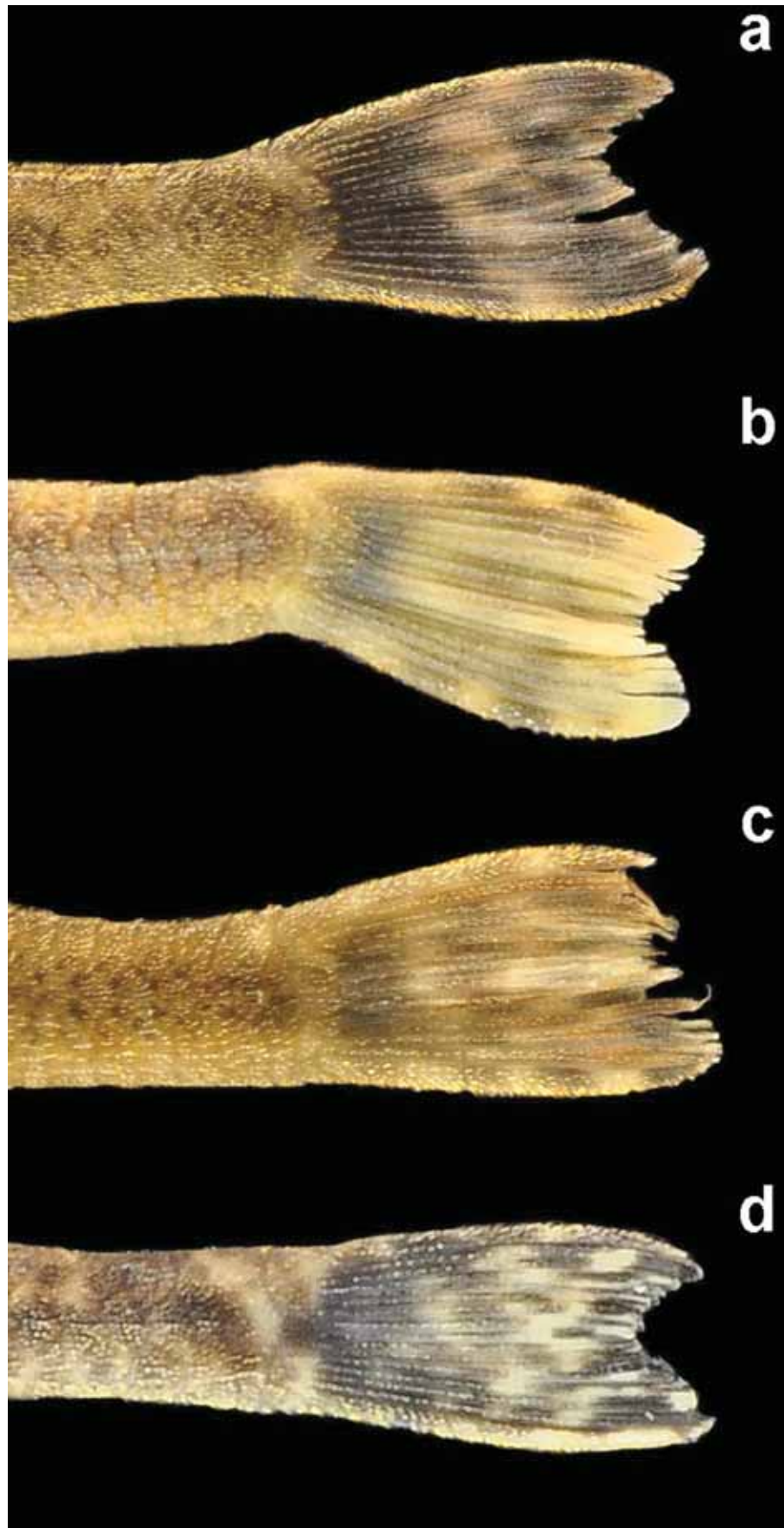
FIGURE 3. Picture showing the different color pattern of the caudal-fin in species of *Pareiorhina*. (a) *Pareiorhina cepta*, (b) *P. rudolphi*, (c) *P. brachyrhyncha*, (d) *P. carrancas*.

on anterior end of first infraorbital; pore io2 located in medial region between first and second infraorbitals; pore io3 located in medial region between second and third infraorbitals; pore io4 located in medial region between third and fourth infraorbitals; pore io5 located in medial region between fourth and fifth infraorbitals, and pore io6 located between sixth and sphenotic. Preopercular canal with two pores; pore pm3 located between cheek plate and preopercle; pore pm4 located between preopercle and compound pterotic. Two postotic pores; pore po2 located just above branchial slit; and po3 located on region overlying opening of swim-bladder capsule.

Head osteology in Fig. 2b. Tip of snout with four rostral plates (r). Three pairs of prenasal plates behind rostral plates (pn1–pn3) all with varied sizes and shapes and a pair of nasal plates (na). Three internasal plates (pni) between nasal plates. Five infraorbital plates (io1-io5) on lateral surface of head; all covered by latero-sensory canal system. First infraorbital largest and fifth smallest. Preopercle (pop) just below fifth infraorbital elongated, which is also covered by latero-sensory canal. Lateral of head covered by postrostral plates, (pr1–pr4); pr4 largest, triangular-shaped plate. Suprarostral plates (sprs) just below io3 and io4. Last lateral plate series in subocular cheek plates (cp1–cp2) and opercle (op). Posterior portion of skull formed by prefrontal (pf), frontal (f), sphenotic (sp), compound pterotic (cpt) and parieto-supraoccipital (soc). Several fenestrae in lateral margin of compound pterotic.

Color in alcohol: Ground color of dorsal surface of head and body light brown to yellowish brown. Ventral surface of body and head lighter than dorsal and covered by dark spots of chromatophores widely settled. Three dark bars on dorsal surface of trunk (in some specimens not evident), anterior most poorly defined. Lateral portion of body with poorly defined dark stripe from head to caudal-fin. Fins with irregularly and poorly defined bars: three on anal fin, four on pectoral and pelvic-fins, and five on dorsal-fin. Caudal-fin completely dark with one hyaline bar.

Sexual dimorphism: Males of *Pareiorhina cepta* have a conspicuous urogenital papilla posterior to urogenital opening (attribute absent in females).

Distribution: Known only from Córrego do Lavapés and Córrego Maria da Prata, which are two small headwater streams in the upper Rio São Francisco basin, near Serra da Canastra, Minas Gerais State, Brazil (Figs. 4 and 5).

Habitat: *Pareiorhina cepta* was found at an altitude of about 810–1065 m.a.s.l. The streams were narrow (about 3–5 m width) and shallow (about 0.25–1.5 m deep). The stream bottom was formed of small to medium-sized rocks, loose stones, gravel and sand. The water in the sampled site was clear, cold (16.6 °C–19.5 °C), highly acidic (pH 2.78–2.87), poorly conductive (μs/cm 0.013–0.014), highly oxygenated (5.91–13.05 mg/l OD) and moderate to fast flowing. Margins of the stream were covered by vegetation, which shadows a wide portion of its bed. In that stream, specimens of *Pareiorhina cepta* sympatric with other species, such as *Astyanax rivularis, Neoplecostomus franciscoensis, Trichomycterus macrotrichopterus* and *Characidium fasciatum* were captured.

Etymology: The specific name *cepta* is derived from CEPTA (an acronym for Centro de Pesquisa Treinamento em Aquacultura—former Centro Nacional de Pesquisa e Conservação de Peixes Continentais) from municipality of Pirassununga, São Paulo State, Brazil.
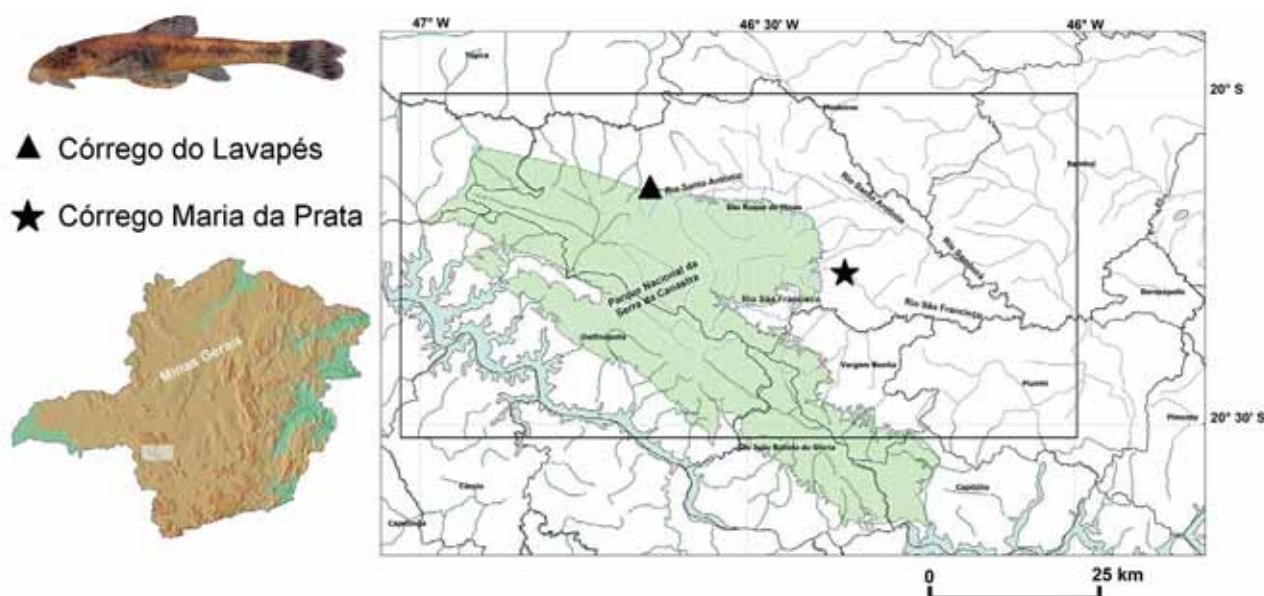
FIGURE 4. Map showing the collecting site of *Pareiorhina cepta* in two streams in Rio São Francisco basin, Minas Gerais State. Triangle—20°08'36"S 46°38'21"W, star—20°15'51"S 46°20'57"W.

