UNIVERSIDADE ESTADUAL PAULISTA

INSTITUTO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: GENÉTICA

Fábio Fernandes Roxo

Análise das relações entre gêneros da subfamília Neoplecostominae (Siluriformes: Loricariidae) com base em seqüências de DNA

ORIENTADOR: Prof. Dr. Claudio de Oliveira

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Dissertação apresentada em formato de artigo ao Programa de Pós-Graduação em Ciências Biológicas: Genética, para obtenção do título de Mestre.

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SUMÁRIO

Agradecimentos..... 4

Capítulo I – Molecular systematic and biogeography of the armored neotropical catfish Neoplecostominae (Siluriformes: Loricariidae)…… . 6

Capítulo II – Three new species of the loricariid genus *Neoplecostomus* (Teleostei: Siluriformes) from upper rio Paraná basin, southeastern Brazil…… 37

Anexos.....63

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“Se você aceitar que as características humanas são variáveis, que essa variação é hereditária, e que há uma luta pela existência, então a evolução pela seleção natural prosseguirá.”

Charles Darwin

Capítulo 1

Molecular systematic and biogeography of the armored neotropical catfish Neoplecostominae (Siluriformes: Loricariidae)

Fábio Fernandes Roxo[1]

[1]*Universidade Estadual Paulista, UNESP, Departamento de Morfologia, Laboratório de Biologia e Genética de Peixes, Botucatu, SP, Brazil.*

Key words: *Neoplecostomus*, mitochondrial DNA, nuclear gene, phylogenetic analysis

Resumo

Estudos morfológicos e moleculares direcionados a indivíduos da família Loricariidae, popularmente conhecidos com cascudos, têm revelado incertezas nos padrões de relacionamentos de algumas de suas subfamílias. Baseado nestes questionamentos foi realizado uma análise filogenética incluindo representantes de todos os gêneros da subfamília Neoplecostominae. Tal análise, baseada em Máxima Parcimônia, Análise Bayesiana e Análise de Distancia Genética (Neighbor-joining) foi executada em uma matriz de 4676 caracteres com sequências parciais dos genes COI, CytB, 16S rRNA, 12S rRNA e F-4 Reticulon. Nesta matriz 1155 caracteres apresentaram-se informativos nas análises de parcimônia. Como grupos externos foram utilizadas as espécies *Hemipsilichthys gobio* e *Hemipsilichthys papilatus*, subfamília Delturinae, amostras de *Hypostomus nigromaculatus* (subfamília Hypostominae), *Hypoptopoma inexpectatum* (subfamília Hypoptopomatinae) e *Corumbataia cuestae* (subfamília Otothyrinae). Segundo proposta recente de relações na família Loricariidae o gênero *Pseudotocinclus* foi incluído nas análises. Os resultados mostraram que a subfamília Neoplecostominae é monofilética, com sua atual composição, incluindo *Pseudotocinclus*. Três subgrupos foram reconhecidos no grupo interno. O primeiro é formado pelas espécies *Pareiorhina carrancas*, uma nova espécie de *Pareiorhina* (*Pareiorhina* sp. 1), um novo gênero de Neoplecostominae e as espécies do gênero *Neoplecostomus* exceto *Neoplecostomus ribeirensis*. O segundo pelos gêneros

Kronichthys, *Isbrueckerichthys*, *Pareiorhaphis* e *Neoplecostomus ribeirensis*, sendo o gênero *Kronichthys* irmão de *Pareiorhaphis* e estes dois, irmãos de *Isbrueckerichthys* mais *Neoplecostomus ribeirensis*. O terceiro grupo mais basal a Neoplecostominae é formado pelas espécies *Pareiorhina rudolphi*, *Pareiorhina* sp. 2, *Pseudotocinclus juquiae* e *Pseudotocinclus tietensis*. Assim, os gêneros *Pareiorhina* e *Neoplecostomus* não apareceram como monofiléticos, e alguns caracteres morfológicos levantados no presente trabalho justificam essas relações. Inferências biogeográficas também foram propostas para justificar as relações internas na subfamília Neoplecostominae.

Abstract

Morphological and molecular studies of samples of the family Loricariidae revealed that the relationship between it members are not well resolved. Based on this fact, in the present work we realized an analysis including samples of all genera of the subfamily Neoplecostominae. The analysis based in Maxima Parsimonious, Bayesian Analysis and Genetic Distance (Neighbor-joining) in a matrix of 4676 characters with partial sequences of the genes COI, CytB, 16S rRNA, 12S rRNA and F-4 Reticulon. In this matrix 1155 characters was parsimonious informative. We used as outgroups samples of the species *Hemipsilichthys gobio* and *Hemipsilichthys papilatus* (subfamily Delturinae), *Hypostomus nigromaculatus* (subfamily Hypostominae), *Hypoptopoma inexpectatum* (subfamily Hypoptopomatinae), and *Corumbataia cuestae* (subfamily Otothyrinae). Following recent results about the relationship of the family Loricariidae the genus *Pseudotocinclus* was included in the present analysis. The results showed that the subfamily Neoplecostominae is monophyletic, including *Pseudotocinclus*. Three subgroups were recognized. The first one is composed by the species *Pareiorhina carrancas*, *Pareiorhina* sp. 1 (new species), a new genus of Neoplecostominae and all species of *Neoplecostomus* genus except *Neoplecostomus ribeirensis*. The second group is composed by *Kronichthys*, *Isbrueckerichthys* and *Pareiorhaphis* genera and the species *Neoplecostomus ribeirensis*. *Kronichthys* formed sister group with *Pareiorhaphis* and these two formed sister group with *Isbrueckerichthys* plus *Neoplecostomus ribeirensis*. The third group is the most basal found in the subfamily Neoplecostominae, composed by the species *Pareiorhina rudolphi*, *Pareiorhina* sp. 2, *Pseudotocinclus juquiae* and *Pseudotocinclus tietensis*. Thus, the genera *Pareiorhina* and *Neoplecostomus* do not appeared monophyletic, and we found some morphological

characters that support these relationships. Biogeography inferences were proposed to justify the relationships in the subfamily Neoplecostominae.

Introduction

Loricariidae, a Neotropical endemic freshwater fish family, is the largest catfish family with 716 species and 96 genera (Ferraris Jr. 2007). Phylogenetic hypothesis of relationships based on morphological data of loricariid species were proposed by Howes (1983), Schaefer (1987), and Armbruster (2004). The great diversity of this group could be explained mainly through the biogeographic history of the South America. This continent can be divided into different crustal provinces: the elevated areas such the stable South American Platform, the Patagonian massif, the Andean orogenic belt and low areas like the foreland basins (Ribeiro 2006b). All these orogenic processes created severed specific environments that made speciation possible and created this great diversity of fish species.

Schaefer (1987) recognized six subfamilies in Loricariidae: Lithogeneinae, Neoplecostominae, Hypoptopomatinae, Loricariinae, Ancistrinae, and Hypostominae. Armbruster (2004), in a broad study of loricariids, mainly focused in Hypostominae and Ancistrinae found that this latter subfamily is actually part of Hypostominae and proposed its classification as the tribe Ancistrini. In this same study, other four tribes of Hypostominae were recognized: Hypostomini, Corymbophanini, Pterygoplichthini, and Rhinelepini. A sixth subfamily, Delturinae, was recently described by Reis et al. (2006). This subfamily has two genera, *Delturus* and *Hemipsilichthys* and is the sister group of all loricariids except Lithogeneinae, as early proposed by Montoya-Burgos et al. (1998) based on molecular data. Chiachio et al. (2008) proposed a seventh subfamily, Otothyrinae, erecting the former tribe Otothyrini, and keeping Hypoptopomatinae as composed only by members of the former tribe Hypoptopomatini.

The subfamily Neoplecostominae is composed of small-sized species. Its representatives are restricted to the southern and southeastern regions of Brazil, and are found in small to medium-sized streams with clear, shallow water (commonly less than 1m deep), moderate to strong currents, and loose stones and, sometimes, sand as bottom substrate (Langeani 1990; Pereira & Reis 2002). Until recently, *Neoplecostomus* was the only genus in Neoplecostominae (Ferraris Jr. 2003). Gosline (1947) was the first to propose Neoplecostominae as a large group, including *Corymbophanes*, *Delturus*,

Hemipsilichthys, *Kronichthys*, *Neoplecostomus*, *Pareiorhaphis*, *Pareiorhina*, *Pogonopoma*, *Pogonopomoides*, *Pseudorinelepis*, *Rhinelepis*, and *Upsilodus*. Among these genera, *Pogonopoma*, *Pogonopomoides*, *Pseudorinelepis*, and *Rhinelepis* do not share any apomorphic character with other Neoplecostominae genera (Armbruster 1998, 2004), and recently, these genera were proposed to belong to a new tribe, Rhinelepini, inside Hypostominae (Armbruster 2004). In the same study, *Corymbophanes* was placed in the tribe Corymbophanini, the sister group of all other Hypostominae (Armbruster 2004). Finally, *Delturus* and *Hemipsilichthys* (including *Upsilodus* as a junior synonym of *Hemipsilichthys*) constitute the new subfamily Delturinae (Reis et al. 2006). Thus, until 2007 Neoplecostominae was composed of *Isbrueckerichthys*, *Pareiorhaphis*, *Kronichthys*, *Neoplecostomus,* and *Pareiorhina* (Ferraris Jr. 2007). The first molecular study of these last five genera showed that they belong to a monophyletic assemblage and suggested that *Pseudotocinclus* (formerly classified in the subfamily Hypoptopomatinae) could also belong to the same group (Montoya-Burgos et al. 1998). This hypothesis was corroborated by Chiachio et al. (2008) that moved *Pseudotocinclus* to the subfamily Neoplecostominae.

Until several years ago, species of *Neoplecostomus* were basically classified according to their hydrographic basin distribution. In the Rio Paraná Basin, for example, only one nominal species, *Neoplecostomus paranensis*, existed (Langeani 1990). New studies based on external morphology and molecular biology identified several new species, mainly in the upper Rio Paraná Basin (Zawadzki et al. 2004, 2008).

Almost all the information available about the biogeography of Neotropical freshwater fishes comes from phylogenetic data derived from revisionary studies of particular groups (Ribeiro et al. 2006). This happens mainly because “the difficulties of biologists in understanding and synthesizing geological processes constitute a serious limitation to our knowledge of the biogeography of Neotropical freshwater fishes” (Ribeiro et al. 2006). Fortunately in recent years several studies have increased the amount of information on the family Loricariidae. Chiachio et al. (2008) provided biogeographic inference about the subfamilies Neoplecostominae, Otothyrinae and Hypoptopomatinae and tested two Dispersal-Extinction-Cladogenesis models for inferring the distribution range evolution of the new subfamilies. This last work found Neoplecostominae (including the genus *Pseudotocinclus* sister group of *Pareiorhina*) monophyletic and forming sister group to the new subfamily Otothyrinae. The genus *Pareiorhina* plus *Pseudotocinclus* formed sister group to (*Neoplecostomus*,

(*Isbrueckerichthys*, *Pareiorhaphis*)). Using morphological characters Pereira (2008), in an unpublished Ph.D. thesis, also analyzed most of the species in subfamily Neoplecostominae. In this work, the genera *Pareiorhaphis*, *Isbrueckerichthys*, *Pareiorhina*, *Kronichthys*, *Isbrueckerichthys* and *Neoplecostomus* are monophyletic. The genera *Kronichthys* and *Pareiorhina* also are monophyletic but were closer related with the subfamily Hypoptopomatinae. Based in his data Pereira (2008) suggested the transference of the last two genera to Hypoptopomatinae.

Considering the observations above the evolutionary relationship, the historical biogeography, and the ecological characteristics in the subfamily Neoplecostominae and the underlying forces which have driven the huge diversification in the family Loricariidae and specifically in the subfamily Neoplecostominae are still poorly known. In this study, an overview about the biogeography and the systematics of the subfamily Neoplecostominae is provided to clarify the relationship and the geographic distribution of the subfamily Neoplecostominae in southeast Brazil.

Material and methods

Specimens and DNA sequencing

The sequenced samples of Neoplecostominae (ingroup) and samples of the subfamilies Delturinae, Hypoptopomatinae, Otothyrinae and Hypostominae (outgroups) are listed in Table 1. Almost all sequences were submitted to GeneBank, and the sequences not submitted will be sending soon. *Hemipsilichthys gobio* and *Hemipsilichthys papilatus* were used to rooted the trees following their basal position among Loricariidae (Montoya-Burgos et al. 1998; Reis et al. 2006). Vouchers of all samples sequenced are deposited at the Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brasil, at the Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura (NUP), Universidade Estadual de Maringá, Paraná, Brasil, or at the Museum of Natural History of the City of Geneva (MHNG), Genebra, Switzerland. The geographic distribution of the samples is present in figure 1.

Total DNA was extracted from ethanol preserved muscle, fin and liver samples with the protocol described by Aljanabi and Martinez (1997) and the Wizard Genomic DNA Purification Kit (Promega). Partial sequences of the genes 16S rRNA (700 pb),

12S rRNA (900 pb), cytochrome c oxidase subunit I (COI, 700 pb), cytochrome b (Cytb, 900 pb) and F-4 reticulon (1900 pb) were amplified by polymerase chain reaction (PCR) with the primers described in the Table 2. Primer concentration was 5 pmol/µl. Mitochodrion genes were amplified with a total volume of 25 µl for 35 cycles (30s at 95°C, 45s at 48-54°C, and 80s at 72°C). The nuclear gene was amplified in two polymerase chain reaction. The first one using the primers Freticul4-D and Freticul4-R with a total volume of 12,5 µl for 37-40 cycles (30s at 95°C, 30s at 48°C, and 135s at 72°C). The PCR was amplified using the commercial kit Gotaq (Promega). After that the products were identified on a 1% agarose gel. The second one using the primers Freticul4 D2, Freticul4 R2 and Freticul4 iR with a total volume of 12,5 µl for 37-40 cycles (30s at 95°C, 30s at 53-54°C, and 135s at 72°C). The PCR products were purified using ExoSap-IT® (USB Corporation) following instructions of the manufacturer. The purified PCR products were used to make a sequencing PCR using the Kit "Big DyeTM Terminator v 3.1 Cycle Sequencing Ready Reaction" (Applied Biosystems). After that, the amplified DNA was purified again and loaded on an automatic sequencer 3130-Genetic Analyzer (Applied Biosystems) presented in Laboratório de Biologia e Genética de Peixes.

Table 1. Species included in the present study. LBP = Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista. NUP = Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura, Universidade Estadual de Maringá. MHNG = Museum of Natural History of the City of Geneva.

Collection No.	Fish No.	Genbank (16S/COI/CytB/12S/Reticulon)	Especies	Locality (river, city, state, hydrographic Basin)	*Geographic Position*
LBP2732	17441	FJ434509/FJ434532/FJ965514/FJ965470	*Neoplecostomus paranensis*	Córrego Mocoquinha/Cajuru/SP/Rio Grande Basin	S 21°19'37" W 47°14'19"
LBP2732	17444	FJ965495/FJ965505/FJ965515/FJ965471	*Neoplecostomus paranensis*	Córrego Mocoquinha/Cajuru/SP/Rio Grande Basin	S 21°19'37" W 47°14'19"
LBP6544	32377	Sequences not submitted	*Neoplecostomus* sp. 1	Córrego Sem Nome/Ouro Fino/MG/Rio Grande Basin	S 22°11'36" W 46°22'44"
LBP7469	33440	Sequences not submitted	*Neoplecostomus* sp. 2	Córrego Espraiado/Borda da Mata/MG/Rio Grande Basin	Not available
LBP7462	33403	Sequences not submitted	*Neoplecostomus* sp. 3	Córrego Monjolinho/S. Bto Sapucaí/MG/Rio Grande Basin	S 22°42'24" W 45°42'57"
LBP6334	29832	Sequences not submitted	*Neoplecostomus* sp. 4	Afluente do Rio Sapucaí/Campos do Jordão/SP/Rio Grande Basin	S 22°42'24" W 45°42'57"
LBP2861	18616	Sequences not submitted	*Neoplecostomus* sp. 5	Paraitinguinha/Salesópolis/SP/Rio Tietê Basin	S 22°42'24" W 45°42'57"
LBP7467	33428	Sequences not submitted	*Neoplecostomus* sp. 6	Casca D'antas/Rio Jaguarí/Rio Tietê/Rio Grande Basin	S 22°46'08" W 45°59'25"
LBP4959	10248	Sequences not submitted	*Neoplecostomus* sp. 7	Córrego do Sapateiro/Barbacena/MG/Rio Grande Basin	S 21°16'26" W 43°38'37"
LBP7466	33421	Sequences not submitted	*Neoplecostomus* sp. 8	Córrego Tamborete/Furnas/MG/Rio Grande Basin	S 20°38'54" W 46°09'52"
LBP5901	27990	Sequences not submitted	*Neoplecostomus* sp. 9	Córrego São Domingos/Muzambinho/MG/Rio Grande Basin	S 21°17'37" W 46°29'06"
LBP6426	29991	Sequences not submitted	*Neoplecostomus* sp. 10	Rio Mourão/Campo Mourão/PR/Rio Tibagi Basin	S 24°06'16" W 52°19'31"
LBP1096	10268	Sequences not submitted	*Neoplecostomus* sp. 11	Rio Chopotó/Desterro de Melo/MG/Rio Doce Basin	S 21°08'56" W 43°23'58"
LBP709	6049	Sequences not submitted	*Neoplecostomus* sp. 12	Córrego Hortelã/Botucatu/SP/Rio Paranapanema Basin	S 22°55'00" W 48°30'00"
LBP7525	34832	Sequences not submitted	*Neoplecostomus* sp. 12	Cachoeira Véu da Noiva/Botucatu/SP/Rio Paranapanema Basin	S 22°59'25" W 48°25'37"
NUP3560	9701	FJ434506/FJ434529/FJ965516/FJ965472	*Neoplecostomus yapo*	Rio Tibagi/Fortaleza/PR/Rio Paranapanema Basin	S 24°25'30" W 50°13'55"
LBP645	7593	FJ434508/FJ434531/FJ965518/FJ965484	*Neoplecostomus microps*	Ribeirão Cajarana/Pindamonhangaba/SP/Rio Paraíba do Sul Basin	S 22°46'00" W 45°27'00"
LBP894	9735	Sequences not submitted	*Neoplecostomus ribeirensis*	Rio Iporanga/Iporanga/SP/Rio Ribeira do Iguape Basin	Not available
LBP7384	34837	Sequences not submitted	*Neoplecostomus ribeirensis*	Rio Água Doce/Tapiraí/SP/Rio Ribeira do Iguape Basin	S 22°27'02" W 49°14'26"
LBP2551	15243	FJ434507/FJ434530/ FJ965512/FJ965485	*Neoplecostomus espiritosantensis*	Rio Jucu/Domingos Martins/ES/oriental coastal Basin	S 22°49'51" W 44°51'53"
NUP2528	9423	FJ434520/FJ434543/FJ965513/FJ965469	*Neoplecostomus corumba*	Rio Corumbá/GO/Rio Paranaíba Basin/upper Rio Paraná Basin	S 17°43'37" W 48°32'54"
LBP6537	31681	FJ965494/FJ965503/FJ965519/FJ965483	*Neoplecostomus franciscoensis*	Rio das Velhas/Brumadinho/MG/Rio São Francisco Basin	S 20°00'37" W 43°58'08"
LBP7383	34843	FJ965496/FJ965506/FJ965517/FJ965473	*Neoplecostomus selenae*	Ribeirão das Batéias/Riacho Grande/SP/Rio Paranapanema Basin	S 24°12'02" W 48°25'06"
LBP7472	32387	Sequences not submitted	New genus	Córrego Guarda Mor/Guarda Mor/MG/Rio São Francisco Basin	S 17°46'18" W 47°05'43"
LBP902	7989	FJ434514/FJ434537/FJ965532/FJ965480	*Pareiorhaphis steindachneri*	Rio Itapucu/Jaraguá do Sul/SC/oriental coastal Basin	S 26°26'48" W 49°09'54"
LBP1161	8935	FJ434512/FJ434535/FJ965530/FJ965479	*Pareiorhaphis vestigipinnis*	Rio Caveiras/Painel/SC/Rio Uruguai Basin	S 27°55'12" W 50°06'25"
LBP701	7363	FJ434513/FJ434536/FJ965531/FJ965482	*Pareiorhaphis hystrix*	Rio Tainhas/Tainhas/RS/oriental coastal Basin	S 29°24'06" W 50°27'01"
LBP748	8257	FJ625811/FJ625820/FJ965524/FJ965481	*Pareiorhaphis splendens*	Rio São João/Guaruva/PR/oriental coastal Basin	S 25°58'39" W 48°52'59"
LBP7373	34853	FJ965490/FJ965506/FJ965525/FJ965477	*Isbrueckerichthys alipionis*	Rio Betari/Iporanga/SP/Rio Ribeira do Iguape Basin	S 24°33'42" W 48°40'05"
LBP6427	29996	FJ965488/FJ965497/FJ965527/FJ965476	*Isbrueckerichthys* sp. 1	Rio Charqueada/Campo Mourão/PR/Rio Paranapanema Basin	Not available
LBP7385	34852	FJ965491/FJ965502/FJ965526/FJ965478	*Isbrueckerichthys epaknos*	Rio Água Doce/Tapiraí/SP/Rio Ribeira do Iguape Basin	S 22°27'02" W 49°14'26"
LBP6389	29765	FJ965489/FJ965503/FJ965529/FJ965474	*Isbrueckerichthys cf. calvus*	Rio Taquará/California/PR/Rio Paranapanema Basin	S 23°40'55" W 51°18'55"
LBP2650	17402	FJ625812/FJ625821/FJ965528/FJ965475	*Isbrueckerichthys duseni*	Rio Pulador/Campinhos/PR/Rio Ribeira de Iguape Basin	S 25°02'47" W 49°05'34"
LBP1766	12886	FJ965493/FJ965501/FJ965521/FJ965466	*Kronichthys* sp. 1	Rio Sítio do Meio/Mongaguá/SP/Oriental Costal Basin	S 24°05'11" W 46°43'59"
LBP795	8304	FJ434503/FJ434526/FJ965522/FJ965463	*Kronichthys lacerta*	Rio Marumbi/Morretes/PR/oriental coastal Basin	S 25°29'12" W 48°49'58"
LBP2122	15096	FJ434502/FJ434525/FJ965520/FJ965464	*Kronichthys heylandi*	Rio Parati-Mirim/Parati/RJ/oriental coastal Basin	S 25°29'12" W 48°49'58"
LBP515	6334	FJ965492/FJ965500/FJ965523/FJ965465	*Kronichthys subteres*	Rio Betari/Iporanga/SP/Rio Ribeira do Iguape Basin	S 24°33'44" W 48°40'10"
LBP4391	24189	FJ434517/FJ434540/FJ965510/FJ965462	*Pareiorhina* sp. 2	Ribeirão Guaxinduva/Jundiaí/SP/Rio Tietê Basin	S 23°15'00" W 46°58'00"
LBP8368	37559	Sequences not submitted	*Pareiorhina carrancas*	Córrego Beijinho/Carrancas/MG/Rio Grande Basin	S 21°26'39" W 44°36'08"
LBP8368	37560	Sequences not submitted	*Pareiorhina carrancas*	Córrego Beijinho/Carrancas/MG/Rio Grande Basin	S 21°26'39" W 44°36'08"
LBP1087	10256	Sequences not submitted	*Pareiorhina cf. carrancas*	Córrego do Sapateiro/Barbacena/MG/Rio Grande Basin	S 21°16'26" W 43°38'36"
LBP8380	37564	Sequences not submitted	*Pareiorhina* sp. 1	Rio Pomba/Santa Barbara do Tugúrio/MG/Rio Paraíba do Sul Basin	S 21°14'07" W 43°30'50"
LBP8380	37565	Sequences not submitted	*Pareiorhina* sp. 1	Rio Pomba/Santa Barbara do Tugúrio/MG/Rio Paraíba do Sul Basin	S 21°14'07" W 43°30'50"
LBP8044	37775	Sequences not submitted	*Pareiorhina rudolphi*	Ribeirão Piquete/Piquete/SP/Rio Paraíba do Sul Basin	S 22°35'00" W 45°10'08"
LBP8347	37571	Sequences not submitted	New genus	Rio Piçarrão/Ferros/MG/Rio Doce Basin	S 19°40'53" W 43°00'50"
LBP616	7564	FJ625810/FJ625819/FJ965511/FJ965461	*Pseudotocinclus juquiae*	Rio Juquiá/Juquitiba/SP/Rio Ribeira do Iguape Basin	S 23°59'49" W 46°56'01"
LBP2931	18994	Sequences not submitted	*Pseudotocinclus tietensis*	Rio Paraitinga/Salesópolis/SP/Rio Tietê Basin	S 23°31'36" W 45°49'11"
LBP2001	12191	FJ965487/FJ965499/FJ965509/FJ965466	*Corumbataia cuestae*	Rio Alambari/Botucatu/SP/Rio Tietê Basin	S 22°56'08" W 48°19'15"
MHNG2678.015	PR12	FJ965486/FJ965498/FJ965508/FJ965468	*Hypoptopoma inexpectatum*	Rio Paraná/Santa Fé/Argentina	Not available
LBP3510	21309	FJ625809/FJ625818/FJ965533/FJ965458	*Hypostomus nigromaculatos*	Córrego Hortelã/Botucatu/SP/Rio Paranapanema Basin	S 22°56'28" W 48°35'03"
LBP2368	15363	FJ434499/FJ434524/ FJ965535/FJ965460	*Hemipsilichthys gobio*	Rio Macaquinho/Bairro dos Macacos/SP/Rio Paraíba do Sul Basin	S 22°49'51" W 44°51'53"
LBP4956	10241	FJ625808/FJ625817/FJ965534/FJ965459	*Hemipsilichthys papilatus*	Ribeirão da Jacutinga/Bom Jardim de Minas/MG/Rio Paraíba do Sul Basin	S 22°02'27" W 44°09'43"

Table 2. Primers used in the present study to amply partial sequences of 12S and 16S rRNA genes, cytochrome oxidase subunit I (COI), cytochrome B (CytB) and F-Reticulon 4.

Region and Fragment Length	Name	References	Primer Sequence
F-4 Reticulon (1900 bp)	Freticul4-D	Chiachio et al. (2008)	5'-AGG CTA ACT CGC TYT SGG CTT TG-3'
	Freticul4-R		5'-GGC AVA GRG CRA ART CCA TCT C-3'
	Freticul4 D2		5'-CTT TGG TTC GGA ATG GAA AC-3'
	Freticul4 R2		5'-AAR TCC ATC TCA CGC AGG A-3'
	Freticul4 iR		5'-AGG CTC TGC AGT TTC TCT AG-3'
12S rRNA (900 bp)	Phe-L941	Present study	5`-AAA TCA AAG CAT AAC ACT GAA GAT G-3`
	Val-H2010		5`-CCA ATT TGC ATG GAT GTC TTC TCG G-3`
16S rRNA (700 bp)	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 (700 bp)	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 (900 bp)	L14841	Present study	5`-CCA TCC AAC ATC TCA GCA TGA TGA AA 3`
	H15915b		5`-AAC CTC CGA TCT TCG GAT TAC AAG AC 3`

Figure 1. Geographical distribution of the Loricariidae samples sequenced in the present study across the southern of South American.

Sequencing alignment and phylogenetic analysis

Individual sequences of each species were initially analyzed with the software BioEdit 5.0.9 (Hall 1999) and a consensus sequence was obtained for each DNA segment of each species. After that, all sequences were independently aligned using the software Muscle (Edgar 2004). The convert the formats we used the software DAMBE (Xia & Xie 2001). Nucleotide variation, substitution patterns, and genetic distances were examined using MEGA 4.0 (Tamura et al. 2007).

Maximum-parsimony (MP) based on phylogenetic analysis were performed using the software TNT (Goloboff et al. 2008). No a priori weighing or ordering of character states was used and gaps were treated as a missing data. Phylogenies were constructed under the 'new technology search' methodology (Goloboff 1996, 1999), using all the options 'sectorial search', 'ratched', 'drift', and 'tree fusing' with their default values and employing a driven search with initial levels setting at level 100 and checking level every two hits. Consistency and retention indexes were calculated with

the script 'stats' of TNT. Clade robustness was assessed using 1000 bootstrap (B, Felsenstein, 1985) pseudoreplicates with the same parameters cited above. Bremer support values (BS, Bremer 1988) were calculated with the script 'bremer' of TNT.

The Neighbor-joining analysis was performed using reconstruction method implemented in MEGA 4.0 (Tamura et al. 2007). Sequence divergences were calculated Tamura-Nei (Tamura & Nei 1993). *Bootstrap* resampling (Felsenstein 1985) was applied to assess support for individual nodes using 1000 replicates. The global transition/transversion (Ti/Tv) ratio was estimated using MEGA 4.0 (Tamura et al. 2007).

The Bayesian-likelihood method of phylogenetic analysis (P, Huelsenbeck & Ronquist 2001) was used to evaluate alternative tree topologies through the estimation of posterior probabilities using MrBayes v.3.0 (Ronquist & Huelsenbeck 2003). Four chains were run simultaneously for 5,000,000 generations using MrBayes analysis. Every 100th generation was sampled and the asymptote of likelihood score was detected with the SUMP command. The above procedure was repeated two times. All sampled topologies beneath the asymptote (3,000,000 generations in the present study) were discarded from the population of trees considered in the subsequent majority-rule consensus. The frequency with which a particular clade appeared in the population of retained topologies was interpreted as its posterior probability. Posterior probabilities were interpreted as a measure of how likely the clade appears in the optimal topology rather than accuracy of the node with respect to species relationships or clade stability. Consensus trees were produced with the software TreeExplorer implemented in MEGA 4 (Tamura et al. 2007).

The TVM nucleotide substitution model (Transversional model), including invariable sites (+I) and rate variation among sites (+G) was utilized for all likelihood analyses based on a hierarchical hypothesis test of alternative models implemented with Modeltest 3.7 (Posada & Crandall 1998).

Biogeography Analysis

The divergence between the species was estimated based on the Neighbor-joining method. A molecular clock was not calibrated because a specific time of a geological event or a fossil of the group was not found in the literature. Records on geographic distribution of species and data on the tectonic evolution of the South

American continent were obtained from the literature, mainly from the papers of Ribeiro (2006b), Ribeiro et al. (2006), and Menezes et al. (2008).

Interpretation of biogeographic patterns was made base on the construction of an area cladogram (Morrone & Crisci, 1995) and follow Nelson (1985) concept of biogeography, as being the interrelationships or synthesis between biology and geology. Two vicariance model was discussed and used to explain the geographic distribution of the species of the subfamily Neoplecostominae.