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FIGURE 5. Field picture showing type locality of *Pareiorhina cepta*, Córrego do Lavapés, Rio São Francisco basin, municipality of São Roque de Minas, district of São João Batista, Minas Gerais State, Brazil.

Key to species of *Pareiorhina* modified from Chamon *et al.* (2005)

la. Postdorsal surface of trunk with median low ridge formed by 10–13 azygous plates extending between dorsal and caudal-fin . . . .*P. carrancas*

lb. Postdorsal surface of trunk without median ridge. 2

2a. Teeth unicuspid . . . . *P. rudolphi*

2b. Teeth with minute lateral cusp. 3

3a. Tip of snout covered with plates; abdomen completely naked . . . . *P. brachyrhyncha*

3b. Tip of snout naked; small plates covered with odontodes randomly distributed to abdomen . . . .*P. cepta*

Discussion

In a molecular analysis of the subfamily Hypoptopomatinae and Neoplecostominae, Cramer *et al.* (2011) identified *Pareiorhina brachyrhyncha* and *P. carrancas* as sister taxa, but this clade occurred in a polytomy with other Neoplecostominae genera. Chiachio *et al.* (2008) identified *Pareiorhina rudolphi*, the type species of the genus, as a sister-group to other undescribed *Pareiorhina,* with both forming a sister-group to the genus *Pseudotocinclus*. Thus, these analyses suggest that *Pareiorhina* may not be monophyletic. Currently, no exclusive synapomorphic character has been presented to diagnose the genus. As a result of the conflict between morphological taxonomy and molecular phylogenies, we compared the new species with other Neoplecostominae genera *Isbrueckerichthys*, *Kronichthys*, *Neoplecostomus*, *Pareiorhaphis*, *Pareiorhina* and *Pseudotocinclus* (*sensu* Armbruster 2004 and Chiachio *et al.* 2008) in an attempt to identify its correct taxonomic position.

According to Langeani (1990) the genus *Neoplecostomus* is diagnosed by inferior lips with three or four papilla series just above the dentary, abdomen partially covered by a shield of plates, presence of the canal bearing plate and pectoral spine mechanism absent. *Pareiorhina cepta* share the canal bearing plate with species of *Neoplecostomus*, however this character is also present in species of *Kronichthys*, *Isbrueckerichthys,* *Pareiorhaphis* and other *Pareiorhina*. The abdomen of *Pareiorhina cepta* is covered by randomly distributed small plates (Fig. 2a), but these do not form a shield as in *Neoplecostomus* species. The other remaining characters used to distinguish *Neoplecostomus* listed above are absent in *Pareiorhina cepta*.

The genus *Isbrueckerichthys* was proposed by Derijst (1996) and despite lacking diagnostic characters (Pereira & Oyakawa 2003), it can be distinguished from other Neoplecostominae by having a small naked area behind the compound pterotic, abdomen with small platelets imbedded in skin between the pectoral girdle and pelvic-fin insertions, dorsal fin with one spine and seven branched rays, and caudal peduncle ovoid in cross-section. *Pareiorhina cepta* has an abdomen with a wide naked area between randomly distributed small plates (Fig. 2a), while in *Isbrueckerichthys* species the abdominal plates form a shield without wide naked areas, moreover, the caudal peduncle is not ovoid but flatted in the dorsal and ventral portion (Fig. 1). The new species shares with *Isbrueckerichthys* the dorsal-fin I,7 and the presence of a small naked area behind the compound pterotic, however these characters are also found in other Neoplecostominae and Hypoptopomatinae species.

According to Gosline (1947) *Kronichthys* is characterized by having a more or less cylindrical body, head rather high and quadrangular, the inner ends of both tooth rows in both jaws turning sharply inwards and running almost parallel to one another, and the presence of an adipose fin. *Pareiorhina cepta* does not have any of these characters, however it shares with species of *Kronichthys* a lateral cusp in the teeth. According to Schaefer (1987) this is a typical character present in species of Loricariidae and Astroblepidae.

Pereira (2005) resurrected the genus *Pareiorhaphis* and used the following combination of characters to distinguish it from other Neoplecostominae genera: fleshy lobes on lateral margins of head ornamented with hypertrophied odontodes on nuptial males, caudal peduncle ovoid in cross-section, abdomen usually naked, dorsal-fin spinelet ovoid, and adipose-fin usually present. The new species *Pareiorhina cepta* shares only the shape of dorsa-fin spinelet (ovoid) with species of *Pareiorhaphis*.

According to Takako *et al.* (2005) the genus *Pseudotocinclus* can be diagnosed from other Hypoptopomatinae by the presence of a caudal peduncle that is nearly square in cross-section, by the presence of 26 or more plates along the lateral line, a dorsally positioned eye, an exposed preopercle and an abdomen covered with numerous small plates. The caudal peduncle of *Pareiorhina cepta* is flatted in dorsal and in ventral portions (Fig. 1), but is not completely square in cross-section as in *Pseudotocinclus*. The abdomen of the new species has few and small randomly distributed plates (Fig. 2a) and in species of *Pseudotocinclus* the number of plates covering the abdomen

is much greater in density. Also in *Pareiorhina cepta* the preopercle is present above the bones of the head which is different from that found in species of *Pseudotocinclus*. Considering the other characters listed above the new species *P. cepta* has all of them. However, these are polymorphic between species of Neoplecostominae and Hypoptopomatinae.

The new species *Pareiorhina cepta* has all characters proposed by Bockmann and Ribeiro (2003) to diagnose *Pareiorhina*, except the abdomen completely naked and teeth simple. The presence of minute lateral cusps in the teeth is a character shared with *Pareiorhina brachyrhyncha*, a species very similar to *P. cepta*. Thus, before a more complete elucidation of the generic composition of Neoplecostominae has been performed, we have allocated the new species to the genus *Pareiorhina*, rather than any other Neoplecostominae genus. Finally, a thorough morphological analysis of *Pareiorhina* is necessary to better elucidate the morphological variation of its member species and its relationship with other Neoplecostominae genera.

Comparative material

Hisonotus notatus: LBP 2100, 1, 55.4 mm SL, Morretes Municipality, Paraná State, Coastal Drainage. LBP 3472, 20, 21.0–34.3 mm SL, Macaé Municipality, Rio de Janeiro State, Coastal Drainage. *Isbrueckerichthys alipionis*: LBP 7373, 17, 31.7–81.6 mm SL, Municipality of Iporanga, SP, Coastal Drainage. *Kronichthys* sp.: LBP 2122, 61, 30.1–78.5 mm SL, Parati Municipality, Rio de Janeiro State, Coastal Drainage. *Kronichthys subteres*: LBP 515, 31, 28.4–61.9 mm SL, Iporanga Municipality, São Paulo State, Coastal Drainage. *Neoplecostomus franciscoensis*: LBP 6489, 50, 42.8–55.9 mm SL, São Bartolomeu Municipality, Minas Gerais State, Rio das Velhas basin. *Neoplecostomus paranensis*: holotype, MZUSP 38572, 71.4 mm SL, Cajuru Municipality, São Paulo State, Rio Grande basin, LBP 2732, 1, 70.5 mm SL, Cajuru Municipality, São Paulo State, Rio Grande basin; *Pareiorhaphis steindachneri*: LBP 739, 6, 33.8–49.0 mm SL, Jaraguá do Sul Municipality, Santa Catarina State, Coastal Drainage. *Pareiorhina carrancas*: LBP 8380, 24, 21.3–35.4 mm SL, Carrancas Municipality, Minas Gerais State, Rio Grande basin. *Pareiorhina brachyrhyncha*: LBP 12240, 50, 26.4–36.9 mm SL, Pindamonhangaba Municipality, São Paulo State, rio Paraíba do Sul. *Pareiorhina rudolphi*: LBP 8044, 18, 31.7–48.9 mm SL, Piquete Municipality, São Paulo State, Rio Paraíba do Sul basin. *Pareiorhina* cf. *rudolphi*: LBP 4396, 5, 35.4–45.9 mm SL, Jundiaí Municipality, São Paulo State, Rio Tietê basin. *Parotocinclus prata*: LBP 11683, 3, 18.6–29.6 mm SL, Claro de Minas Municipality, Minas Gerais State, Rio São Francisco basin. *Pseudotocinclus juquiae*: LBP 1081, 2, 29.0–31.9 mm SL, Juquitiba Municipality, São Paulo State, Coastal Drainage. *Pseudotocinclus tietensis*: LBP 2931, 3, 38.6–62.3 mm SL, Salesópolis Municipality, São Paulo State, Rio Tietê basin. *Schizolecis guntheri*: LBP 2123, 21, 28.4–36.3 mm SL, Parati Municipality, Rio de Janeiro State, Coastal Drainage, LBP 3546, 77, 20.9–35.8 mm SL, Ubatuba Municipality, São Paulo State, Coastal Drainage.