Results

Partial sequences of four mitochondrial genes (12S rRNA, 16SrRNA, COI, Cytb) and one nuclear gene (F-4 Reticulon) were obtained from 52 specimens, representing 47 loricariid species (Table 1). The combined sequence data resulted in a matrix with 4676 base pairs (bp), out of which 2676 were conserved and 1155 were parsimony informative characters. Saturation was not observed in transitions or transversions and the transition/transversion rate observed was 3.2.

The analysis with the 'new technology search' (Goloboff 1996, 1999) resulted in a single most parsimonious tree (Figure 2) with a consistence index (CI) = 0.509 and a retention index (RI) = 0.682. All nodes were supported by Bremer (B) indexes with values ranging from 1 to 156 and most of the nodes were also supported by high *bootstrap* (BS) values. A Bayesian analysis was performed using TVM nucleotide substitution model (Transversional model) implemented with Modeltest 3.7 (Posada & Crandall 1998). The analysis resulted in 50,001 trees of which the first 30,001 was discarded and the others 20,000 were used to perform the consensus tree (Figure 3). Neighbor-joining analysis was performed in MEGA 4.0 (Tamura et al. 2007) using the Tamura-Nei (TN) with 1000 *bootstrap* replicates (Figure 4).

Figure 2 (parsimony analysis), Figure 3 (Bayesian analysis) and Figure 4 (area cladogram with distance analysis) show that Neoplecostominae, including *Pseudotocinclus*, is monophyletic with considerable statistical support (BS = 90 and B = 16 in Parsimony analysis, Probabilistic P = 100 in Bayesian analysis). Also three lineages are recognized in Neoplecostominae. The first one (group one) is composed by the genera *Neoplecostomus*, *Pareiorhina* and a new genus (BS = 53 and B = 6 in parsimony analysis, P = 100 in bayesian analysis). The second (group two) is composed

of *Isbrueckerichthys*, *Pareiorhaphis* and *Kronichthys* (BS = 100 and B = 32 in parsimony analysis, P = 100 in bayesian analysis) and the third (group three) is composed by the last *Pareiorhina* and *Pseudotocinclus* (BS = 100 and B = 28 in parsimony analysis, P = 100 in bayesian analysis).

In biogeographic analysis two different dispersion models were discussed. The first one was the headwaters captures responsible for introducing fishes from one geographic basin to the neighbor basin. The second one was a model responsible to the dispersion in the hydrographic basin. This model assumes that in the glacial and interglacial periods the rivers change their flows and fish from small streams could reach lowest parts of the rivers that have less water.

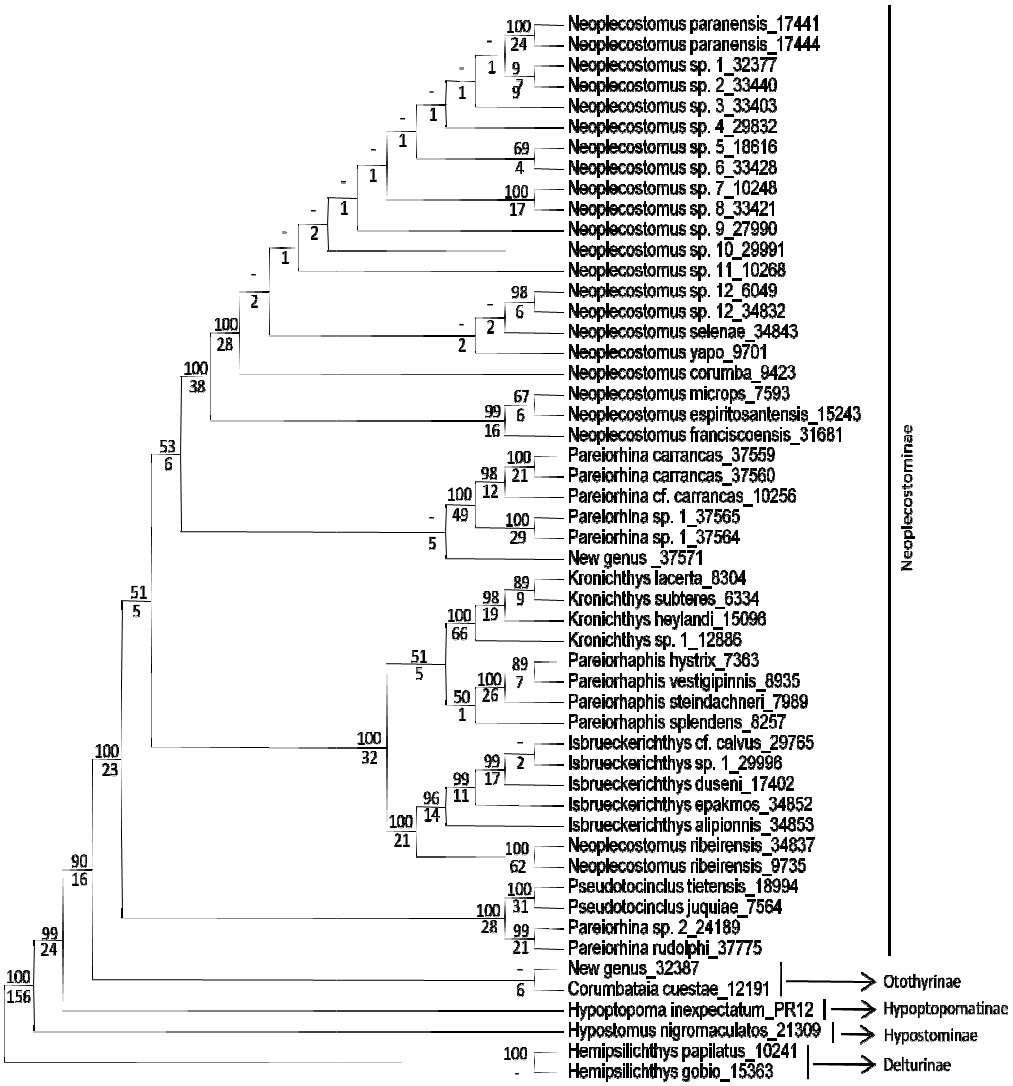


Figure 2. Single most parsimonious tree obtained in the parsimony analysis (CI = 0.509, RI = 0.682). Numbers above branches are bootstrap values from 1000 bootstrap pseudoreplications. Numbers below branches are Bremer decay support values. Bootstrap values below 50% (-) are not shown.

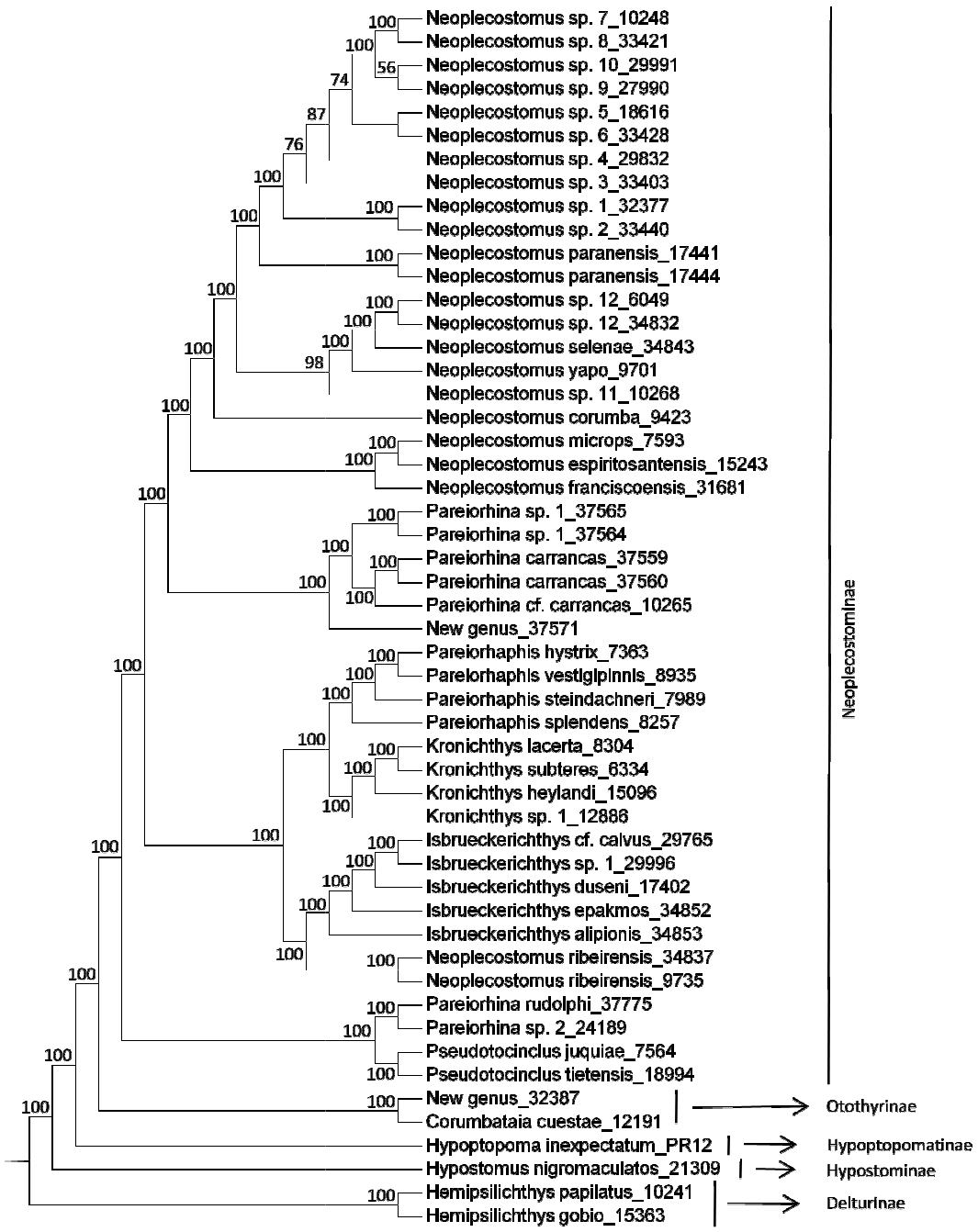


Figure 3. Major rule consensus tree obtained in the Bayesian-likelihood analysis. Numbers are posterior probabilities obtained in the analysis of 3,000,000 generations with four chains.

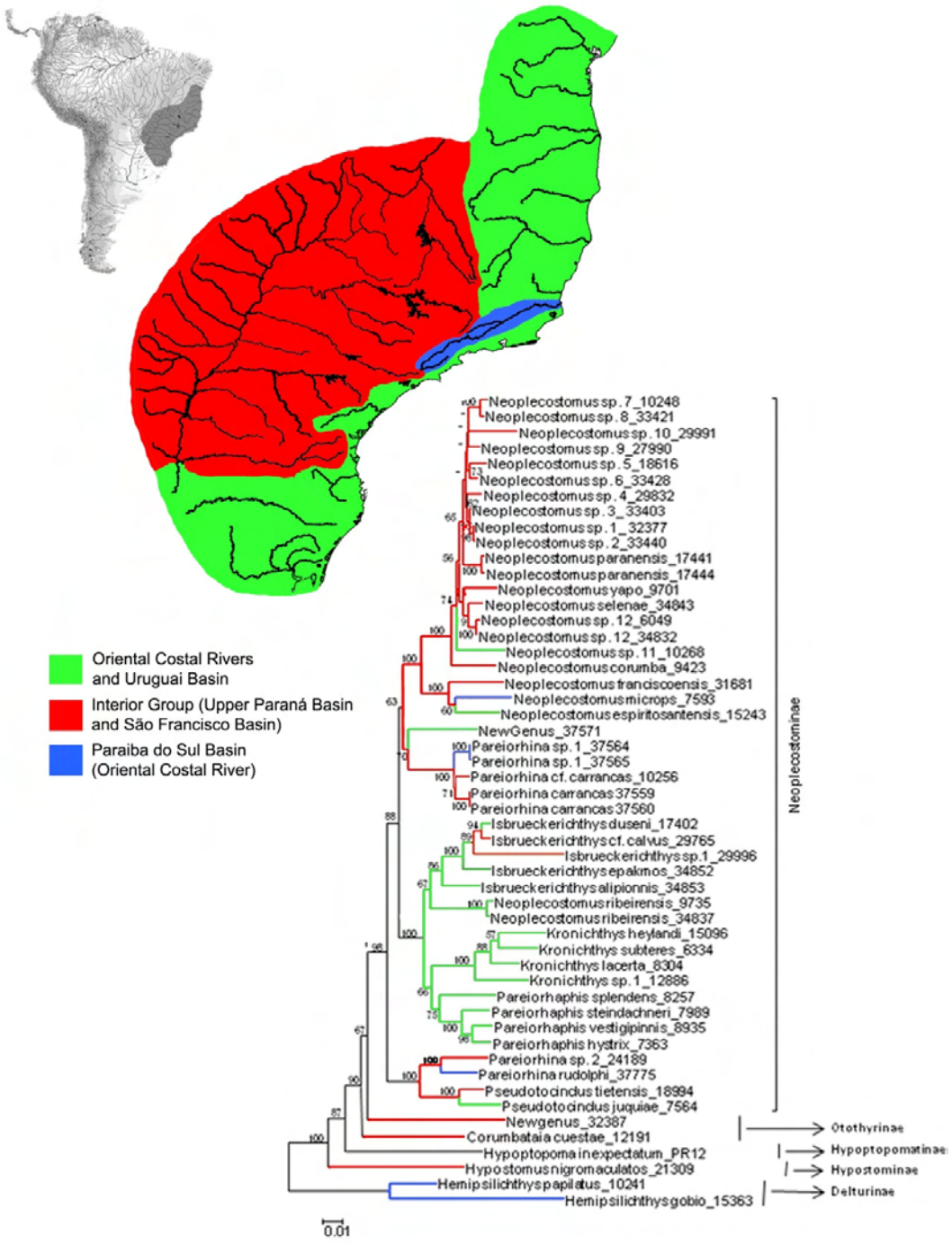


Figure 4. Area cladogram and geographic distribution of Neoplecostominae across the southern of Brazil. The phylogeny was performed by the Neighbor-Joining method. The numbers are bootstrap values higher than 50%. The lengths of the branches represent the genetic distance between all taxa.

Discussion

The species of the subfamily Neoplecostominae are distributed throughout the south and southeast regions of Brazil. In this study all species of the subfamily, sensu Chiachio et al. (2008), belong to a monophyletic group, including *Pseudotocinclus*, which corroborates the studies of Montoya-Burgos et al. (1998), Chiachio et al. (2008) and Cramer et al. (2008), and refutes the paraphyletic hypothesis of Armbruster (2004) and Pereira (2008).

The species *Hemipsilichthys gobio* and *Hemipsilichthys papilatus* (subfamily Delturinae) were used to root the trees in all analyses, according to the work of Monotoya-Burgos et al. (1998), Armbruster (2004), and Reis et al. (2006). The species *Hypostomus nigromaculatus* (from subfamily Hypostominae), *Hypoptopoma inexpectatum* (from subfamily Hypoptopomatinae), *Corumbataia cuestae* and New genus_32387 (from subfamily Otothyrinae) also were used as outgroups.

In our area cladogram we divided the Neoplecostominae subfamily in three great groups. The first one is the coastal group composed by the genera *Kronichthys*, *Isbrueckerichthys* and *Pareiorhaphis*. The second is the continental group composed by the genera *Neoplecostomus*, *Pareiorhina* and New genus_37571. However, the break in two groups cannot be considered a rule in the evolution of the group, because apparently many headwaters captures occurred in the south and southeast portions of the South America, and some fishes from the littoral are present in the continental group and vice-versa. It is important to note that headwaters captures should represent an important vicariance event in Neoplecostominae. According to Ribeiro (2006b) headwater captures is a geological event that is present in faults regions that allow geological movements and permit the uplift of a complex structure and portions of rivers change the course to a different hydrographic basin. We believe that some genera may be examples of this event, such as *Hypostomus* (Montoya-Burgos 2003), *Oligossarcus* (Ribeiro 2006a) and *Harttia* (Costa-Silva 2009).

A third group composed of *Pareiorhina rudolphi*, *Pareiorhina* sp. 2 and two species of *Pseudotocinclus* appeared as the first divergent group in our analysis. The genus *Pareiorhina* appeared as a paraphyletic cluster, different from the results of Pereira (2008) which consider this genus monophyletic. However the last study did not find a diagnostic character supporting the genus *Pareiorhina*. In our analysis *Pareiorhina rudolphi* (type species of the genus) and *Pareiorhina* sp. 2 (from Ribeirão

Guaxinduva, upper Rio Paraná basin, Jundiaí, São Paulo) form a sister group of the genus *Pseudotocinclus* corroborating the works of Montoya-Burgos et al. (1998) and Chiachio et al. (2008).

A morphological aspect that can support the monophyly of *Pareiorhina* excluding *Pareiorhina carrancas* and *Pareiorhina* sp. 1 is the lack of a ridge in the postdorsal surface of the trunk (Figure 5). In *Pareiorhina carrancas* and *Pareiorhina* sp. 1 (from Rio Pomba/Santa Barbara do Tugúrio/MG/bacia do Rio Paraíba do Sul) this character is present (Figure 6). Thus, it can be an evidence that *Pareiorhina carrancas* and *Pareiorhina* sp 1. belong to a new genus. *Pareiorhina brachyrhyncha* was not analyzed in the molecular data but also does not have the ridge in the postdorsal portion suggesting that this species is closely related with the type species *Pareiorhina rudolphi*. The genus *Pseudotocinclus* is divided into species from two geographic regions, *Pseudotocinclus tietensis* in the interior drainages and *Pseudotocinclus juquiae* from along the coast. Thus, with only these two species it is difficult to understand the biogeography of the genus. However, based in this evidence, we believe that the ancestor of the genus existed before the geological events that divided the groups between the continent and the littoral.

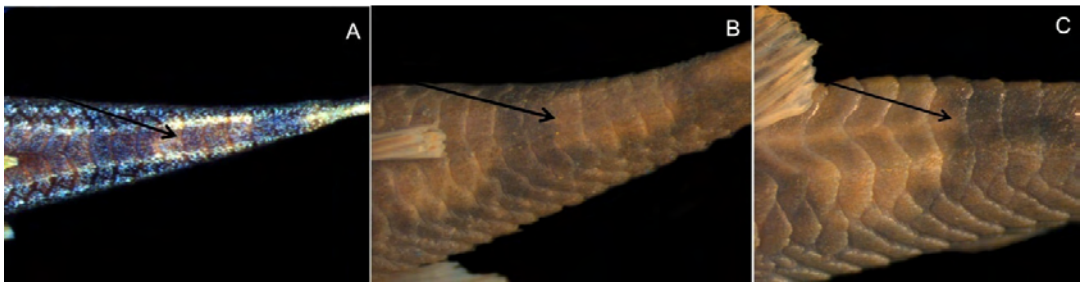


Figure 5. Species without a ridge in the postdorsal portion. A – *Pareiorhina rudolphi*, B – *Pareiorhina brachyrhyncha* C – *Pareiorhina* sp. 2.

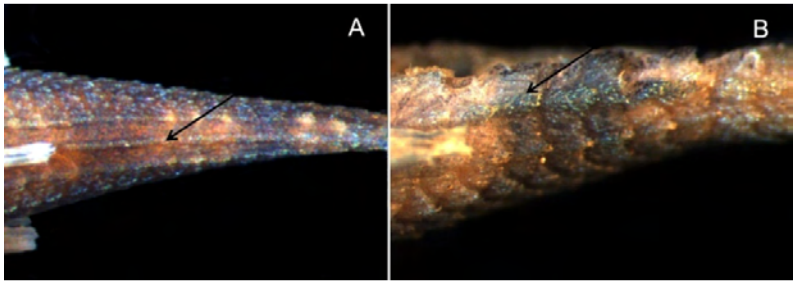


Figure 6. Species with a ridge in the postdorsal portion.A – *Pareiorhina carrancas*, B – *Pareiorhina* sp. 1.

Considering the littoral group, it is interesting to observe that the genera *Isbrueckerichthys*, *Pareiorhaphis* and *Kronichthys* occur almost exclusively in the oriental costal basin (Figure 4) and have a close relationship between them. It is possible that the ancestor of these groups occurred in the coastal drainages and some event during Serra do Mar formation divided the ancestor of this basin from the ancestor that we call the interior group, composed by all species of *Neoplecostomus* (except *Neoplecostomus ribeirensis*), *Pareiorhina carrancas*, *Pareiorhina* sp.1 and the New genus_37571. However, we acknowledge the close relationship between these two groups. In our phylogeny one example of this may be the group composed of the genus *Isbrueckerichthys* and the species *Neoplecostomus ribeirensis*. Both are from oriental coastal basin, while most of members of genus *Neoplecostomus* are from the interior drainages. This fact also shows the paraphyly of the *Neoplecostomus*, and reinforces the hypothesis of the break between the coastal and the interior groups.

The genus *Isbrueckerichthys* is represented in this study by four of the five described species (Jerep et al. 2006) and a new undescribed species. The genus is a monophyletic group in our analysis. *Isbrueckerichthys alipionis* and *I. duseni* already appeared as a monophyletic group in the analysis of Armbruster (2004) but as a paraphyletic group in Chiachio et al. (2008). The three species from Rio Ribeira de Iguape Basin(*Isbrueckerichthys alipionis*, *I. duseni*, and *I. epakmos*) do not belong to a natural group because *I. duseni* is more related to the Upper Rio Paraná Basin species. On the other hand, the presence of the two most basal species of *Isbrueckerichthys* in the Rio Ribeira de Iguape Basin is evidence that this genus originated there.

Forming sister group with *Isbrueckerichthys* we found *Neoplecostomus ribeirensis*, resulting in a paraphyletic *Neoplecostomus*. Initially we thought that this result was strange and possibly because of contamination. However, the inclusion of more samples from different geography regions and the repetition of sequencing proved that the result is correct. Another possibility could be introgression of mitochondrial from the ancestor of *Isbrueckerichthys* in the ancestor of *Neoplecostomus ribeirensis*. But we do not believe in this possibility. The nuclear gene was independently analyzed when we were assembling the total matrix and, as a result, *Neoplecostomus ribeirensis* always appeared close related with *Isbrueckerichthys* species. Then based on our analysis we believe that these two groups are closely related, supporting the hypothesis of the proximity of the species from the oriental coastal basin, and that, some geological events divided the group from littoral from the interior of the continent.

The four species of *Kronichthys*, the three already recognized species for the genus (Ferraris Jr. 2007) plus a new undescribed species, forms a monophyletic group. The first divergent species is *Kronichthys* sp 1. from Mongaguá (Brazilian coastal basin). *Kronichthys lacerta* is the sister group of *K. subteres* and these two species are the sister species to *K. heylandi*. Two hypotheses can explain the relationship between *Kronichthys* and remaining loricariids. Montoya-Burgos et al. (1998) suggested that *Kronichthys* is the sister group of an unidentified Hypostominae (possibly Neoplecostominae species) and closely related to *Isbrueckerichthys* and *Pareiorhaphis* (identified as *Hemipsilichthys* in their study). A closer relationship to *Pareiorhaphis* than to *Isbrueckerichthys* plus *Neoplecostomus* was indeed observed here. According to Armbruster (2004) *Kronichthys* is the sister group of Hypoptopomatinae, which is refuted in this study because this genus appeared to be the sister group to the genus *Pareiorhaphis*.

The four *Pareiorhaphis* species of the 18 species recognized for this genus (Pereira, Vieira & Reis 2007) also form a monophyletic group, corroborating the studies of Pereira (2008). This genus is the sister group of the genus *Isbrueckerichthys* and is not directly related to *Hemipsilichthys gobio* and *H. papillatus*. This result agreed with Pereira (2005), who resurrected *Pareiorhaphis*, including all species previously assigned to *Hemipsilichthys*, except *H. gobio*, *H. papillatus,* and *H. nimius*. The close relationship between *Pareiorhaphis* and *Isbrueckerichthys* was proposed by Chiachio et al. (2008), although *Isbrueckerichthys* was found as a polyphyletic group in that study. Montoya-Burgos et al. (1998) found that *Pareiorhaphis* (identified as *Hemipsilichthys*) was polyphyletic but one species, *Pareiorhaphis* sp. n., appeared as the sister group of *I. duseni,* the only *Isbrueckerichthys* species used in their study. The close relationship between *Pareiorhaphis* and *Isbrueckerichthys* was not observed in this study corroborating the works of Armbruster (2004) and Pereira (2008). In this last work *Isbrueckerichthys* is sister group of *Neoplecostomus*.

According to Cobbold et al. (2001), the obliquely rifted margin of southeastern Brazil, responsible for headwater captures, is characterized by a tectonic context that contrasts with the traditional view in which the Atlantic margin of Brazil is a passive margin. This interpretation also supports our theory of the numerous speciations events observed in the subfamily Neoplecostominae. These authors interpreted the reactivation of older structures, attributing them to the combined effect of far-field stress (related to the Andean uplift) and hot-spot activity (the Trindade hot-spot).

In the same way headwater captures are not the only event responsible for isolating Neoplecostominae populations and subsequent speciation. We suggested that the sea level may be another important fact in speciation. Animals that lived in the coastal rivers during the glacial age should have contacted with each other, because in glacial age, the sea retreated can connect other coastal rivers. However, during interglacial age the sea level increase and the populations were again isolated. This hypothesis can help explain the great diversity of species distributed throughout southeast Brazil.

Interestingly the species found in the northern part of coastal basin co-occur with continental species. This region contains numerous geological faults, which caused several of headwaters captures in the geological history of the area. Thus, these events of headwaters captures allowed the genus *Neoplecostomus* to be dispersed in the area. Possibly two genera, *Isbrueckerichthys* and *Kronichthys*, do not crossed the Rio Paraíba do Sul Basinto the northeast basin (Rio Doce, Rio Jequitinhonha and others), although the genus *Isbrueckerichthys* is present in this basin, suggesting that the Rio Paraíba do Sul is a barrier to the species of these genera. This river is one of the biggest rivers of the oriental coastal basin by water volume, and these genera are restricted to headwaters with fast flow and rocky buttons. As a result, a great volume of water may be a barrier to these species.

During the glacial age Rio Paraíba do Sul Basin was a forest refuge (Ab´Saber 1979). This is evidence that, in glacial ages, this river maintained high water flow when compared with other rivers of the same size, in the same period. Thus, during the glacial age when the sea level was lower many drainages merged with each other. Rio Paraíba do Sul functioned as a barrier and species that lived in small streams could not cross its riverbed.

However the genus *Pareiorhaphis* occurs through almost all portions of the oriental coastal basin and some species are found in the Upper Rio Paraná Basin (*Pareiorhaphis parmula*), Rio São Francisco Basin (*Pareiorhaphis mutuca*), the Uruguay Basin (*Pareiorhaphis eurycephalus*, *Pareiorhaphis hystrix* and *Pareiorhaphis vestigipinnis*) and the Amazon Basin (*Pareiorhaphis regani*). However, the species we believe that reached these others basins reached then through headwaters captures. In this occasion the Rio Paraíba do Sul was not a barrier to this genus, and it reached the northern portion of the oriental coastal basin.

In our analysis, the three genera *Isbrueckerichthys*, *Pareiorhaphis* and *Kronichthys* appeared as sister groups to the genera *Neoplecostomus*, *Pareiorhina* (probably a new genus) and a species from a genus (new genus_37571). This relationship was not observed in previous studies (Montoya-Burgos et al. 1998; Chiachio et al. 2008; Cramer et al. 2008, Pereira 2008).

Another vicariance model proposed to explain the speciation in subfamily Neoplecostominae is based on elevation and depression of the sea level and on changes in the water flow during glacial and interglacial cycles. The last glacial period occurred in the Pleistocene and there were several changes between glacier advance and retreat. In South America this process can explain the diversification of some fish species into the oriental coastal basin and the Upper Rio Paraná Basin. As was said before, a large volume of water could be a barrier to many species in the Neoplecostominae subfamily, because most of the species need of high oxygen concentration in water to survive. In the riverbed of great rivers have lower oxygen concentration than small streams, in this case large rivers act as a barrier to these species.

However in glacial periods great rivers decrease water flows just as sea level also declined. Lower rainfall could have caused lower water flow (Ab'Sáber 1979). Species that live in this characteristically ambient go to the riverbed of great rivers, and this was not a barrier. As a consequence many species, able to reproduce, can mix their genes and a homogeneous population can emerge. During the next interglacial period the species migrated to the headwaters randomly and remained isolated by the large flows of the principal rivers again. The need for high oxygen levels limits members of the genus to fast flowing streams. This process can be an important vicariance model to explain the dispersion of small fishes of the subfamily Neoplecostominae. It also may explain the low genetic divergence in *Neoplecostomus* species in upper Rio Paraná basin, mainly in Rio Grande basin. In this region many species stay isolated in small streams at the top of Serra do Mar Mountains. If this process happened with *Neoplecostomus* from the Upper Rio Paraná Basin in the last glacial age, only 18,000 years can be a short period to bring about the morphological and genetic diversification found between these species (see Figure 7).

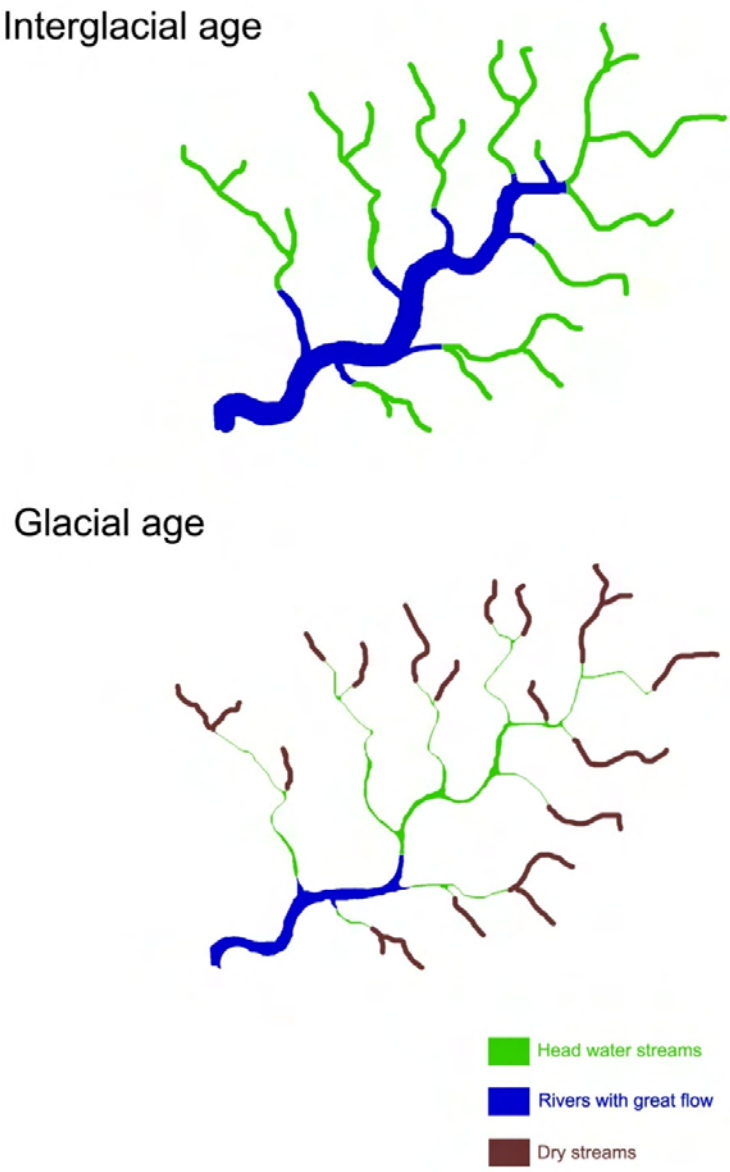


Figure 7. Hypothetical differences of flows in great rivers between glacial and interglacial periods (present study).