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Appendix 2

Ichthyological Exploration of Freshwaters

An international journal for field-orientated ichthyology

Ichthyological Exploration of Freshwaters
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Hisonotus bocaiuva, a new species
from the rio São Francisco basin, Brazil
(Teleostei: Loricariidae)

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Hisonotus bocaiuva, new species, is described from the rio São Francisco basin, municipality of Bocaiúva, Minas Gerais State, Brazil. It is distinguished from other members of the Otothyrinae by the following characters: a conspicuous tuft of enlarged odontodes on the posterior tip of parieto-supraoccipital; the presence of a rostral plate with posterior notch articulated with mesethmoid; the snout completely covered by odontodes; the abdomen partially covered by plates; the number of lateral median plates; the absence of adipose fin; and by morphometric characters. The new species is additionally distinguished by having three vertical dark bands on the caudal fin and the absence of broader light stripes on the dorsolateral surface of the head.

Introduction

The Loricariidae is one of the most diverse and widespread families of the Neotropical freshwater ichthyofauna and is composed of seven subfamilies which are, in alphabetical order: Delturinae, Hypoptopomatinae, Hypostominae, Lithogeninae, Loricariinae, Neoplecostominae, and Otothyrinae (Armbruster, 2004; Reis et al., 2006; Chiachio et al., 2008). Actually, Otothyrinae (sensu Chiachio et al., 2008) is composed of about 75 species classified in 10 genera (Eschmeyer, 2012): *Corumbataia*, *Epactionotus*, *Eurycheilichthys*, *Hisonotus*, *Microlepidogaster*, *Otothyris*, *Otothyropsis*, *Parotocinclus*, *Pseudotothyris* and *Schizolecis*.

The genus *Hisonotus* was established by Eigenmann & Eigenmann (1889) to accommodate *Hisonotus notatus*, the type species collected in Santa Cruz by the Brazilian emperor Dom Pedro II and in Juiz de Fora during the Thayer Expedition. Actually, *Hisonotus* includes 30 valid species (Carvalho & Reis, 2009; Carvalho & Reis, 2011; Carvalho & Datovo, 2012; Martins & Langeani, 2012) and is widespread in drainages of Southern and Southeastern Brazil, from the rio Uruguay basin, upper rio Paraná basin, Laguna dos Patos and coastal drainages extending from Rio Grande do Sul to Rio de Janeiro and the Amazon basin.

Regan (1904) studied the osteology of the members of the family Loricariidae and consid-

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ered *Hisonotus* and *Parotocinclus* to be synonyms of *Otocinclus*. Thenceforth, Eigenmann (1910) maintained the generic distinctiveness of *Parotocinclus*, but *Hisonotus* continued to be regarded as a synonym of *Otocinclus* until its resurrection by Schaefer (1998). Recently, during a collecting trip in tributaries of rio São Francisco basin we found an undescribed species of Otothyrinae, which meets the characteristics of *Hisonotus*. The present work deals with the description of this species.

Material and methods

Measurements and counts were taken from the left side. Body plate and bone nomenclature follows Schaefer (1997) and measurements follow Carvalho & Reis (2009). All measurements were taken point to point to the nearest 0.1 mm with digital calipers. The animals were anesthetized with benzocaine and fixed in 10 % formalin for at least two weeks, then transferred to 70 % ethanol for permanent storage. All samples (holotype, 18 paratypes and 4 c&s paratypes) are deposited at: DZSJRP, Departamento de Zoologia e Botânica, Universidade Estadual Paulista Júlio de Mesquita Filho, São José do Rio Preto, São Paulo; LBP, Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, São Paulo; MZUSP, Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo; NUP, Coleção Ictiológica do Nupélia, Universidade Estadual de Maringá, Maringá, Paraná; MNRJ, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro. Specimens were cleared and double stained (c&s) according to the method of Taylor & Van Dyke (1985). Vertebrae counts include five vertebrae of the Weberian Apparatus.

Hisonotus bocaiuva, new species
(Fig. 1)

Holotype. MZUSP 112204, male, 24.2 mm SL; Brazil: Minas Gerais State: Bocaiúva, córrego Cachoeira: rio Jequitaí drainage: rio São Francisco basin, 17°08'55"S 43°49'32"W; C. Oliveira, G. J. C. Silva, F. F. Roxo & T. N. A. Pereira, 14 May 2010.

Paratypes. LBP 9817, 9, 4 c&s, 18.3–23.2 mm SL; DZSJRP 16461, 3, 22.0–25.6 mm SL; NUP 12331, 4, 21.7–22.3 mm SL; MNRJ 40368, 2, 18.6–20.6 mm SL; collected with holotype.

Diagnosis. *Hisonotus bocaiuva* differs from most members of Otothyrinae, except *Corumbataia britskii*, *Hisonotus carreiro*, *H. francirochai*, *H. iota*, *H. leucophrys*, *H. prata*, *Parotocinclus arandai*, *P. cesarpintoi* and *P. cristatus* in having a conspicuous tuft of enlarged odontodes on posterior tip of parieto-supraoccipital in specimens with 17.9–25.6 mm (vs. absent) or with a conspicuous tuft of enlarged odontodes on posterior tip of parieto-supraoccipital restricted to juveniles. The new species differs from *Corumbataia britskii* by having a rostral plate with posterior notch articulated with mesethmoid (vs. snout tip naked and lacking rostral plate), by the caudal fin with three vertical dark bands (vs. mostly dark-brown with two unpigmented spots on caudal-fin lobes; Ferreira & Ribeiro, 2007: fig. 5a). *Hisonotus bocaiuva* differs from *Hisonotus carreiro* and *H. prata* in having the anterior portion of the snout completely covered with odontodes (vs. anterior portion of the snout with a narrow odontode-free band; Carvalho & Reis, 2011: figs. 29, 35); from *H. iota* in having the abdomen partially covered by plates, which are surrounded by naked areas (vs. abdomen completely covered with plates not surrounded by naked areas; Carvalho & Reis, 2009: fig. 6a) and fewer lateral median plates (18–20 vs. 20–22); from *H. leucophrys* in having the abdomen partially covered by plates, which are surrounded by naked areas (vs. the abdomen completely covered with plates not surrounded by naked areas; Carvalho & Reis, 2009: fig. 6b) and absence (vs. presence) of broader light stripes on dorso-lateral surface of the head from snout tip to posterior end of the compound pterotic; from *H. francirochai* in having three vertical dark bands on the caudal fin (vs. the caudal fin completely dark except for two hyaline rounded areas on upper and lower lobes and in a small area on the distal tip of each lobe), fewer lateral median plates (18–20 vs. 23–26), a higher suborbital depth (20–24 % HL vs. 16–20) and a deeper head (depth 50–57 % HL vs. 39–50); from *Parotocinclus arandai*, *P. cesarpintoi* and *P. cristatus* in absence (vs. presence) of an adipose fin.

Description. Morphometric and meristic data presented in Table 1. Adult size small to moder-

Fig. 1. *Hisonotus bocaiuva*, MZUSP 112204, holotype, male, 24.2 mm SL; Brazil: Minas Gerais: rio São Francisco basin: rio Jequitaí drainage.

ate (maximum 25.6 mm SL) for a species of Otothyrinae. Dorsal profile strongly convex from snout tip to dorsal-fin origin; descending from dorsal-fin origin to insertion of caudal fin. Ventral profile almost straight from snout tip to anal-fin origin. Caudal peduncle ascending from origin of anal-fin base to caudal-fin origin. Greatest body depth at dorsal-fin origin. Greatest body width at opercular region; lateral profile in dorsal view strongly convex from snout tip to end of dorsal fin; straight to caudal fin. Body progressively narrowing from opercular region to caudal fin. Cross-section of body between pectoral and pelvic fins dorsally rounded and ventrally flat; cross-section of caudal peduncle ellipsoid. Eyes small (16–19 % HL), dorsolaterally positioned. Spinelet present. Dorsal fin II,7; its origin slight-

ly posterior to pelvic-fin origin. Tip of adpressed dorsal fin surpassing vertical through end of anal-fin base. Dorsal, pectoral and pelvic fins without locking mechanism. Pectoral fin I,6; its tip reaching middle of pelvic-fin length when depressed. Pectoral axillary slit absent. Pelvic fin I,5; its tip almost reaching anal-fin origin when depressed. Anal fin i,5. Caudal fin i,7–7,i. Adipose fin and azygous plates absent. Total vertebrae 25 (in 4 c&s specimens).

Body almost entirely covered by plates, except in ventral portion of head, region between pectoral girdle and lower lip and area around anus; abdomen partially covered by plates randomly distributed and surrounded by naked areas. Lateral median plates 18–20; truncated, not reaching posterior end of caudal peduncle. Lateral line

incomplete, with gap along mid-length of body (Fig. 2a). Coracoid and cleithrum exposed, covered with odontodes. Arrector fossae partially enclosed by ventral lamina of coracoids. Odontodes randomly arranged on head and body. Snout completely covered with well-developed odontodes. Head without conspicuous crests, except conspicuous tuft of enlarged odontodes on parieto-supraoccipital posterior tip. Premaxillary teeth 14–24; dentary teeth 14–20. Teeth bifid, major (medial) cusp large and rounded, minor (lateral) cusp minute and pointed. Accessory patch of teeth absent on dentary and premaxilla. Oral disk roundish, covered with papillae.