In the continental group that is a sister group with *Pareiorhina carrancas*, *Pareiorhina cf. carrancas* and *Pareiorhina* sp. 1 is what we consider to be a new genus (New genus_37571). All these species form sister groups to the genus *Neoplecostomus*.

The genus *Neoplecostomus* consists of small fishes that Ferraris Jr. (2003) stated were scarcely known, and very few studies have been conducted beyond its original descriptions. In our study we analyzed nine of the eleven described species of the genus

(Langeani 1990, Bizerril 1995, Zawadzki et al. 2008) and twelve more species that we think are new species. The morphological traits to differentiate species of the genus are limited. Thus molecular tools have been helpful to distinguish these species, and population studies based mainly on allozyme have also been carried out (Zawadzki et al. 2004, Philippsen et al. 2009).

In this work the genus *Neoplecostomus* appeared as paraphyletic with *Neoplecostomus ribeirensis* forming a sister group with *Isbrueckerichthys*. This result did not corroborate the hypothesis that *Neoplecostomus* is a monophyletic group (Pereira 2008). This author found three diagnostic features to the genus: dorsal profile of the unbranched pectoral-fin ray with strong curvature; lateropterygium bearing small expantion on distal portion and lower lip with papillae forming conspicuous series situated posteriorly to dentary. Additionally, *Neoplecostomus* is distinguished from the remaining neoplecostomines by having joined platelets forming a hexagonal scute between the insertion of pectoral and pelvic fins (absent in the other Neoplecostominae). However, Pereira (2008) also found some morphological characters that differs *Neoplecostomus ribeirensis* from the other *Neoplecostomus* species. These characters are the basiopterygium laterally projected in *N. ribeirensis* and anteriorly projected in the remaining *Neoplecostomus* species. Another aspect is the three different shapes of the lateropterygium: (0) clearly enlarged on distal portion in *Astroblepus*; (1) narrow and constant along its extension; and (2) narrow and slightly expanded on its distal portion, in *Neoplecostomus* (Pereira 2008). The second shape was considered as a sinapomorphy to the species of *Neoplecostomus*, but reverted in *N. ribeirensis*. In our analysis we find a character, the presence of a different form of the papilla (diagnostic character of the *Neoplecostomus*) in *Neoplecostomus ribeirensis*, when compared with the other *Neoplecostomus* species. In *Neoplecostomus ribeirensis* the papillas forms only two rows and the first one do not have buds and all others species of *Neoplecostomus* have three rows (Figure 8), all with buds.

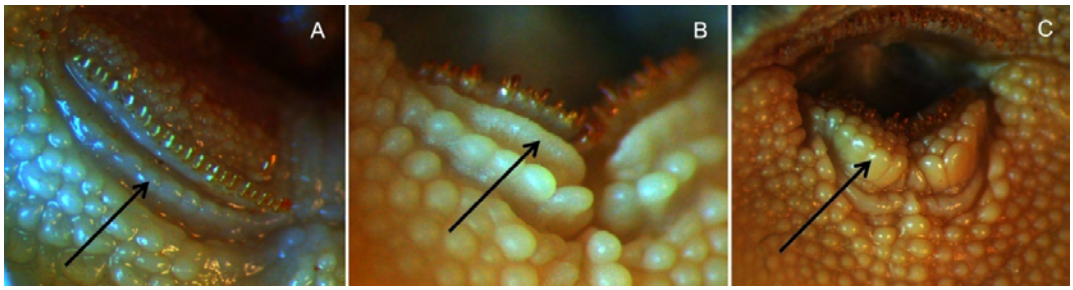


Figure 8. Different forms and numbers of papilla lines in Neoplecostominae. (A) *Isbrueckerichthys saxicola*, (B) *Neoplecostomus ribeirensis*, (C) *Neoplecostomus microps*.

Most of the relationships between the species of Neoplecostomus have low bootstrap support, however, some groups can be recognized with good statistic support, considering the parsimony analysis. The first divergent group is composed of *N. franciscoensis*, sister group of *N. microps* (type species of the genus) and *N. espiritosantensis*. This group is composed of fishes of the oriental coastal basin, however do not corroborate the work of Pereira (2008). In the last work *N. franciscoensis* formed a sister group with *N. ribeirensis*, but in this study it is a sister group to the genus *Isbrueckerichthys*. *Neoplecostomus espiritosantensis* is the first divergent species of the genus and sister of all other *Neoplecostomus*, and *N. microps* is a sister species to *Neoplecostomus* P sp. n (from Rio Perequê-Açu, Parati, RJ).

In the group formed by the upper Rio Paraná basin, *N. corumba* appeared to be the first divergent taxa. This species inhabits the western portion of Upper Rio Paraná Basin and can be isolated by a long period of time. The separation between these closely related basins suggests that the splitting between Upper Rio Paraná Basin and oriental coastal basin is older than the fragmentation of the oriental coastal basin. The proximity between *N. microps* and *N. espiritosantensis* could be explained by this same processes.

After *Neoplecostomus corumba* the first divergent species in Upper Rio Paraná Basin was formed by the group represented by *N. yapo* sister group of *N. selenae* plus *Neoplecostomus* sp. 12, which, in turn, form sister group with *Neoplecostomus selenae*. These last species are all from Rio Paranapanema basin. *Neoplecostomus* species from this river are paraphyletic because *Neoplecostomus* sp. 10 is not closely related with the other species, but form a sister group with the species from Rio Grande basin. The species from this basin also formed a paraphyletic cluster because the species *Neoplecostomus* sp. 5 is from Rio Tietê Basin. The only described species between all the analyzed species from Rio Grande Basin is *Neoplecostomus paranensis*. But almost

all new species proposed herein are from this basin. As Rio Grande Basinspans a large area in the Serra do Mar hills, numerous orogenetic processes that formed this basin must have isolated the populations of *Neoplecostomus*. Thus the speciation process differentiated the endemic population, but the morphological traits that distinguish them are limited. *Neoplecostomus paranensis* appeared to be closely related to *Neoplecostomus* sp. 1 (from Ouro Fino/MG) and *Neoplecostomus* sp. 2 (from Borda da Mata/MG). The only species of *Neoplecostomus* analyzed from Rio Doce Basin(*Neoplecostomus* sp. 10) was genetically similar to those of Upper Rio Paraná Basin in parsimonious analysis, but formed a sister group with *Neoplecostomus* sp. 12 (Rio Paranapanema basin), *Neoplecostomus yapo* (Rio Paranapanema basin) and *Neoplecostomus selenae* (Rio Paranapanema basin) in probabilistic analysis. Although we do not have a statistically supported position to *Neoplecostomus* sp. 11, the headwater captures between the Upper Rio Paraná Basin and Rio Doce Basincan be an explanation to the close relationship between these two basins.

When we analyzed the area cladogram (Figure 4) most of the species of *Neoplecostomus* appeared in the interior portion (upper Rio Paraná basin). The species found in the oriental coastal basin may be there due headwaters captures, mainly the captures between Rio Paraíba do Sul and the surrounds basin (Upper Rio Paraná Basin, Rio São Francisco Basin and Rio Doce Basin). This process are called the Taubaté Graben basin, and it was reported by Ab'Sáber (1957, 1998). The origin of the Taubaté Graben probably resulted in the capture of several other adjacent rivers, such as headwaters of the rivers Tietê, Grande, São Francisco, and probably Doce.

References

Ab'Saber AN (1957) O problema das conexões antigas e da separação da drenagem do Paraiba e Tietê. Boletim Paulista de Geografia **26:**38–49.

Ab'Sáber AN (1979) Os mecanismos de desintegração das paisagens tropicais no Pleistoceno: efeitos paleoclimáticos do período Würm-Wisconsin no Brasil. São José do Rio Preto, Instituto de Biociências, Letras e Ciências Exatas da UNESP.

Ab'Saber AN (1998) Megageomorfologia do Território Brasileiro. *In*: Cunha SB, AJT Guerra (Eds.), Geomorfologia do Brasil. BERTRAND, Rio de Janeiro, pp. 71–106.

Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res **22:**4692–4693.

Armbruster JW (1998) Phylogenetic relationships of the sucker-mouth armored catfishes of the *Rhinelepis* group (Loricariidae: Hypostominae). Copeia **3:**620–636.

Armbruster JW (2004) Phylogenetic relationships of the sucker-mouth armored catfishes (Loricariidae) with particular emphasis on the Hypostominae and the Ancistrinae. Zool J Linn Soc **141:**1–80.

Bizerril CRSF (1995) Descrição de uma nova espécie de *Neoplecostomus* (Loricariidae, Neoplecostominae) com uma sinópse da composição taxonômica dos Loricariidae no leste brasileiro. Arq Biol Tecno **38:**693–704.

Bremer K (1988) The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution **42:**795–803.

Chiachio MC, Oliveira C, Montoya-Burgos JI (2008) Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). Mol Phylogenet Evol **49:**606–617.

Cobbold PR, Meisling KE, Mount VS (2001) Reactivation of an obliquely rifted margin, Campos and Santos basins, southeastern Brazil. The American Association of Petroleum Geologists Bulletin **85:**1925–1944.

Costa Silva GJ (2009) Análise filogenética entre gêneros da subfamília Loricariinae (Siluriformes: Loricariidae) com ênfase no gênero *Harttia*, baseada em caracteres moleculares. Unpublished Dissertation. Universidade Estadual Paulista Julio de Mesquita Filho.

Cramer CA, Liedke AMR, Bonatto LS, Reis RE (2008) The phylogenetic relationship of the Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae) as inferred from mitochondrial cytochrome c oxidase I sequences. Bull Fish Biol **9:**51–59.

Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics **5:**113.

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

Ferraris Jr CJ (2003) Subfamily Neoplecostominae. *In*: Reis RE, Kullander SO, Ferraris Jr CJ (Eds.), Check list of the freshwater fishes of South and Central America. EDIPUCRS, Porto Alegre, pp 319–320.

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

Goloboff P (1996) Methods for faster parsimony analysis. Cladistics **12:**199–220.

Goloboff P (1999) Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics **15:**415–428.

Goloboff PA, Farris JS, Nixon KC (2008) TNT, a free program for phylogenetic analysis. Cladistics **24:**774–786.

Gosline WA (1947) Contributions to the classification of the loricariid catfishes. Arq Mus Nac Rio de Janeiro **41:**79–134.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser **41:**95–98.

Howes GJ (1983) The cranial muscles of loricarioid catfishes, their homologies and value as taxonomic characters (Teleostei: Siluroidei). Bull British Mus Nat Hist, Zool Ser **45:** 309–345.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics **17:**754-755.

Jerep FC, Shibatta OA, Pereira EHL, Oyakawa OT (2006) Two new species of *Isbrueckerichthys* Derijst, 1996 (Siluriformes: Loricariidae) from the rio Paranapanema basin, Brazil. Zootaxa **1372:**53–68.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution **16:**111-120.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Päbo S, Villablanca FX, Wilson A (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc Nati Acad Sci **86:**6196–6200.

Langeani F (1990) Revisão do gênero *Neoplecostomus*, com a descrição de quatro espécies novas do sudeste brasileiro (Ostariophysi, Siluriformes, Loricariidae). Comun Mus Ciênc PUCRS, Sér Zool **3:**3–31.

Menezes NA, Ribeiro AC, Weitzman S, Torres RA (2008) Biogeography of Glandulocaudinae (Teleostei: Characiformes: Characidae) revisited: phylogenetic patterns, historical geology and genetic connectivity. Zootaxa **1726:** 33–48.

Montoya-Burgos JI, Muller S, Weber C, Pawlowski J (1998) Phylogenetic relationships of the Loricariidae (Siluriformes) basen on mitochondrial rRNA gene sequences. *In*: Malabarba LR, Reis RE, Vari RP, Lucena ZM, Lucena CAS (Eds), Phylogeny and Classification of Neotropical Fishes. EDIPUCRS, Porto Alegre, pp 363–375.

Montoya-Burgos JI (2003) Historical biogeography of the catfish genus *Hypostomus* (Siluriformes: Loricariidae), with implications on the diversification of Neotropical ichthyofauna. Molecular Ecology **12:**1855–1867.

Morrone J, Crisci JV (1995) Historical Biogeography: Introduction to Methods. Annual Review in Ecology and Systematics **26:**373–401.

Pereira EHL, Reis RE (2002) Revision of the loricariid genera *Hemipsilichthys* and *Isbrueckerichthys* (Teleostei: Siluriformes) with descriptions of five new species of *Hemipsilichthys*. Ichthyol Explor Freshw **13:**97–146.

Pereira EHL (2005) Resurrection of *Pareiorhaphis* Miranda Ribeiro, 1918 (Teleostei: Siluriformes: Loricariidae), and description of a new species from the rio Iguaçu basin, Brazil. Neotrop Ichthyol **3:**271–276.

Pereira EHL, Vieira F, Reis RE (2007) A new species of sexually dimorphic *Pareiorhaphis* Miranda Ribeiro, 1918 (Siluriformes: Loricariidae) from the Rio Doce basin, Brazil. Neotrop Ichthyol **5:**443–448.

Pereira EHL (2008) Relações Filogenéticas de Neoplecostominae Regan, 1904 (Siluriformes: Loricariidae). Unpublished Ph.D. Thesis. Pontifícia Universidade Católica do Rio Grande do Sul.

Philippsen JS, Renesto E, Gealh AM, Artoni RF, Shibatta OA, Zawadzki CH (2009) Genetic variability in four samples of *Neoplecostomus yapo* (Teleostei: Loricariidae) from the rio Paranapanema basin, Brazil. Neotropical Ichthyology **7:**25-30.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics **14:**817-818.

Reis RE, Pereira EHL, Armbruster JW (2006) Delturinae, a new loricariid catfish subfamily (Teleostei, Siluriformes), with revisions of *Delturus* and *Hemipsilichthys*. Zool J Linn Soc **147:**277–299.

Ribeiro AC (2006a) Filogenia e biogeografia do gênero *Oligosarcus* Günther, 1864 (Ostariophysi; Characidae). Unpublish Phd Thesis.

Ribeiro AC (2006b) Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil: an example of faunal evolution associated with a divergent continental margin. Neotrop Ichthyol **4:**225–246

Ribeiro AC, Lima FCT, Riccomini C, Menezes NA (2006) Fishes of the Atlantic Rainforest of Boracéia: testimonies of the Quaternary fault reactivation within a Neoproterozoic tectonic province in Southeastern Brazil. Ichthyol Explor Freshw **17:**157–164.

Ronquist F, JP Huelsenbeck (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:**1572-1574.

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

Swofford DL, Olsen GJ, Waddell PJ, Hillis DM (1996) Phylogenetic inference, *In*: Hillis DM; Mable BK, Moritz C (Eds.). Molecular Systematics. Sunderland, Sinauer pp 407-514.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol Biol Evol **24:**1596–1599.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. Phil Trans R Soc B **360:**1847–1857.

Xia X, Xie Z (2001) DAMBE: Data analysis in molecular biology and evolution. Journal of Heredity **92:**371–373.

Zawadzki CH, Alves AL, Renesto E, Oliveira C (2004) Biochemical evidence of a possible new species of *Neoplecostomus* (Teleostei: Loricariidae) from the upper Rio Paraná basin, Brazil. Bioc Syst Ecol **32:**573–582.

Zawadzki CH, Pavanelli CS, Langeani F (2008) *Neoplecostomus* (Teleostei: Loricariidae) from the upper Rio Paraná basin, Brazil, with description of three new species. Zootaxa **1757:**31–48.

Capítulo 2

Three new species of the loricariid genus *Neoplecostomus* (Teleostei: Siluriformes) from Upper Rio Paraná Basin, southeastern Brazil

Fábio Fernandes Roxo[1]

[1]Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, IB-UNESP, Campos de Botucatu, 18618-000 Botucatu, SP, Brazil.

Key words: catfish, freshwater fish, molecular identification, Neoplecostominae, neotropical fishes, taxonomy.

Resumo

Neoplecostomus paranensis até pouco tempo era a única espécie descrita para o gênero *Neoplecostomus* na bacia do alto Rio Paraná. Nos últimos anos os esforços de coletas dos ictiologistas nas cabeceiras de riachos aumentaram e houve o reconhecimento e descrição de três novas espécies para o gênero, *N. corumba*, *N. selenae* e *N. yapo*, diagnosticadas basicamente pelo desenvolvimento da nadadeira adiposa. No presente estudo descrevemos mais três espécies para o gênero *Neoplecostomus: N.* sp. *nov. 1* de Salesópolis, São Paulo, *N.* sp. *nov. 2* de Muzambinho, Minas Gerais e *N.* sp. *nov. 3* de Botucatu, São Paulo, todas da bacia do Alto rio Paraná. As novas espécies são diferenciadas umas das outras e das outras espécies já descritas por sequências de DNA, morfologia e caracteres merísticos, e também por aspectos de coloração.

Abstract

Neoplecostomus paranensis was the only species assigned to *Neoplecostomus* in the upper Rio Paraná basin. In recent years the increasing efforts of ichthyological surveys in the headwaters of this basin allowed the recognition and description of three new species of *Neoplecostomus*, *N. corumba*, *N. selenae* and *N. yapo*, diagnosed by a developed adipose fin. In this study we describe three new *Neoplecostomus* species*: N.* sp. *nov. 1* from Salesópolis, São Paulo, *N.* sp. *nov. 2* from Muzambinho, Minas Gerais, and *N.* sp. *nov. 3* from Botucatu, São Paulo, all from the Upper Parana River Basin. The new species are differentiated from congeners by DNA sequences, morphometric and meristic traits, in addition to color pattern.

Introduction

Neoplecostomus, *Isbrueckerichthys* and *Pareiorhaphis* are three genera of the subfamily Neoplecostominae which form a monophyletic clade according to recent findings on molecular analysis (Chiachio et al. 2008; Cramer et al. 2008;). Notwithstanding, *Neoplecostomus* and *Pareiorhaphis* are clearly differentiated from the remaining Neoplecostominae genera by presenting a supraopercle and small platelets covering the abdomen between pectoral and pelvic-fin origins vs. supraopercle absent and abdomen entirely naked in *Isbrueckerichthys* (Armbruster 2004). *Neoplecostomus* is easily distinguished from *Isbrueckerichthys* by its conspicuous series of enlarged papillae just after the dentary teeth larger than those on the remaining portions of the lower lip, and by having abdominal platelets closely attached vs. absent series of large papillae after the dentary teeth and abdominal platelets surrounded by naked areas.

In the Upper Rio Paraná Basin *sensu* Britski & Langeani (1988) *N. paranensis*, diagnosed by the lack or reduction of the adipose fin, was the only species assigned to *Neoplecostomus*. In recent years the increasing efforts of ichthyological surveys in the headwaters of this basin allowed the recognition and description of three new species: *N. corumba*, *N. selenae,* and *N. yapo* (Zawadzki et al. 2008). These species clearly contrast with *N. paranensis* by the possession of a well-developed adipose fin.

Additional studies have also been conducted trying to better understand this fish group. Souza Braga et al. (2008) described by the first time aspects of feeding and reproduction in *Neoplecostomus microps* from Ribeirão Grande (Serra da Mantiqueira, rio Paraíba do Sul Basin) and found that the diet of this species are based on Diptera larvae (Simulium, Chironomus), Plecoptera nymphs and Coleoptera aquatic larvae (Psephenus). Additionally, some populational studies of *Neoplecostomus* based on allozyme has been carried out trying to identify new species on Rio Paraná Basin (Zawadzki et al. 2004; Philippsen et al. 2009). Zawadzki et al. (2004) compared a sample identified as *N. paranensis* from the Ribeirão Hortelã (rio Pardo Basin, Botucatu, São Paulo) with a sample of *N. corumba* (*Neoplecostomus* sp. in that work) from the Rio Corumbá in the Rio Paranaíba Basin (Caldas Novas, Goiás). These authors found 12 diagnostic loci among the 22 surveyed ones as well as zero heterozygosity values in the population from Ribeirão Hortelã. Such a small value usually indicates low population number, endogamy and possibly, isolation from nearby populations, factors which triggers speciation.

In the present work we used combined morphological and molecular tools to recognize, as well as to describe three new species of the genus *Neoplecostomus* from Upper Rio Paraná Basin.

Material and methods

Measurements and counts were taken from left side. Body plate nomenclature follows Schaefer (1997) and measurements follow Langeani (1990) modified by Zawadzki et al. (2008) and are taken in Table 2. All measurements were taken point to point with digital calipers to the nearest 0.1 mm and when the sample was youngest we didn't measure or identify the sex. All samples analyzed are deposited at the, DZSJRP, Departamento de Zoologia e Botânica, Universidade Estadual Paulista, São José do Rio Preto (SP); LBP, Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista, Botucatu (SP); MZUSP, Museu de Zoologia da Universidade de São Paulo, São Paulo (SP); NUP, Coleção Ictiológica do Nupélia, Universidade Estadual de Maringá, Maringá (PR). Abbreviations used in the text are HL (head length), SL (standard length), CP = caudal-peduncle, IO = interorbital length, OD = orbital diameter, PDS = predorsal, SL = standard length, ad = adipose fin, an = anal fin, cd = caudal fin, ds = dorsal fin. To analyze the correct position of each measurement one specimen of each species was cleaned and double stained according to the method of Taylor & Van Dyke (1985).

Genetic analysis: The samples of all species analyzed are listed in Table 1. The Voucher specimens are deposited at the Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil, or at the Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura (NUP), Universidade Estadual de Maringá, Paraná, Brazil.

Total DNA was extracted from ethanol preserved muscle, fin and liver samples with the protocol described by Aljanabi & Martinez (1997) and the Wizard Genomic DNA Purification Kit (Promega). Partial sequences of the gene Cytochrome Oxidase subunit I were amplified by polymerase chain reaction (PCR) with the following primer: FishF1 5'- TCA ACC AAC CAC AAA GAC ATT GGC AC -3' and FishR1 5'- TAG ACT TCT GGG TGG CCA AAG AAT CA -3' (Ward et al. 2005). Primer concentration was 5 pmol/µl. Amplifications were performed in a total volume of 25 µl for 35 cycles (30s at 95°C, 60s at 50-60°C, and 120s at 72°C). The PCR products were identified on a 1% agarose gel. The PCR products were purified using ExoSap-IT®

(Exonuclease I: Recombinant SAP: Pandalus borealis - USB Corporation) following instructions of the manufacturer. The purified PCR products were used to make a sequencing PCR using the Kit "Big DyeTM Terminator v 3.1 Cycle Sequencing Ready Reaction" (Applied Biosystems). After that, the amplified DNA was purified again and loaded on an automatic sequencer 3130-Genetic Analyzer (Applied Biosystems) presented in our laboratory.

Phylogenetic analysis and calculation of genetic distances: Individual sequences of each species were initially analyzed with the software BioEdit 5.0.9 (Hall 1999) and a consensus sequence was obtained for each DNA segment for each species. After that, all sequences were aligned using the software DAMBE (Xia & Xie 2001). The alignment was checked manually and corrected where necessary. Nucleotide variation, substitution patterns, and genetic distances between species were examined using MEGA 4.0 (Tamura et al. 2007).

Phylogenetic analyses were performed using the Neighbor-Joining reconstruction method implemented in MEGA 4.0 (Tamura et al. 2007). Sequence divergences were calculated using the Kimura two-parameter (K2P) model of base substitution (Kimura 1980) and with shape parameter of the gamma distribution (Swofford et al. 1996). Bootstrap resampling (Felsenstein 1985) was applied to assess support for individual nodes using 1000 replicates. The global transition/transversion (Ti/Tv) ratio was estimated using MEGA 4.0 (Tamura et al. 2007).

Table1. Species included in the present study. LBP = Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista. NUP = Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura, Universidade Estadual de Maringá.

Collection Number	Sample	Species	GenBank	Collecting Localities	Coordenates
LBP2861	18612	*Neoplecostomus sp. nov. 1*	GQ214795	Rio Paraitinga, Salesópolis, São Paulo, Upper Rio Paraná Basin	23°31'37"S 45°45'43"W
LBP2861	18613	*Neoplecostomus sp. nov. 1*	GQ214793	Rio Paraitinga, Salesópolis, São Paulo, Upper Rio Paraná Basin	23°31'37"S 45°45'43"W
LBP2861	18614	*Neoplecostomus sp. nov. 1*	GQ214796	Rio Paraitinga, Salesópolis, São Paulo, Upper Rio Paraná Basin	23°31'37"S 45°45'43"W
LBP2861	18615	*Neoplecostomus sp. nov. 1*	GQ214794	Rio Paraitinga, Salesópolis, São Paulo, Upper Rio Paraná Basin	23°31'37"S 45°45'43"W
LBP2861	18616	*Neoplecostomus sp. nov. 1*	FJ434534	Rio Paraitinga, Salesópolis, São Paulo, Upper Rio Paraná Basin	23°31'37"S 45°45'43"W
LBP6173	29260	*Neoplecostomus sp. nov. 2*	GQ214801	Córrego da Prata, Muzambinho, Minas Gerais, Upper Rio Paraná Basin	21°21'41"S 46°34'36"W
LBP6195	29290	*Neoplecostomus sp. nov. 2*	GQ214797	Rio Muzambinho, Muzambinho, Minas Gerais, Upper Rio Paraná Basin	21°22'15"S 46°32'35"W
LBP6195	29291	*Neoplecostomus sp. nov. 2*	GQ214798	Rio Muzambinho, Muzambinho, Minas Gerais, Upper Rio Paraná Basin	21°22'15"S 46°32'35"W
LBP5901	27990	*Neoplecostomus sp. nov. 2*	GQ214800	Rio São Domingos, Muzambinho, Minas Gerais, Upper Rio Paraná Basin	21°17'37"S 46°29'06"W
LBP5901	27991	*Neoplecostomus sp. nov. 2*	GQ214799	Rio São Domingos, Muzambinho, Minas Gerais, Upper Rio Paraná Basin	21°17'37"S 46°29'06"W
LBP7525	34832	*Neoplecostomus sp. nov. 3*	GQ214784	Ribeirão Água de Madalena, Rio Pardo, Botucatu, São Paulo, Upper Rio Paraná Basin	22°59'25" 48°25'37"W
LBP7525	34833	*Neoplecostomus sp. nov. 3*	GQ214786	Ribeirão Água de Madalena, Rio Pardo, Botucatu, São Paulo, Upper Rio Paraná Basin	22°59'25"S 48°25'37"W
LBP7525	34834	*Neoplecostomus sp. nov. 3*	GQ214788	Ribeirão Água de Madalena, Rio Pardo, Botucatu, São Paulo, Upper Rio Paraná Basin	22°59'25"S 48°25'37"W
LBP7525	34835	*Neoplecostomus sp. nov. 3*	GQ214785	Ribeirão Água de Madalena, Rio Pardo, Botucatu, São Paulo, Upper Rio Paraná Basin	22°59'25"S 48°25'37"W
LBP7525	34836	*Neoplecostomus sp. nov. 3*	GQ214787	Ribeirão Água de Madalena, Rio Pardo, Botucatu, São Paulo, Upper Rio Paraná Basin	22°59'25"S 48°25'37"W
LBP2732	17440	*Neoplecostomus paranensis*	FJ965505	Córrego Mocoquinha, Cajuru, São Paulo, Upper Rio Paraná Basin	21°19'37"'S 47°14'19"W
LBP2732	17441	*Neoplecostomus paranensis*	FJ434532	Córrego Mocoquinha, Cajuru, São Paulo, Upper Rio Paraná Basin	21°19'37"S 47°14'19"W
LBP2732	17446	*Neoplecostomus paranensis*	GQ214803	Córrego Mocoquinha, Cajuru, São Paulo, Upper Rio Paraná Basin	21°19'37"S 47°14'19"W
LBP2732	17448	*Neoplecostomus paranensis*	GQ214802	Córrego Mocoquinha, Cajuru, São Paulo, Upper Rio Paraná Basin	21°19'37"S 47°14'19"W
NUP6104	29279	*Neoplecostomus paranensis*	GQ214814	Rio Verde, Caldas, Minas Gerais, Upper Rio Paraná Basin	21°56'35"S 46°25'57"W
LBP5034	24681	*Neoplecostomus yapo*	GQ214781	Represa Três Pontes, Londrina, Paraná, Upper Rio Paraná Basin	Not available
LBP5035	24682	*Neoplecostomus yapo*	FJ434533	Ribeirão Atlântico, Mandaguaçu, Paraná, Upper Rio Paraná Basin	23º18'11"S 52º01'54"W
NUP3560	9699	*Neoplecostomus yapo*	GQ214783	Rio Fortaleza, Tibagi, Paraná, Upper Rio Paraná Basin	24°25'30"S 50°13'55"W
NUP3560	9700	*Neoplecostomus yapo*	GQ214782	Rio Fortaleza, Tibagi, Paraná, Upper Rio Paraná Basin	24°25'30"S 50°13'55"W
NUP3560	9701	*Neoplecostomus yapo*	FJ434529	Rio Fortaleza, Tibagi, Paraná, Upper Rio Paraná Basin	24°25'30"S 50°13'55"W
LBP7383	34842	*Neoplecostomus selenae*	GQ214792	Ribeirão das Batéias, Riacho Grande, São Paulo, Upper Rio Paraná Basin	24°12'02"S 48°25'06"W
LBP7383	34843	*Neoplecostomus selenae*	FJ965506	Ribeirão das Batéias, Riacho Grande, São Paulo, Upper Rio Paraná Basin	24°12'02"S 48°25'06"W
LBP7383	34844	*Neoplecostomus selenae*	GQ214791	Ribeirão das Batéias, Riacho Grande, São Paulo, Upper Rio Paraná Basin	24°12'02"S 48°25'06"W
LBP7383	34845	*Neoplecostomus selenae*	GQ214790	Ribeirão das Batéias, Riacho Grande, São Paulo, Upper Rio Paraná Basin	24°12'02"S 48°25'06"W