Tip of snout with a large rostral plate, prenasal plates behind rostral plate. Lateral surface of head formed by postrostral plates; fourth postrostral plate (pr4) largest and triangular-shaped. Infraorbital series with five plates, all bearing branch of latero-sensory system. Preopercle positioned below fifth infraorbital, bearing branch of latero-sensory system. Subocular cheek plate present below preopercle plate (Fig. 2b).

Coloration. Yellowish brown ground color. Dorsal surface of head dark brown, except for yellowish areas on snout tip. Four dark brownish saddles crossing dorsum, reaching lateral line: first at dorsal-fin origin, inconspicuous, second at end of dorsal-fin base, third at typical adipose fin region, and fourth at end of caudal peduncle. Saddles inconspicuous in some specimens. Ventral region of body with few clusters of small dark chromatophores. Dorsal, pectoral, and pelvic fins with dark chromatophores forming irregular sets of bars: three on dorsal fin, one on pectoral fin, and one on pelvic fin. Anal fin exhibits few and sparse chromatophores, sometimes forming bands; caudal fin with three vertical dark bands.

Sexual dimorphism. Males possess a papilla posterior to the urogenital opening, which is not present in females. Adult males possess an expanded flap of skin on dorsal surface of the first pelvic-fin ray, which is absent in juvenile males and in females.

Table 1. Morphometric and meristic data of 23 specimens of *Hisonotus bocaiuva*. SD = Standard deviation.

	holotype	range	mean	SD
Standard length mm	24.2	17.9–25.6	20.8	2.1
Percents of standard length				
Head length	34.2	34.1–37.0	35.7	1.0
Predorsal length	44.7	44.6–48.5	46.3	0.9
Dorsal-fin spine length	24.2	21.8–26.4	24.3	1.3
Anal-fin unbranched ray length	17.3	17.3–20.4	18.5	0.8
Pectoral-fin spine length	23.3	22.3–27.2	24.6	1.1
Pelvic-fin unbranched ray length	20.9	17.4–21.0	19.3	1.0
Cleithral width	25.6	23.0–28.7	26.4	1.3
Thoracic length	17.7	15.3–18.9	17.3	0.9
Abdominal length	21.5	19.1–24.9	21.8	1.4
Body depth at dorsal-fin origin	20.0	18.3–21.5	19.6	0.8
Caudal-peduncle length	35.7	29.7–36.8	33.5	1.5
Caudal-peduncle depth	9.6	8.4–10.1	9.5	0.4
Percents of head length				
Snout length	48.4	44.5–49.9	46.7	1.3
Orbital diameter	18.4	15.8–19.0	17.5	0.7
Interorbital width	38.6	35.5–39.1	37.7	0.9
Head depth	56.5	50.4–56.5	54.0	1.7
Suborbital depth	24.1	20.2–24.1	22.4	1.0
Mandibular ramus	9.7	8.8–11.3	10.3	0.6
Counts			mode	
Premaxillary teeth	17	14–24	20	2.4
Dentary teeth	17	14–20	17	1.7
Lateral median plates	19	18–20	20	1.2
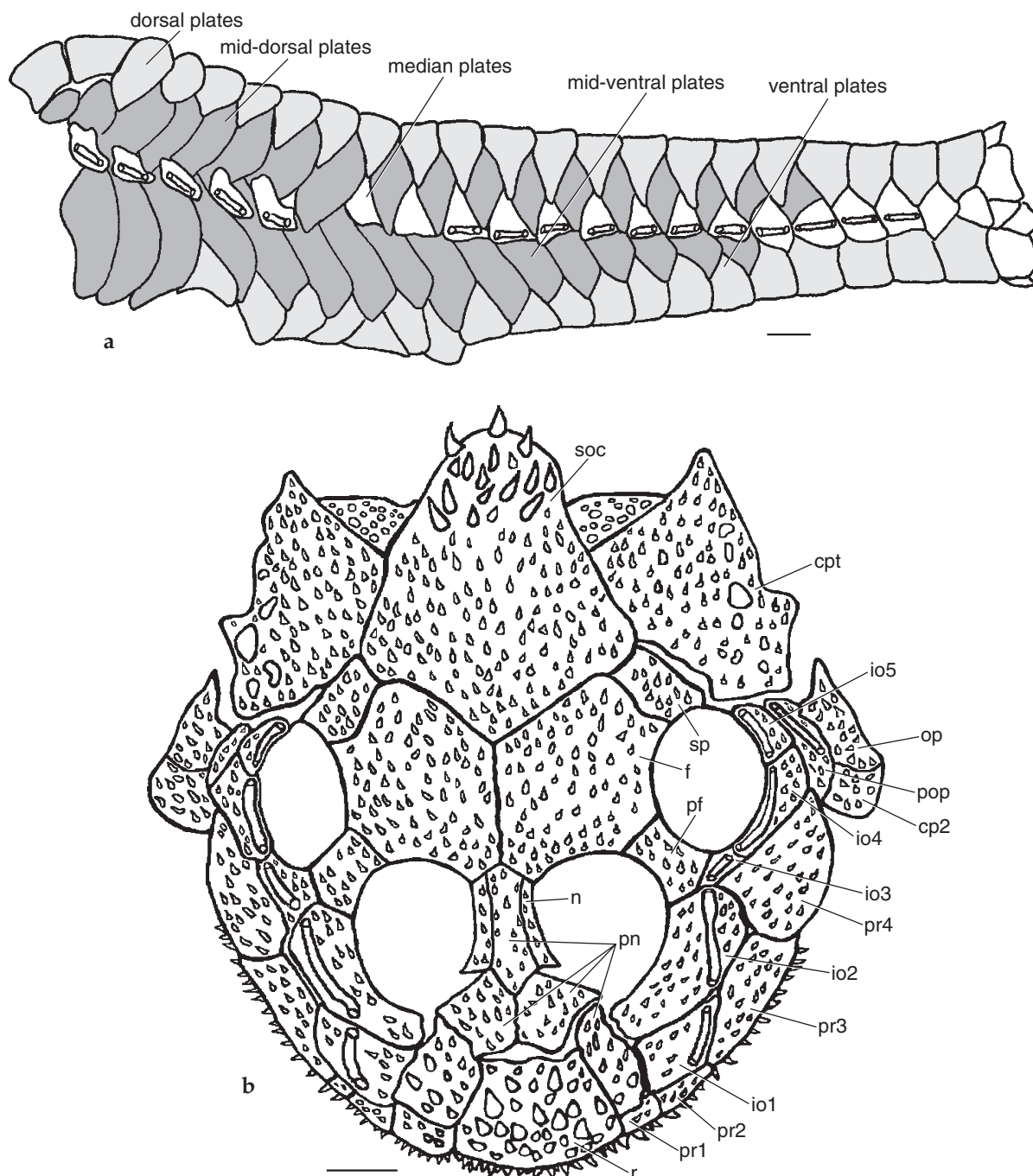
Fig. 2. *Hisonotus bocaiuva*, LBP 9817, 22.1 mm SL. **a,** Lateral view of plates series; **b,** dorsal view of head plates (cp1 is not visible). Scale bar 1 mm. Abbreviations of bones follow Schaefer (1997): **cpt**, compound pterotic; **f**, frontal; **io1–5**, infraorbitals; **n**, nasal; **op**, opercle; **pr1–4**, postrostral plates; **pf**, prefrontal; **pn**, prenasal; **pop**, preopercle; **r**, rostral plates; **sp**, sphenotic; **cp2**, subocular cheek plate 2; **soc**, parieto-supraoccipital.

Etymology. Named after the municipality of Bocaiúva, the city where the specimens were collected. A noun in apposition.

Distribution. *Hisonotus bocaiuva* is known only from the drainages of córrego Cachoeira, rio Jequitaí drainage, rio São Francisco basin, municipality of Bocaiúva, Minas Gerais State, Brazil (Fig. 3).

Discussion

Hisonotus bocaiuva has the four characters proposed by Schaefer (1998) to support the monophyly of his tribe Otothyrini (actually subfamily Otothyrinae sensu Chiachio et al., 2008): presence of metapterygoid channel; ventral margin of preopercle medially reflected; rostral plate with posterior notch articulated with mesethmoid and
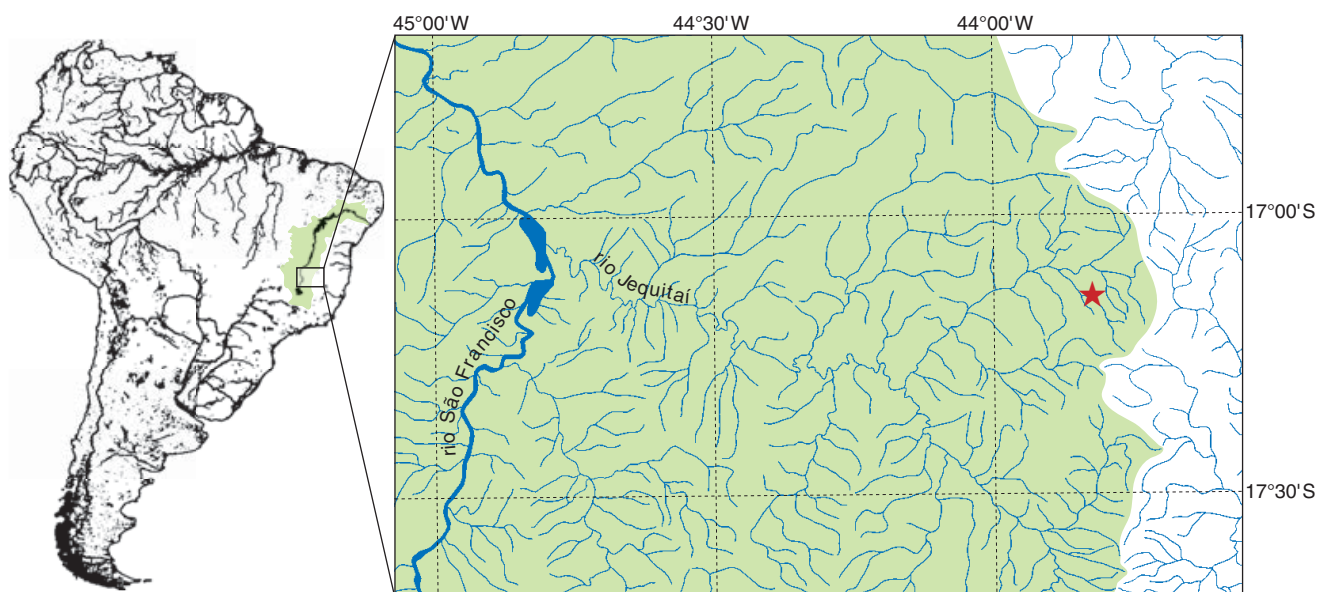
Fig. 3. Collection site of *Hisonotus bocaiuva* (★, 17°08'55" S 43°49'32" W). The green coloration represents the rio São Francisco basin.

fourth infraorbital expanded ventrally. Additionally, Otothyrinae, sensu Chiachio et al. (2008), can be diagnosed by the almost complete fusion of pectoral dermal bony plates forming a strong pectoral armor, with the exception of the two small anteromedian apertures, a character also present in *Hisonotus bocaiuva*. Thus, the new species is a typical Otothyrinae species.

Schaefer (1998) re-established *Hisonotus* considering that fishes of this genus share the characters: (1) reduced or absent snout plates anterior to the nostril, (2) rostrum with enlarged odontodes, and (3) thickened plates forming the lateral rostral margin. *Hisonotus bocaiuva* has the rostrum with enlarged odontodes. Additionally, Schaefer (1998) suggested that the median plate series truncated is an additional synapomorphy for *Hisonotus*, a character also present in *H. bocaiuva*. However, Britski and Garavello (2007) considered the last character polymorphic among Hypoptopomatinae (sensu Schaefer, 1998). *Hisonotus bocaiuva* also shares two characters with most species of *Hisonotus*: a single rostral plate on the tip of the snout and an arrector fossae partially enclosed by ventral lamina of coracoids. The last character was used by Schaefer (1998) as synapomorphy of all Otothyrini except his New Taxon 3.

The main character useful to distinguish *H. bocaiuva* from other Otothyrinae species is a conspicuous tuft of enlarged odontodes on the posterior tip of the parieto-supraoccipital present in all 23 specimens available. This character is also present in *Corumbataia britskii*, *Hisonotus carreiro*, *H. francirochai*, *H. iota*, *H. leucophrys*, *H. prata*, *Parotocinclus arandai*, *P. cesarpintoi* and *P. cristatus*. Britski & Garavello (2009) found that young specimens of *P. bahiensis* (up to about 24 mm SL) have a pair of crests on parieto-supraoccipital posterior portion and an unpaired crest on posterior tip of bone. In specimens larger than 24 mm SL up to 30.3 mm SL the crests are reduced or absent. Additionally, Ferreira & Ribeiro (2007) showed that this character can vary in intensity in *C. cuestae* and *C. tocantinensis*, where it decreases with growth, to almost absent in adults (Fig. 3, Ferreira & Ribeiro, 2007). However, among all available specimens of *H. bocaiuva* (23 specimens, 17.9–25.6 mm SL) the conspicuous tuft of enlarged odontodes on the posterior tip of the parieto-supraoccipital is always well-developed. In species of the genus *Otothyris* and *Pseudotothyris* the posterior portion of parieto-supraoccipital is formed by three crests: an anterior pair short and an unpaired one of median length covered by odontodes. *Hisonotus bocaiuva* has only the median crest which consists of a conspicuous tuft of odontodes.

The subfamily Otothyrinae is one of the richest and most diverse group of the family Loricariidae, however the generic classification of most groups remains yet unsettled due to the absence of certain diagnose characters as cited above. Thus, a phylogenetic analysis of Otothy-

rinae is necessary to better understand its generic composition.

Comparative material. *Corumbataia cuestae*: LBP 3688, 3, 28.5–29.9 mm SL; upper rio Paraná basin. *C. britskii*: LBP 9590, 50, 17.9–28.8 mm SL; upper rio Paraná basin.

Epactionotus sp.: LBP 12358, 6, 29.2–43.0 mm SL; coastal drainage.

Hisonotus carreiro: MCP 40943, 3, 33.6–35.8 mm SL; coastal drainage. *H.* cf. *charrua*: LBP 13147, 1, 30.7 mm SL; coastal drainage. *H. chromodontus*: LBP 7964, 25, 24.0–28.3 mm SL; rio Teles Pires basin. – LBP 7974, 46, 17.9–25.7 mm SL; rio Teles Pires basin. *H. francirochai*: LBP 5026, 1, 34.6 mm SL; NUP 67, 5, 24.2–31.4 mm SL; rio Tietê basin. – LBP 10213, 4, 29.6–34.6 mm SL; rio Paraná basin. – LBP 13923, 22, 25.7–35.7 mm SL; rio Grande basin. *H. heterogaster*: LBP 3335, 39, 20.8–30.1 SL; coastal drainage. *H. iota*: LBP 13072, 5, 32.3–33.0 mm SL; rio Uruguay basin. *H. laevior*: LBP 3377, 1, 25.2 mm SL; LBP 6037, 8, 33.4–47.0 mm SL; LBP 13187, 7, 19.4–45.8 mm SL; coastal drainage. *H. leucofrenatus*: LBP 2085, 7, 38.3–50.6 mm SL; coastal drainage. – LBP: 6837, 36, 35.1–43.5 mm SL; rio Ribeira de Iguape basin. *H. leucophrys*: LBP 13065, 6, 17.2–33.6 mm SL; LBP 13073, 1, 36.8 mm SL; rio Uruguay basin. *H. megaloplax*: LBP 13108, 6, 36.4–37.8 mm SL; rio Uruguay basin. *H. montanus*: LBP 13055, 5, 24.8–31.9 mm SL; rio Uruguay basin. *H. nigricauda*: LBP 579, 16, 34.1–40.1 mm SL; LBP 4783, 10, 16.3–35.8 mm SL; coastal drainage. *H. notatus*: LBP 3472, 20, 21.0–34.3 mm SL; coastal drainage. *H. prata*: MCP 40492, 18, 19.5–33.2 mm SL; LBP 9918, 14, 21.7–32.6 mm SL; coastal drainage.

Microlepidogaster dimorpha: LBP 10683, 2, 28.8–35.6 mm SL; rio Paraná basin.

Otothyris travassosi: LBP 1971, 13, 14.0–27.2 mm SL; coastal drainage.

Otothyropsis marapoama: LBP 4698, 6, 23.9–36.3 mm SL; rio Tietê basin.

Parotocinclus cf. *bahiensis*: LBP 7182, 3, 27.9–35.6 mm SL; coastal drainage. *P. maculicauda*: LBP 2869, 15, 20.2–44.7 mm SL; LBP 3181, 1, 40.3 mm SL; rio Ribeira de Iguape basin. *P. prata*: LIRP 1136, 38, 19.8–41.9 mm SL; rio São Francisco basin. *P. robustus*: LBP 8258, 33, 18.5–39.5 mm SL; rio São Francisco basin.

Pseudotothyris obtusa: LBP 6822, 70, 22.5–31.7 mm SL; coastal drainage.

Rhinolekos britskii: 21.9–34.7 mm SL; rio Paranaíba basin.

Schizolecis guntheri: LBP 2123, 21, 28.4–36.3 mm SL; LBP 3546, 77, 20.9–35.8 mm SL; coastal drainage.

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Appendix 3

RESEARCH ARTICLE

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Pareiorhina hyptiorhachis, a new catfish species from Rio Paraíba do Sul basin, southeastern Brazil (Siluriformes, Loricariidae)

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Abstract

Pareiorhina hyptiorhachis is described from Ribeirão Fernandes and Rio Pomba, Rio Paraíba do Sul basin, Brazil. The new species is distinguished from its congeners (*P. brachyrhyncha*, *P. carrancas*, *P. cepta*, and *P. rudolphi*) by the presence of a conspicuous ridge on the trunk posterior to the dorsal fin (postdorsal ridge), simple teeth, a completely naked abdomen, a round dorsal profile of the head, greater suborbital depth and greater head width. We discuss the distributional pattern of the new species and its congeners and hypothesize that headwater capture is responsible for the distribution of *Pareiorhina* species across different watersheds in southeastern of Brazil.