LBP7383	34846	*Neoplecostomus selenae*	GQ214789	Ribeirão das Batéias, Riacho Grande, São Paulo, Upper Rio Paraná Basin	24°12’02”S 48°25’06”W
LBP7464	33410	*Neoplecostomus corumba*	GQ214779	Rio Taquarí, Carandaí, Minas Gerais, Upper Rio Paraná Basin	17°29’29”S 48°22’13”W
LBP7464	33411	*Neoplecostomus corumba*	GQ214780	Rio Taquarí, Carandaí, Minas Gerais, Upper Rio Paraná Basin	17°29’29”S 48°22’13”W
LBP7464	33413	*Neoplecostomus corumba*	GQ214778	Rio Taquarí, Carandaí, Minas Gerais, Upper Rio Paraná Basin	17°29’29”S 48°22’13”W
LBP7464	33415	*Neoplecostomus corumba*	GQ214777	Rio Taquarí, Carandaí, Minas Gerais, Upper Rio Paraná Basin	17°29’29”S 48°22’13”W
NUP2528	9423	*Neoplecostomus corumba*	FJ434543	Córrego Gameleira, Corumbaíba, Goiás, Upper Rio Paraná Basin	17°43’37”S 48°32’54”W
LBP2551	15243	*Neoplecostomus espiritosantensis*	FJ434530	Rio Jucu, Domingos Martins, Espírito Santos, Oriental Coastal Basin	20°24’30”S 40°54’55”W
LBP2551	17102	*Neoplecostomus espiritosantensis*	GQ214810	Rio Jucu, Domingos Martins, Espírito Santos, Oriental Coastal Basin	20°24’30”S 40°54’55”W
LBP2551	17103	*Neoplecostomus espiritosantensis*	GQ214809	Rio Jucu, Domingos Martins, Espírito Santos, Oriental Coastal Basin	20°24’30”S 40°54’55”W
LBP2551	17104	*Neoplecostomus espiritosantensis*	GQ214808	Rio Jucu, Domingos Martins, Espírito Santos, Oriental Coastal Basin	20°24’30”S 40°54’55”W
LBP6489	31533	*Neoplecostomus franciscoensis*	GQ214804	Rio das Velhas, São Bartolomeu, Minas Gerais, Rio São Francisco Basin	20°18’43”S 43°34’02”W
LBP6489	31535	*Neoplecostomus franciscoensis*	GQ214812	Rio das Velhas, São Bartolomeu, Minas Gerais, Rio São Francisco Basin	20°18’43”S 43°34’02”W
LBP6493	31549	*Neoplecostomus franciscoensis*	GQ214811	Afluente rio das Velhas, Ouro Preto, Minas Gerais, Rio São Francisco Basin	20°17’14”S 43°37’53”W
LBP6537	31681	*Neoplecostomus franciscoensis*	FJ965503	Afluente rio das Velhas, Ouro Preto, Minas Gerais, Rio São Francisco Basin	20°00’37”S 43°58’08”W
LBP645	7593	*Neoplecostomus microps*	FJ434531	Ribeirão Cajarana, Pindamonhangaba, São Paulo, Rio Paraiba do Sul Basin	22°46’00”S 45°27’00”W
LBP1094	10232	*Neoplecostomus microps*	GQ214807	Ribeirão Fernandes, Sta. Bárbara do Tugúrio, Minas Gerais, Rio Paraiba do Sul Basin	21°14’47”S 43°34’07”W
LBP6231	29382	*Neoplecostomus microps*	GQ214806	Ribeirão Água Santa, São José dos Barreiros, São Paulo, Rio Paraíba do Sul Basin	22°39’19”S 44°34’44”W
LBP6319	29383	*Neoplecostomus microps*	GQ214805	Ribeirão Água Santa, São José dos Barreiros, São Paulo, Rio Paraíba do Sul Basin	22°39’19”S 44°34’44”W
LBP6319	29385	*Neoplecostomus microps*	GQ214813	Ribeirão Água Santa, São José dos Barreiros, São Paulo, Rio Paraíba do Sul Basin	22°39’19”S 44°34’44”W
LBP7965	9735	*Neoplecostomus ribeirensis*	GQ214814	Rio Betari, Iporanga, São Paulo, Rio Ribeira do Iguape Basin	24°33’44”S 48°40’09”W
LBP7384	34837	*Neoplecostomus ribeirensis*	GQ214776	Rio Água Doce, Tapiraí, São Paulo, Rio Ribeira do Iguape Basin	22°27’02”S 49°14’26”W
LBP7384	34838	*Neoplecostomus ribeirensis*	GQ214775	Rio Água Doce, Tapiraí, São Paulo, Rio Ribeira do Iguape Basin	22°27’02”S 49°14’26”W
LBP7384	34839	*Neoplecostomus ribeirensis*	GQ214774	Rio Água Doce, Tapiraí, São Paulo, Rio Ribeira do Iguape Basin	22°27’02”S 49°14’26”W
LBP7384	34841	*Neoplecostomus ribeirensis*	GQ214773	Rio Água Doce, Tapiraí, São Paulo, Rio Ribeira do Iguape Basin	22°27’02”S 49°14’26”W

Results

Neoplecostomus sp. nov. 1, new species

Holotype: MZUSP xx (1 male 109.9 mm SL), Brazil, São Paulo State, Municipality of Salesópolis, rio Paraitinguinha, rio Tietê Basin, 23°31'25"S 43°53'22"W, 14 Sep 2006, R. Devidé, J. Carlos, L. R. Paiva.

Paratypes: All from Brazil, São Paulo State, Municipality of Salesópolis, rio Paraitinguinha, rio Tietê basin. DZSJRP xx (1 male 88.0 mm SL, 2 female 60.5-83.4 mm SL) collected with holotype. LBP 2861 (10 male 82.2-106.4 mm SL, 16 unsexed

Figure 1. *Neoplecostomus sp. nov. 1*, holotype from Rio Paraitinguinha in Salesópolis municipality, SL=109.99mm.

not measured) 23º31'37"S 45º45'53"W, 20 Mai 2005, E. R. M. Martinez et al. LBP 3578 (1 female 41.3 mm SL, 1 unsexed not measured), 23º30'40"S 45º51'32"W, 21 Jul 2008, R. Devidé et al. LBP 3921 (2 male 58.7-94.9 mm SL, 4 female 46.0-60.9 mm SL, 7 unsexed not measured), 23º31'25"S 43º53'22"W, 14 Sep 2006, R. Devidé et al. LBP 4993 (2 female 40.8-74.3 mm SL, 1 unsexed not measured), 23°30'40"S 45°51'32"W, R. Devidé et al. MZUSP 59117 (1 male 46.1 mm SL, 1 female 56.0 mm SL), 23º31'37"S 45º45'52"W, 17 Dec 1999, L. R. Malabarba et al. MZUSP 59118 (1 female 58.2 mm SL), 23°35'02"S 45°46'43"W, 17 Dec 1999, L. R. Malabarba et al. MZUSP 59139 (1 unsexed not measured), 23º31'37"S 45º45'52"W, 17 Dec 1999, L. R. Malabarba et al. MZUSP 87141 (6 unsexed not measured), no coordinates available, 15 May 1999, M. R. Britto et al. NUP 6103 (1 male 101.7 mm SL, 1 female 74.3 mm SL, 18 unsexed not measured); 23º31'37"S 45º45'52"W, 17 Dec 1999, L. R. Malabarba et al.

Diagnosis: *Neoplecostomus sp. nov. 1* is distinguish from all other congeners by having plates from the mid-ventral and ventral series of plates widely spaced (usually the skin area between two adjacent plates are similar in length to each plate vs skin area between each adjacent plates absent or smaller than the length of the plates. Additionaly, *N.* sp. *nov. 1* is distinguished from all other congeners by the odontodes along snout margin and ridge over eyes moderately enlarged than the remaining odontodes on head. From *N. selenae* and *N. yapo* it is distinguished by the lack of an evident swollen skin around the enlarged odontodes on snout margin and ridge over eyes.

Description: Counts and measurements are presented in Table 2. Body elongated and depressed. Greatest width at cleithrum, narrowing to caudal peduncle. Dorsal body profile gently convex, elevating from snout tip to dorsal-fin origin and descending to first caudal-fin procurrent spine. Greatest body depth at dorsal-fin origin. Trunk and caudal peduncle dorsally rounded in cross-section; body ventrally flattened to anal-fin origin, flattened to slightly rounded to caudal fin. Dorsal body surface completely covered by dermal plates, excepting for a naked area around dorsal-fin base. Snout tip naked. Ventral head surface naked except by a plate bearing odontodes in front of gill openings. Abdomen with conspicuous, small dermal platelets between insertions of pectoral and pelvic fins, forming a thoracic shield surrounded by naked areas. Head wide and depressed. Head and snout rounded in dorsal view. Interorbital space straight to slightly concave in frontal view. Median ridge rising from snout tip to area between

nares, more evident on larger specimens. A pronounced ridge from nares to superior margin of orbit. Snout convex in lateral profile. Mature males with moderately enlarged odontodes and distinct swollen skin along lateral margins of snout. Eye moderately small (6.7-10.8 of HL), dorsolaterally placed. Lips well developed and rounded. Lower lip far from reaching pectoral girdle and covered with papillae, wider anteriorly; two to three irregular and conspicuous rows of large and transversally flattened papillae, just posterior to dentary teeth; posterior row of papillae distributed along whole dentary ramus. Maxillary barbel short, coalesced, usually its tip not free from lower lip. Teeth long, slender and bicuspid; mesial cusp longer than lateral. Dentary rami forming an angle of approximately 125-130º. Dorsal-fin origin slightly posterior to vertical passing through pelvic-fin origin; nuchal plate not covered by skin; dorsal-fin spinelet half-moon shaped and wider than dorsal-fin spine base; dorsal-fin locking mechanism absent. Dorsal fin with one flexible spine, followed by seven branched rays; its posterior margin slightly rounded, not reaching vertical through end of pelvic-fin rays when adpressed. Well-developed and always present adipose fin, preceded by azygous plate. Pectoral fin with one spine and six branched rays; spine depressed and curved inward (more pronounced in larger specimens), shorter than longest branched ray, its posterior margin slightly convex, reaching or almost reaching pelvic-fin insertion when adpressed. Pelvic fin with one spine and five branched rays; its posterior margin nearly straight, reaching to almost reaching anal-fin insertion when adpressed. Pelvic-fin spine ventrally flattened, with dermal flap on its dorsal surface in males. Anal fin with one flexible spine and five branched rays; its posterior margin nearly straight. Caudal fin furcate; lower lobe longer than upper; 14 branched rays. Pectoral and pelvic-fin spines with odontodes on lateral and ventral portions. Anal-fin spine with odontodes only ventrally.

Color in alcohol: Ground color of dorsal surface of head and body yellowish. Head, dorsum, flanks and fins covered by some inconspicuous darker dots or blotches of variable shapes and sizes. Dorsal color pattern, even in mature larger individuals, retains the generic juvenile color pattern of five transverse dark bars: the first through supraoccipital, the second anterior to dorsal fin, the third posterior to dorsal fin, the fourth at adipose fin, and the last at caudal-peduncle posterior portion. Head usually with two light, short and parallel, lines anterior to nares, bordering the naked area on snout tip. A clear spot on naked area of snout tip. Orbital margin lighter, mainly on its superior portion. Small clear spot on interorbital space, inconspicuous in some

specimens. Body lateral portion with an upper darker region and a lower lighter one, just below lateral line, not easily visualized in large specimens. Dorsal fin with irregular series of dark dots on rays. Caudal fin irregularly dark at base and distal portion of rays, leaving two lighter areas on median portion and rays tips, in some specimens. Pectorals, pelvics, anal and adipose fins with dark dots forming irregular bands usually diffuse. Ventral surface of head and abdomen mostly depigmented, except lateral margins of body and from pelvic fin to caudal-fin base; upper lip dark brown, except for its light narrow margin.

Sexual dimorphism: the samples of this species have sexual dimorphism. The male has a papilla in the cloaka and a membrane in ventral fin absente in the female.

Distribution: The specie is known only from the type-locality.

***Neoplecostomus sp. nov. 2*, new species**

Holotype: MZUSP xx, (1 male 85.5 mm SL), Brazil, Minas Gerais, Muzambinho, rio São Domingos, tributary to rio Muzambinho, rio Grande Basin, 21°23'22"S 46°28'40"W, 7 Jan 2008, F. F. Roxo, K. T. Abe, J. M. Henriques, G. J. Costa e Silva, L. H. G. Pereira.

Paratypes: All from Brazil, Minas Gerais, Muzambinho, rio Muzambinho, rio Grande basin. DZSJRP xx (5 female 44.8-63.9 mm SL) 21°19'36"S 46°27'27"W, 10 Jan 2008, F. F. Roxo et al. DZSJRP xx (2 male 69,0-70,6 mm SL) 21°23'22"S 46°28'40"W, 7 Jan 2008, F. F. Roxo et al. LBP 5870 (21, unsexed not measured); 21°20'47"S 46°28'08"W, 9 Jan 2008, F. F. Roxo et al. LBP 5873 (1, unsexed not measured); 21°19'36"S 46°27'27"W, 9 Jan 2008, F. F. Roxo et al. LBP 5878 (2 male not measured 7 female not measured); 21°23'53"S 46°28'45"W, 9 Jan 2008, F. F. Roxo et al. LBP 5886 (2 male not measured, 4 female not measured, 14 unsexed not measured), 21°18'08"S 46°28'33"W, 9 Jan 2008, F. F. Roxo et al. LBP 5901 (2 male 61.3-65.0 mm

Figure 2. *Neoplecostomus sp. nov. 2,* holotype from rio Muzambinho drainages in Muzambinho municipality, SL=85.05mm.