Resumo

Pareiorhina hyptiorhachis é descrita do ribeirão Fernandes e do rio Pomba, bacia do rio Paraíba do Sul, Brasil. A nova espécie é distinguida de suas congêneres (*P. brachyrhyncha*, *P. carrancas, P. cepta,* e *P. rudolphi*) por caracteres relacionados a presença de uma quilha conspícua na superfície pós-dorsal do tronco, dentes simples, abdomen completamente nú, perfil dorsal da cabeça arredondado, altura do sub-orbital e largura da cabeça. Nós discutido o padrão de distribuição da nova espécie e de seus congêneres e a hipótese de que a captura de cabeceira é responsável pela distribuição das espécies de *Pareiorhina* através das diferentes bacias hidrográficas do sudeste do Brasil.

Introduction

The genus *Pareiorhina* was proposed by Gosline (1947) to include *Rhinelepis rudolphi* Miranda-Ribeiro, 1911 and is currently included in the subfamily Neoplecostominae (sensu Chiachio et al. 2008; Roxo et al. 2012a, 2012b). Recently, three new species have been described: *P. carrancas* by Bockmann and Ribeiro (2003); *P. brachyrhyncha* by Chamon et al. (2005); and *P. cepta* by Roxo et al. (2012c). In their description of *P. carrancas*, Bockmann and Ribeiro (2003) proposed a combination of characters to separate *Pareiorhina* from other genera of Loricariidae: the lateral borders of the head lacking hypertrophied odontodes; unicuspid teeth; a naked abdomen; dorsal plates meeting along the mid-dorsal line between the dorsal and caudal fins; adipose fin absent; ventral plates covering the mid-ventral line behind the anal-fin base; and the dorsal portion of the body behind the dorsal fin flattened. However, no exclusive synapomorphies were presented to diagnose the genus. Recently, the molecular studies of Cramer et al. (2011) and Roxo et al. (2012a, 2012b) have found that *Pareiorhina* may not be monophyletic.

An examination of the fish collections at the Laboratório de Biologia e Genética de Peixes de Botucatu (LBP) and Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul (MCP) revealed the existence of an undescribed *Pareiorhina* species from the Rio Paraíba do Sul basin, Brazil. This new species is formally described herein.

Material and methods

All measurements were taken from point to point to the nearest 0.1 mm using digital calipers (except the postdorsal ridge depth, which was measured using a stereomicroscope and analyzed using the software Axio Vision Release 4.8.2). Counts were taken from the left side when possible. In the description, counts are followed by their frequencies in parentheses. The measurements followed Bockman and Ribeiro (2003), except for the folded dorsal-fin length and the snout-opercle length that were not included in that publication. We added the following measurements from Carvalho and Reis (2009): mandibular ramus, suborbital depth and unbranched anal-fin ray length. We also added the measurement of postdorsal ridge depth (from the base of the postdorsal ridge to its upper portion). Osteology was performed on specimens cleared and double-stained (c&s) according to the procedures of Taylor and Van Dyke (1985). The osteological and the body-plate

nomenclature followed Schaefer (1997). Vertebral counts were obtained from cleared-and-stained specimens and included the first five vertebrae modified into the Weberian apparatus. The compound caudal centrum (PU1 + U1; Lundberg and Baskin 1969) was counted as one vertebra. The pores nomenclature followed Arratia and Huaquin (1995). Asterisks in the text refer to the holotype. After collection the animals were anesthetized using 1% benzocaine in water and fixed in 10% formalin for at least two days, then transferred to 70% ethanol for permanent storage for morphological studies.

All examined material was housed at the following Brazilian institutions: LBP (Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu - SP); MCP (Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre - RS); MZUSP (Museu de Zoologia da Universidade de São Paulo, São Paulo - SP); and NUP (Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Maringá - PR).

Results

Pareiorhina hyptiorhachis, sp. n.

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http://species-id.net/wiki/Pareiorhina_hyptiorhachis
Figure 1; Table 1

"*Pareiorhina* sp. 1" - Roxo et al. 2012a:2443 [phylogenetic relationships]. - Roxo et al. 2012b:38 [phylogenetic relationships].

Holotype. MZUSP 111956, female, 33.6 mm SL, Brazil, Minas Gerais State, municipality of Santa Bárbara do Tugúrio, Ribeirão Fernandes, a tributary of Rio Pomba, Rio Paraíba do Sul basin, 21°14'47"S, 43°34'07"W, 19 Jun 2011, Ferreira AT, Roxo FF, Silva GSC.

Paratypes. Brazil, Minas Gerais State, municipality of Santa Bárbara do Tugúrio, Rio Paraíba do Sul basin. LBP 12248, 2 males, 4 females, 1 c&s, 26.6–34.8 mm SL, collected with holotype. NUP 14331, 1 female, 29.6 mm SL, collected with holotype. LBP 1093, 1 male, 33.4 mm SL, Ribeirão Fernandes, 21°14'47"S, 43°34'07"W, 12 Oct 2001, Oliveira JC, Alves AL, Sato LR. LBP 8368, 5 females, 27.9–34.4 mm SL, Rio Pomba, 21°14'07"S, 43°30'50"W, 19 May 2009, Oliveira C, Silva GJC, Roxo FF, Pereira TNA. LBP 12257, 1 female, 27.2 mm SL, Rio Pomba, 21°14'07"S, 43°30'50"W, 19 Jun 2011, Ferreira AT, Roxo FF, Silva GSC. MCP 29432, 3 male, 1 female, 2 unsexed, (1 juvenile not measured) 23.8–39.0 mm SL, Ribeirão Fernandes, 21°14'47"S, 43°34'07"W, 12 Aug 2001, Oliveira JC, Alves AL, Sato LR.

Diagnosis. *Pareiorhina hyptiorhachis* is distinguished from its congeners, except for *P. carrancas*, by the presence of a postdorsal ridge (vs. the absence of a postdorsal ridge). The new species differs from *P. carrancas* by having a more elevated postdorsal

Figure 1. *Pareiorhina hyptiorhachis*, sp. n., MZUSP 111956, 33.6 mm SL, holotype from Ribeirão Fernandes, Rio Paraíba do Sul basin, municipality of Santa Barbara do Tugúrio.

ridge, (Fig. 2; 16.7–26.8% of CP depth vs. 4.47–9.03%; table 1). Additionally, the new species can be distinguished from *P. cepta* by having a naked abdomen (vs. having small plates covered with odontodes irregularly distributed on the abdomen); from *P. brachyrhyncha* and *P. cepta* by having unicuspid teeth (vs. teeth with a minute lateral

Table 1. Morphometric data for *Pareiorhina hyptiorhachis*. SD = Standard Deviation, IO = Interorbital, OD = Orbital Diameter, CP = Caudal Peduncle.

	Pareiorhina hyptiorhachis n=21			
	Holotype	Range	Mean	SD
Standard length (SL)	33.6	26.6–38.8	31.0	3.0
Percents of Standard length (SL)				
Predorsal length	44.2	41.5–48.8	44.7	1.6
Preanal length	59.2	56.1–65.9	60.5	2.4
Head length	31.7	28.6–35.5	31.8	1.5
Cleithral width	32.8	30.4–36.9	33.5	1.8
Dorsal-fin unbranched ray length	21.2	20.3–24.1	22.2	1.1
Base of dorsal fin length	15.4	14.3–18.3	16.5	1.1
Thorax length	18.1	15.1–19.6	17.0	1.3
Pectoral-fin unbranched ray length	20.5	20.5–26.0	22.6	1.5
Abdomen length	27.0	22.6–30.1	26.2	1.6
Pelvic-fin unbranched ray length	22.5	17.7–26.6	22.9	2.1
Anal-fin length	15.3	13.7–17.8	15.6	0.9
Ventral unbranched caudal ray	24.9	20.3–30.5	25.2	2.8
Caudal-peduncle depth	9.0	8.3–11.0	9.39	0.7
Postanal length	34.9	31.6–38.1	33.9	1.4
Anal width	15.4	11.3–16.0	14.0	1.5
Percents of Head Length (HL)				
Head width	103.8	100.1–108.6	103.8	2.6
Head depth	61.7	53.5–62.8	56.9	2.3
Snout length	63.1	58.0–64.7	61.2	1.9
Interorbital width	37.7	34.8–40.7	38.0	1.4
Orbital diameter	11.4	11.1–15.5	12.7	1.6
Suborbital depth	39.2	35.0–40.5	37.3	1.6
Mandibular ramus	18.1	16.0–23.4	19.9	1.9
Other measurements (expressed as percentages)				
Anal width/cleithral width	47.1	32.0–49.7	42.0	5.1
IO/OD	29.5	21.6–42.1	33.5	4.5
IO/Mandibulary ramus	50.6	44.1–62.5	52.4	5.6
Predorsal length/first ds ray length	47.9	45.7–54.5	49.7	2.6
Postanal length/CP depth	25.8	24.8–31.1	27.6	1.9
Pelvic-fin length/CP depth	40.0	36.0–46.8	41.1	3.0
Ventral unbranched caudal ray/CP depth	36.2	32.1–46.0	37.5	4.3
Postdorsal ridge depth/CP depth	19.0	16.7–26.8	21.5	3.4

cusp); from *P. carrancas* and *P. rudolphi* by having the anterior profile of the head rounded in dorsal view (vs. elliptical; Fig. 3) and by having a greater suborbital depth (35.0–40.5% of HL vs. 27.4–34.2% in *P. carrancas* and 24.5–31.8% in *P. rudolphi*). Moreover *Pareiorhina hyptiorhachis* is distinguished from its congeners by having a wider head (100.1–108.6% of HL vs. 91.7–98.1% in *P. brachyrhyncha*, 82.9–96.2% in *P. carrancas*, 83.4–90.5% in *P. cepta* and 77.8–82.1% in *P. rudolphi*).

Description. Morphometric and meristic data are given in Table 1. In lateral view, dorsal profile of body strongly convex from snout tip to distal margin of supraoccipital;
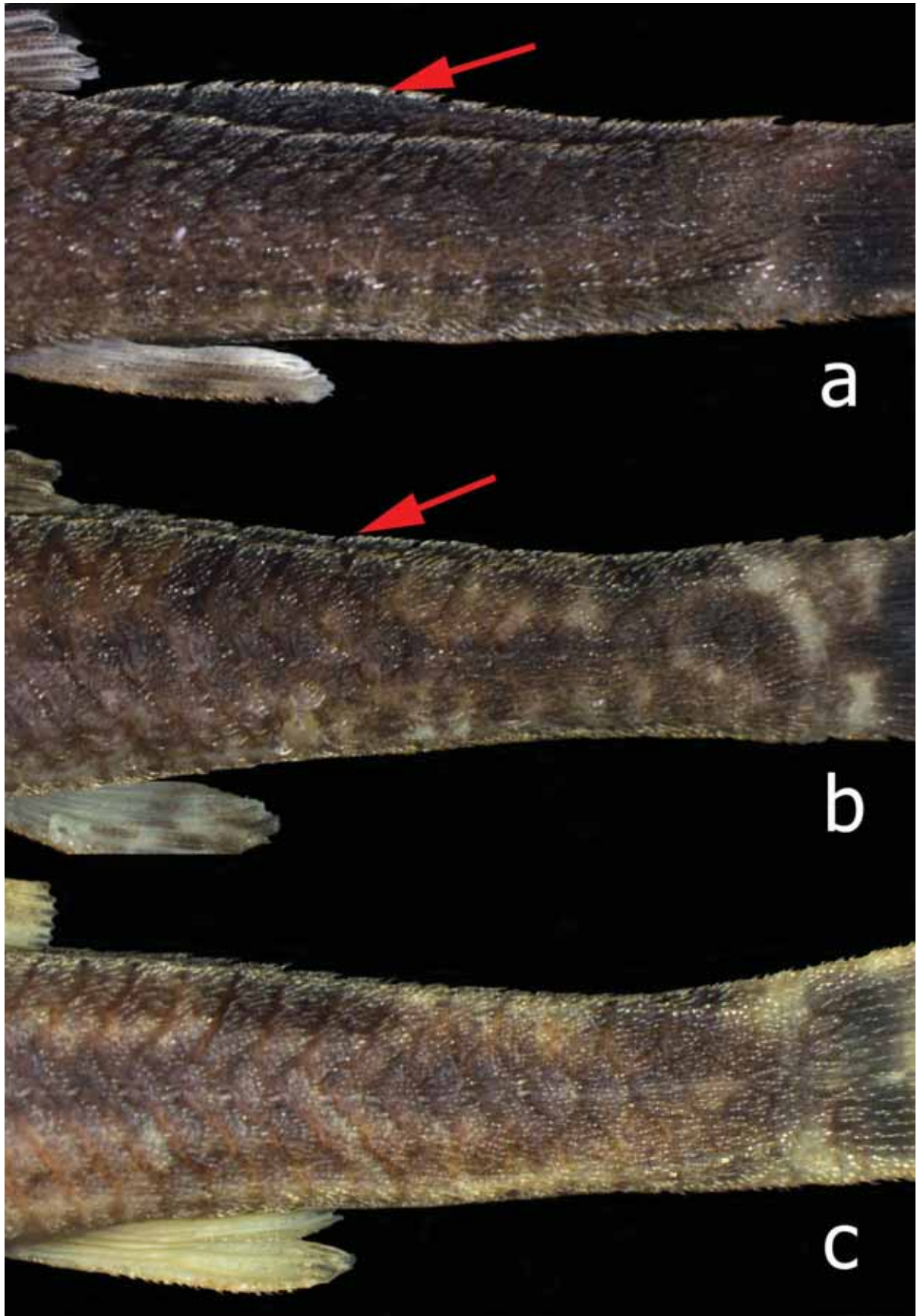
Figure 2. a *Pareiorhina hyptiorhachis*, sp. n., paratype, LBP 12248, 29.2 mm SL, showing the conspicuous postdorsal ridge **b** *Pareiorhina carrancas*, LBP 8380, 38.2 mm SL, showing the poorly-developed postdorsal ridge **c** *Pareiorhina rudolphi*, LBP 8044, 40.5 mm SL, showing the absence of a postdorsal ridge.

straight from supraoccipital to dorsal-fin origin; slightly decreasing to end of caudal peduncle. Ventral surface of body, slightly concave at head portion, straight to convex from posterior end of head to pelvic-fin insertion, and straight but angled to posterior end of 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. Head flat to slightly convex between orbits; superior margin of orbits elevated. Eye small, orbital diameter 11.1–15.5% of HL, situated dorsolaterally just posterior of midpoint of head. Rostral margin of snout with minute, posteriorly-directed odontodes; numerous small odontodes on dorsal portion of head. Opening of swimbladder capsule small. Perforations of compound pterotic distributed on whole bone, greater and more concentrated on its ventral margin; its openings nearly rounded in median region, and irregular along inferior and posterior margins of bone. Lips large; oral disk rounded, papillose; premaxillary teeth 22 (1), 29 (1), 30 (1), 32 (1), 33 (1), 34 (2), 36 (1)*, 37 (2), 38 (1), 39 (2), 40 (2), 42 (2) or 44 (1). Dentary teeth 17 (1), 21 (1), 23 (1), 28 (1), 30 (2), 32 (2)*, 33 (2), 34 (2), 35 (1), 36 (1), 39 (1), or 45 (1). Teeth unicuspid. Maxillary barbel short and free distally.

Dorsal-fin rays ii,7; dorsal-fin originating at vertical through posterior end of pelvic-fin base; distal margin slightly convex. Pectoral-fin rays i,6; distal margin slightly convex; unbranched pectoral-fin ray reaching middle of unbranched pelvic-fin ray; unbranched pectoral-fin ray covered with large and pointed odontodes. 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 well-developed, and uniformly distributed odontodes which are larger at ventral portion. Anal-fin rays i,5; distal margin slightly convex. Caudal fin rays i,7-7,i. Adipose fin absent. Caudal fin truncated with ventral unbranched principal ray longer than dorsal ray.

Body entirely covered by bony plates, except for ventral surface of head, abdomen and region overlaying swimbladder capsule. Dorsal series of plates 24–26, mid-dorsal 17–21, median perforated plates 24–26, mid-ventral 17–22, and ventral 19–22. Trunk with conspicuous, elongated, postdorsal ridge formed by 13–15 raised, unpaired, median plates; ridge continuous posteriorly with procurrent caudal-fin rays. Head lacking crest. Head and body plates covered with minute, uniformly sized and distributed odontodes. Seven pairs of ribs associated with vertebrae 8–14. Ribs slender and poorly ossified. Total vertebrae 29.

Supraorbital sensory canal with four pores; pore s1 located on prenasal plate below nasal plate; pore s3 located on posterior portion of nasal; pore s6+s6 located between frontal plates, on horizontal line through anterior limits of eye; pore s8 located on division between frontals, sphenotic and supraoccipital plates, just above eye. Infraorbital sensory canals with six pores; pore io1 located on anterior portion of first infraorbital; pore io2 located in medial region between first and second infraorbitals; pore io3 located in medial region between second and third infraorbitals; pore io4 located in medial region between third and fourth infraorbitals; pore io5 located in medial region between fourth and fifth infraorbitals and pore io6 located between sixth infraorbital and sphe-
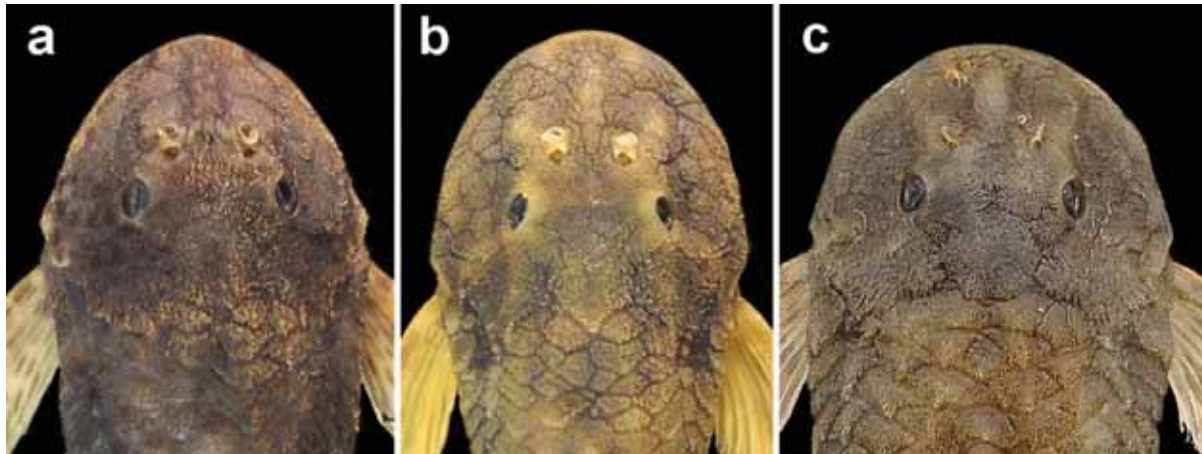
Figure 3. a *Pareiorhina carrancas*, LBP 8380, 36.5 mm SL, showing the elliptical anterior profile of the head elliptical in dorsal view **b** *Pareiorhina rudolphi*, LBP 8044, 42.0 mm SL, showing the elliptical anterior profile of the head in dorsal view **c** *Pareiorhina hyptiorhachis*, new species, holotype, MZUSP 111956, 33.6 mm SL, showing the rounded anterior profile of the head in dorsal view.

Figure 4. Additional coloration pattern of coloration of *Pareiorhina hyptiorhachis*, sp. n., LBP 12257, female, 27.2 mm SL.

notic. Preopercular canal with three pores; pore pm2 located on ventral portion of cheek plate, pore pm3 located between cheek plate and preopercle; pore pm4 located between preopercle and compound pterotic. Two postotic pores; pore po2 located just above of branchial slit; pore po3 located in region of overlying opening of swim-bladder capsule.

Color in alcohol. Two body-coloration patterns observed. First pattern (Fig. 1): Ground color of dorsal surface of head and body yellowish brown. Ventral surface of body and head lighter than dorsal with dark spots of melanophores widely separated. Three dark saddles on dorsal surface of trunk (in some specimens not present), most anterior one inconspicuous. 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. Second pattern (Fig. 4): Ground color of body uniformly dark except, ventral portion of body mostly clear; Fins with inconspicuous, irregularly defined bands: one in anal fin, two in pectoral and pelvic fins. Dorsal and caudal fins entirely dark.

Sexual dimorphism. Males with a papilla at urogenital opening and fewer teeth in premaxillary 22–39 (*vs*. 32–44 females) and 17–32 dentary (*vs*. 30–45 females).

Figure 5. The Rio Paraíba do Sul basin indicating the type locality of *Pareiorhina hyptiorhachis* in Ribeirão Fernandes, a tributary of Rio Pomba, Rio Paraíba do Sul basin, 21°14'47"S, 43°34'07"W.

Etymology. The specific name, *hyptiorhachis* is a combination of Greek, *hyptios* = supine, lying on the back, and *rhachis* = ridge, midrib, and is in reference to the conspicuous postdorsal ridge found in this species.

Distribution and habitat. *Pareiorhina hyptiorhachis* is known from Rio Pomba and one of its tributaries, the Ribeirão Fernandes, in the municipality of Santa Barbara do Tugúrio, Minas Gerais State, Brazil (Fig. 5). This species inhabits moderate to fast-flowing streams, with a substrate of rocks and sand and margins covered by aquatic vegetation. Specimens were collected in association with loose stones, on the streambed. The new species is syntopic throughout its distribution with *Astyanax* sp., *Characidium* sp., *Geophagus brasiliensis*, *Harttia* cf. *carvalhoi*, *Imparfinis* sp., *Neoplecostomus microps*, *Trichomycterus* cf. *alternatus*, and *Trichomycterus* sp.

Discussion

Bockmann and Ribeiro (2003) proposed seven characters to diagnose *Pareiorhina*. The new species described herein, *P. hyptiorhachis*, possesses all of these characters. On the other hand, *Pareiorhina* did not form a monophyletic group in the molecular analysis of Roxo et al. (2012a, 2012b); in that analysis, *P. hyptiorhachis*, cited as *Pareiorhina* sp. 1, appeared as the sister group of *P. carrancas*, and these two species formed the sister group of *Neoplecostomus*. Furthermore, *P. rudolphi*, the type species of *Pareiorhina* was the sister group of *Pseudotocinclus*. Considering that *P. hyptiorhachis* exhibits all of the characters listed by Bockmann and Ribeiro (2003) for *Pareiorhina*, the molecular data conflict with the available morphological data for Neoplecostominae, and new morphological studies in Neoplecostominae are in progress (Edson Henrique Lopes Pereira, pers. comm.), we prefer to include *P.*

hyptiorhachis in *Pareiorhina* rather than in another Neoplecostominae genus or in a new genus.

Pareiorhina hyptiorhachis is similar to *P. carrancas* from the upper Rio Paraná basin. The two species share unicuspid teeth and the presence of a postdorsal ridge of unpaired plates, although the postdorsal ridge is better developed in *P. hyptiorhachis* (all female and male samples) (Fig. 2). Moreover, the new species has more raised median unpaired plates in the postdorsal ridge (13–15 vs. 10–13 in *P. carrancas*). The close relationship between *P. hyptiorhachis* and *P. carrancas* suggested by the molecular data of Roxo et al. (2012b) is thus at least superficially supported by morphology.

Pareiorhina is distributed across three hydrographic basins, with *P. rudolphi*, *P. brachyrhyncha* and *P. hyptiorhachis* from the Rio Paraíba do Sul basin; *P. carrancas* from the upper Rio Paraná basin; and *P. cepta* from the Rio São Francisco basin. Ribeiro et al. (2006) suggested that the activation of old faults in southeastern Brazil during the Miocene and Pliocene resulted in several headwater captures between adjacent drainages of the São Francisco, upper Paraná and Coastal rivers. Roxo et al. (2012a) suggested that the lineage that gave rise to *P. carrancas* and *P. hyptiorhachis* was from the upper Rio Paraná basin and that *P. hyptiorhachis* reached the Rio Paraíba do Sul basin about 6.2 (2.3–11.2) million years ago, probably through headwater captures between the upper Paraná and several coastal drainages (Rio Paraíba do Sul and Ribeira do Iguape basin) during the late Miocene. Chamon et al. (2005) suggested that the evolutionary history of *P. rudolphi* and *P. brachyrhyncha* was linked to Pleistocene and pre-Pleistocene climatic fluctuations that may have temporarily isolated hillside streams at or near the headwaters of the Ribeirão Grande, producing the events that subsequently led to the sympatry of *P. brachyrhyncha* and *P. rudolphi*. However, as suggested by Crammer et al. (2008, 2011), Chiachio et al. (2008) and by Roxo et al. (2012a, 2012b), *P. brachyrhyncha* and *P. rudolphi* do not share an exclusive most recent common ancestor, which negates the hypothesis of Chamon et al. (2005). Additionally, Roxo et al. (2012a) suggested that the origin of the lineages that gave rise to the species of *Pareiorhina* were much older, originating in the Miocene [17.87 (8.24–28.42) million years ago for *Pareiorhina rudolphi* and 6.27 (2.33–11.21) million years ago for *Pareiorhina carrancas* plus *P. hyptiorhachis* (*Pareiorhina* sp. 1 in Roxo et al. 2012a)].

Comparative material

Isbrueckerichthys alipionis: LBP 7373, 17, 31.7–81.6 mm SL, municipality of Iporanga, SP, Rio Ribeira de Iguape basin; LBP 2660, 1, 55.1 mm SL, municipality of Iporanga, SP, Rio Ribeira de Iguape basin. *Kronichthys subteres*: LBP 515, 31, 28.4–61.9 mm SL, municipality of Iporanga, SP, Rio Ribeira de Iguape basin. *Neoplecostomus microps*: LBP 8036, 38, 41.3–65.0 mm SL, municipality of Piquete, SP, Rio Paraíba do Sul basin. *Neoplecostomus franciscoensis*: LBP 6489, 50, 42.8–55.9 mm SL, municipality of São Bartolomeu, MG, Rio São Francisco basin. *Neoplecostomus paranensis*: holotype, MZUSP 38572, 71.4 mm SL, municipality of Cajuru, MG, Rio Grande

basin. *Pareiorhaphis splendens*: LBP 1117, 20, 32.0–100.0 mm SL, municipality of Morretes, PR, Coastal Drainage. *Pareiorhaphis steindachneri*: LBP 739, 6, 33.8–49.0 mm SL, municipality of Jaraguá do Sul, SC, Coastal Drainage. *Pareiorhina brachyrhyncha*: LBP 12240, 50, 26.4–36.9 mm SL, municipality of Pindamonhangaba, SP, Rio Paraíba do Sul basin. *Pareiorhina carrancas*: LBP 8380, 24, 21.3–38.2 mm SL, municipality of Carrancas, MG, Rio Grande basin. *Pareiorhina cepta:* holotype, MZUSP 111095, 41.5 mm SL, municipality of São Roque de Minas, MG, Rio São Francisco basin, paratypes, LBP 10261, 1, 30.2 mm SL, municipality of São Roque de Minas, MG, Rio Paraíba do Sul basin, LBP 10287, 13, 21.5–43.6 mm SL, municipality of São Roque de Minas, MG, Rio Paraíba do Sul basin, LBP 11835, 19, 25.1–44.0 mm SL, municipality of São Roque de Minas, MG, Rio Paraíba do Sul basin. *Pareiorhina rudolphi*: LBP 8044, 18, 31.7–48.9 mm SL, municipality of Piquete, SP, Rio Paraíba do Sul basin. *Pseudotocinclus juquiae*: LBP1081, 2, 29.0–31.9 mm SL, municipality of Juquitiba, SP, Coastal Drainage. *Pseudotocinclus tietensis*: LBP 2931, 3, 38.6–62.3 mm SL, municipality of Salesópolis, SP, Rio Tietê basin.

Acknowledgements

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