SL, 1 not measured, 5 female 54.2-62.9 mm SL, 4 not measured); 21°17'37"S 46°29'06"W, 11 Jan 2008, F. F. Roxo et al. LBP 5915 (1 male 68.3 mm SL, 1 unsexed not measured); 21°21'33"S 46°28'32"W, 8 Jan 2008, F. F. Roxo et al. LBP 5926 (1 male not measured, 3 female not measured) 21°19'59"S 46°27'24"W, 10 Jan 2008, F. F. Roxo et al. LBP 5931 (4 male 51.8-69.6 mm SL, 7 female 48.4-62.8 mm SL, 8 unsexed not measured), 21°23'22"S 46°28'40"W, 07 Jan 2008, F. F. Roxo et al. LBP 5942 (1 unsexed not measured), 21°22'48"S 46°28'29"W, 8 Jan 2008, F. F. Roxo et al. LBP 5947 (2 male 71.6-73.5 mm SL, 6 female 56.6-67.6 mm SL, 32 unsexed not measured), 21°19'36"S 46°27'27"W, 10 Jan 2008, F. F. Roxo et al. LBP 5961 (16 unsexed not measured) 21°22'48"S 46°28'29"W, 8 Jan 2008, F. F. Roxo et al. LBP 6142 (2 male 42.2-45.7 mm SL, 7 female 38.1-49.2 mm SL); 21°24'12"S 46°34'33"W,

15 Apr 2008, F. F. Roxo et al. LBP 6150 (3 male not measured, 1 female not measured), 21°22'43"S 46°33'21"W, 15 Apr 2008, F. F. Roxo et al. LBP 6155 (11 unsexed not measured); 21°23'49"S 46°33'17"W, 15 Apr 2008, F. F. Roxo et al. LBP 6160 (3 unsexed not measured) 21°23'04"S 46°32'22"W, 16 Apr 2008, F. F. Roxo et al. LBP 6173 (5 female 39.7-47.0 mm SL); 21°21'41"S 46°34'36"W, 16 Apr 2008, F. F. Roxo et al. LBP 6179 (4 male not measured, 1 female not measured, 5 unsexed not measured); 21°21'40"S 46°33'22"W, 16 Apr 2008, F. F. Roxo et al. LBP 6183 (1 male 44.4 mm SL); 21°22'13"S 46°32'11"W, 16 Apr 2008, F. F. Roxo et al. LBP 6195 (1 male 68.0 mm SL, 5 female 35.1-68.0 mm SL, 1 unsexed not measured), 21°22'15"S 46°32'35"W, 18 Apr 2008, F. F. Roxo et al. LBP 6210 (1 unsexed not measured). 21º23'31"S 46º30'11"W, 18 Apr 2008, F. F. Roxo et al. LBP 6244 (3 male 68.7-79.0 mm SL); 21°19'44"S 46°30'04"W, 19 Apr 2008, F. F. Roxo et al. MZUSP xx (1 male 85.5 mm SL) 21°17'37"S 46°29'06"W, 11 Jan 2008, F. F. Roxo et al. MZUSP xx (3 male not measured, 7 female not measured); 21°19'36"S 46°27'27"W, 10 Jan 2008, F. F. Roxo et al. NUP 6102 (2 male not measured, 5 female not measured) 21°20'47"S 46°28'08"W, 09 Mar 2008, F. F. Roxo et al.

Diagnosis: *Neoplecostomus sp. nov. 2* is distinguish from *N. sp. nov. 1* by having the skin area between each adjacent plates absent or smaller than the length of the plates vs plates from the mid-ventral and ventral series of plates widely spaced, usually the skin area between tow adjacent plates are similar in length to each plate; *N. sp. nov. 3* and *N. paranensis* by having well-developed adipose fin vs adipose fin reduced or absent; by *N espiritosantensis* and *N. ribeirensis* by having dorsal-fin spinelet larger than dorsal-fin spine vs dorsal-fin spinelet absent to vestigial and not wider that dorsal-fin spine; from *N. granosus*, *N. microps* and *N. varipiictus* by five dark bands on dorsum evident and lacking evident dark spots vs spots evident all over body and fins and dorsal bands not evident; by *N. selenae* by lacking enlarged odontodes and distinct swollen skin along lateral margins of snout and along ridges before the eyes vs having enlarged odontodes and distinct swollen skin along lateral margins of snout and along ridges before the eyes; from *N. corumba* by having smaller eyes, orbital diameter 8.3-11.4 % in head length, 12.9-18.5% in snout length and, 27.0-35.7% in interobital length vs 12.2-13.05%, 18.4-20.1% and, 36.7-41.5% respectively; from *N. yapo* by having interdorsal length 14.8-19.5% in SL vs 20.7-23.0%, caudal peduncle depth 19.8-29.0% in caudal peduncle length vs 17.6-19.6%, orbital diameter 8.3-11.4% in head length and 19.8-29.0% in snout length vs 11.9-21.4% and 19.1-21.4% respectively.

Description: Counts and measurements are presented in Table 2. Body elongated and depressed. Greatest width at cleithrum, narrowing to caudal peduncle. Dorsal body profile gently convex, elevating from snout tip to dorsal-fin origin and descending to first caudal-fin procurrent spine. Greatest body depth at dorsal-fin origin. Trunk and caudal peduncle dorsally rounded in cross-section; body ventrally flattened to anal-fin origin, flattened to slightly rounded to caudal fin. Dorsal body surface completely covered by dermal plates, excepting for a naked area around dorsal-fin base. Snout tip naked. Ventral head surface naked except by a plate bearing odontodes in front of gill openings. Abdomen with conspicuous, small dermal platelets between insertions of pectoral and pelvic fins, forming a thoracic shield surrounded by naked areas; in some specimens also some isolated platelets near pectoral-fin base. Head wide and depressed. Head and snout rounded in dorsal view. Interorbital space straight to slightly concave in frontal view. Slight median ridge rising from snout tip to area between nares, not evident in some specimens. A ridge from nares to superior margin of orbit. Snout gently convex in lateral profile. Mature males with moderately enlarged boomerang-like (curved backward) odontodes, mainly on anterior portion of head, that is, from snout tip to post-orbital region. Hypertrophied odontodes not surrounded by distinct swollen skin along dorsal and ventral lateral margin of snout. Eye moderately small (8.3-11.4 of HL), dorsolaterally placed. Lips well developed and rounded. Lower lip not reaching pectoral girdle and covered with papillae, wider anteriorly; two or three irregular and conspicuous rows of large and transversally flattened papillae, just posterior to dentary teeth; posterior row of papillae distributed along whole dentary ramus. Maxillary barbel short and coalesced with lower lip, its tip not free from lower lip. Teeth long, slender and bicuspid; mesial cusp longer than lateral. Dentary rami forming an angle of approximately 125-130º. Dorsal-fin origin slightly posterior to vertical passing through pelvic-fin origin; nuchal plate not covered by skin; dorsal-fin spinelet short and wider than dorsal-fin spine base; dorsal-fin locking mechanism absent. Dorsal-fin with one flexible spine, followed by seven branched rays; its posterior margin straight or slightly falcate, not reaching vertical through end of pelvic-fin rays when adpressed. Well-developed and always present adipose fin, not preceded by azygous plate. Pectoral fin with one spine and six branched rays; spine depressed and curved inward curved (more pronounced in larger specimens), shorter than longest branched ray, its posterior margin emarginate, reaching about half pelvic-fin spine length when adpressed. Pelvic fin with one spine and five branched rays; its posterior margin straight to nearly straight,

surpassing anal-fin insertion when adpressed. Pelvic-fin spine ventrally flattened, with dermal flap on its dorsal surface in males. Anal fin with one flexible spine and five branched rays; its posterior margin slight emarginated to straight. Caudal fin falcate; lower lobe longer than upper; 14 branched rays. Pectoral and pelvic-fin spines with odontodes on lateral and ventral portions. Anal-fin spine with odontodes only ventrally.

Color in alcohol: Ground color of dorsal surface of head and body yellowish. Head, dorsum, flanks and fins covered by some few inconspicuous darker dots or blotches of variable shapes and sizes. Dorsal color pattern, even in mature larger individuals, retains the generic juvenile color pattern of five transverse dark bars: the first through supraoccipital, the second anterior to dorsal fin, the third posterior to dorsal fin, the fourth at adipose fin, and the last at caudal-peduncle posterior portion. Head usually with two clear, short and parallel inconspicuous lines anterior to nares, bordering the naked area on snout tip. Orbital margin lighter, mainly on its superior portion. Small light spot on interorbital space, inconspicuous in some specimens. Body lateral portion with an upper darker region and a lower lighter one, just below lateral line, not easily visualized in large specimens. Dorsal fin with irregular series of dark dots or bands on rays. Caudal fin with three irregular dark bands at base, at middle portion, and at distal portion of rays, leaving two interspaced lighter areas among dark bands. Pectorals, pelvics, and anal fins with dark dots forming irregular bands usually diffuse. Adipose fin generally dark on spine and pale on the membrane portion. Ventral surface of head and body mostly depigmented, except on lateral body margins and from pelvic fin to caudal-fin base; upper lip dark brown, except for its light narrow margin.

Sexual dimorphism: the samples of this species have sexual dimorphism. The male has a papilla in the cloaka and a membrane in ventral fin absent in female.

Distribution: The specie is known from the drainages of Rio Muzambinho in Minas Gerais in the municipality of Muzambinho.

***Neoplecostomus sp. nov. 3*, new species**

Figure 3. *Neoplecostomus sp. nov. 3,* holotype from Córrego Águas de Madalena in Botucatu municipality, SL=98.41mm.

Neoplecostomus paranensis. - Zawadzki et al. (2008): 36 [photo; Figure 1 a and b comparing absent and ill-developed adipose fin, respectively]; Alves et al. (2005): 128 [citogenetics; ribeirão Hortelã, tributary to rio Pardo].

Holotype: MZUSP xx, (1 male 98.62 mm SL), Brazil, São Paulo, Botucatu, córrego Águas de Madalena, tributary rio Pardo, rio Paranapanema basin, 22°59'25"S 48°25'37"W, 22 Mar 2009, F. F. Roxo, M. F. Pazian, M. N. Mehanna, B. F. Melo.

Paratypes: All from Brazil, São Paulo, Botucatu, Córrego Águas de Madalena, tributary to rio Pardo, rio Paranapanema basin. DZSJRP xx (4 male 62.3-95.9 mm SL, 4 female 56.1-78.1 mm SL) collected with holotype. LBP 2937 (1 unsexed not measured),

22°59'25"S 48°25'37"W, 12 Apr 2005, R. Devidé et al. LBP 2969 (1 male not measured, 1 female not measured), 22°59’25”S 48°25’37”W, 12 Apr 2005, R. Devidé et al. LBP 7525 (5 male 91,0-102,2 mm SL, 1 cs, 98,4 mm SL, 15 female 56,2-98,6 mm SL), 22°59’25”S 48°25’37”W, 15 Nov 2008, F. F. Roxo et al. LBP 8065, (3 male 73.5-88.2 mm SL, 9 female 67.5-84.9 mm SL), 22°59’25”S 48°25’37”W, 22 Mar 2009, F. F. Roxo et al. MZUSP xx (2 male 85.3-89.8 mm SL, 1 female 76.7 mm SL). 22°59’25”S 48°25’37”W, 22 Mar 2009, F. F. Roxo et al. NUP 8015 (1 male 90.7 mm SL, 2 female 71.3-75.8 mm SL), 22°59’25”S 48°25’37”W, 24 Aug 2008, R. Devidé et al. NUP 8016 (1 male 69.8 mm SL), 22°59’25”S 48°25’37”W, 31 Jul 2008, R. Devidé et al. NUP 8118 (1 male 95.9 mm SL) 22°59’25”S 48°25’37”W, 22 Mar 2009, F. F. Roxo et al.

Diagnosis: *Neoplecostomus sp. nov. 3* is distinguished from all other congers, except *N. paranensis* by the lack or reduction of the adipose fin vs. adipose fin moderate to well developed and always present. From *N. paranensis* it is distinguished by dark spots evident all over body and fins and dorsal bands not evident vs five dark band on dorsum evident and lacking evident dark spots; rudimentar maxilary barbel usually present and linked to lower lip by a labial papillae vs maxillary barbell very reduced and lacking labial papillae.

Description: Counts and measurements are presented in Table 2. Body elongated and depressed. Greatest width at cleithrum, narrowing to caudal peduncle. Dorsal body profile gently convex, elevating from snout tip to dorsal-fin origin and descending to first caudal-fin procurrent spine. Greatest body depth at dorsal-fin origin. Trunk and caudal peduncle dorsally rounded in cross-section; body ventrally flattened to anal-fin origin, flattened to slightly rounded to caudal fin. Dorsal body surface completely covered by dermal plates, excepting for a naked area around dorsal-fin base. Snout tip naked. Ventral head surface naked except by a plate bearing odontodes in front of gill openings. Abdomen with conspicuous, small dermal platelets between insertions of pectoral and pelvic fins, forming a thoracic shield surrounded by naked areas; in some specimens also some isolated platelets near pectoral-fin base. Head wide and depressed. Head and snout rounded in dorsal view. Interorbital space slight concave in frontal view. Median ridge slightly rising from snout tip to area between nares evident in specimens up to 70 mm SL. A ridge from snout to superior margin of orbit. Snout gently convex in lateral profile. Mature male with moderately enlarged odontodes and distinct swollen skin on whole head. Eye moderately small (8.0–10.6 of HL),

dorsolaterally placed. Lips well developed and rounded. Lower lip almost reaching pectoral girdle and covered with papillae, wider anteriorly; two or three irregular and conspicuous rows of large and transversally flattened papillae, just posterior to dentary teeth; posterior row of papillae mesially restricted, that is, occupying the mesial half of dentary ramus. Maxillary barbel short and coalesced with lower lip, generally with free tip. Teeth long, slender and bicuspid; mesial cusp longer than lateral. Dentary rami forming an angle of approximately 100–120º. Dorsal-fin origin slightly posterior to vertical passing through pelvic-fin origin; nuchal plate not covered by skin; dorsal-fin spinelet very short, usually composed by a transversally orientated row of single odontodes, absent in some few specimens; dorsal-fin locking mechanism absent. Dorsal-fin with spine flexible, followed by seven branched rays; its posterior margin straight or slightly falcate, not reaching vertical through end of pelvic-fin rays when adpressed. Ill-developed and not always present adipose fin (see Zawadzki et al., 2008 Fig. 1A and 1B). Pectoral fin with one spine and six branched rays; spine depressed and curved inward (more pronounced in larger specimens), shorter than longest branched ray, its posterior margin nearly straight, reaching about one-third pelvic-fin spine length when adpressed. Pelvic fin with one spine and five branched rays; its posterior margin straight to slight concave, not to almost reaching anal-fin insertion when adpressed. Pelvic-fin spine ventrally flattened, with dermal flap on its dorsal surface in males. Anal fin with one flexible spine and five branched rays; its posterior margin straight to slight convex. Caudal fin moderate falcate; lower lobe slight longer than upper; 14 branched rays. Pectoral and pelvic-fin spines with odontodes on lateral and ventral portions. Anal-fin spine with odontodes only ventrally.

Color in alcohol: Ground color of dorsal surface of head and body yellowish. Head, dorsum, flanks and fins covered by numerous conspicuous dark irregular blotches of variable shapes and sizes. Dorsal color pattern, in specimens up to 60.0 mm SL retains the generic juvenile color pattern of five transverse dark bars: the first through supraoccipital, the second anterior to dorsal fin, the third at dorsal-fin end, the fourth at adipose fin, and the last at caudal-peduncle posterior portion; in larger specimens the dark bars are no longer distinguished. Orbital margin lighter, mainly on its superior portion. All fins, with irregular series of dark dots on rays, sometimes forming irregular, diffused, transverse stripes; adipose fin, when present, with a dark spot on distal portion of spine. Ventral surface of head and body mostly depigmented, except for some brown,

faded and scattered cromatophores on lateral margins of body from pelvic fin to caudal-fin base; upper lip dark brown, except for its light narrow margin.

Sexual dimorphism: the samples of this species have sexual dimorphism. The male has a papilla in the cloaka and a membrane in ventral fin absent in female.

Distribution: *Neoplecostomus sp. nov. 3* is only known from the type-locality.

Genetic Analysis

We sequenced the COI gene for 53 samples of 11 species of the genus *Neoplecostomus*. Eight were described species and three of them are the new species, described in the present work. The final matrix has 591 pb and all of the sequences were deposited in Genbank (Table 1). The nucleotide frequencies are 0.237 (A), 0.254 (T/U), 0.317 (C), and 0.192 (G). The overall transition/transversion bias is R = 5.243. Saturation was not observed in transitions or transversions. The presented phylogeny is a 50% majority-rule consensus performed by Neighbor-Joining method, and the clustered frequency is shown at each node.

Genetic distances between *Neoplecostomus* species and between samples of the same localities are shown in Table 3. The distances between the samples of different hydrographic basins and between samples of the same basin are shown in Table 4. We found in the analysis 11 different clusters corroborating the morphological traits.

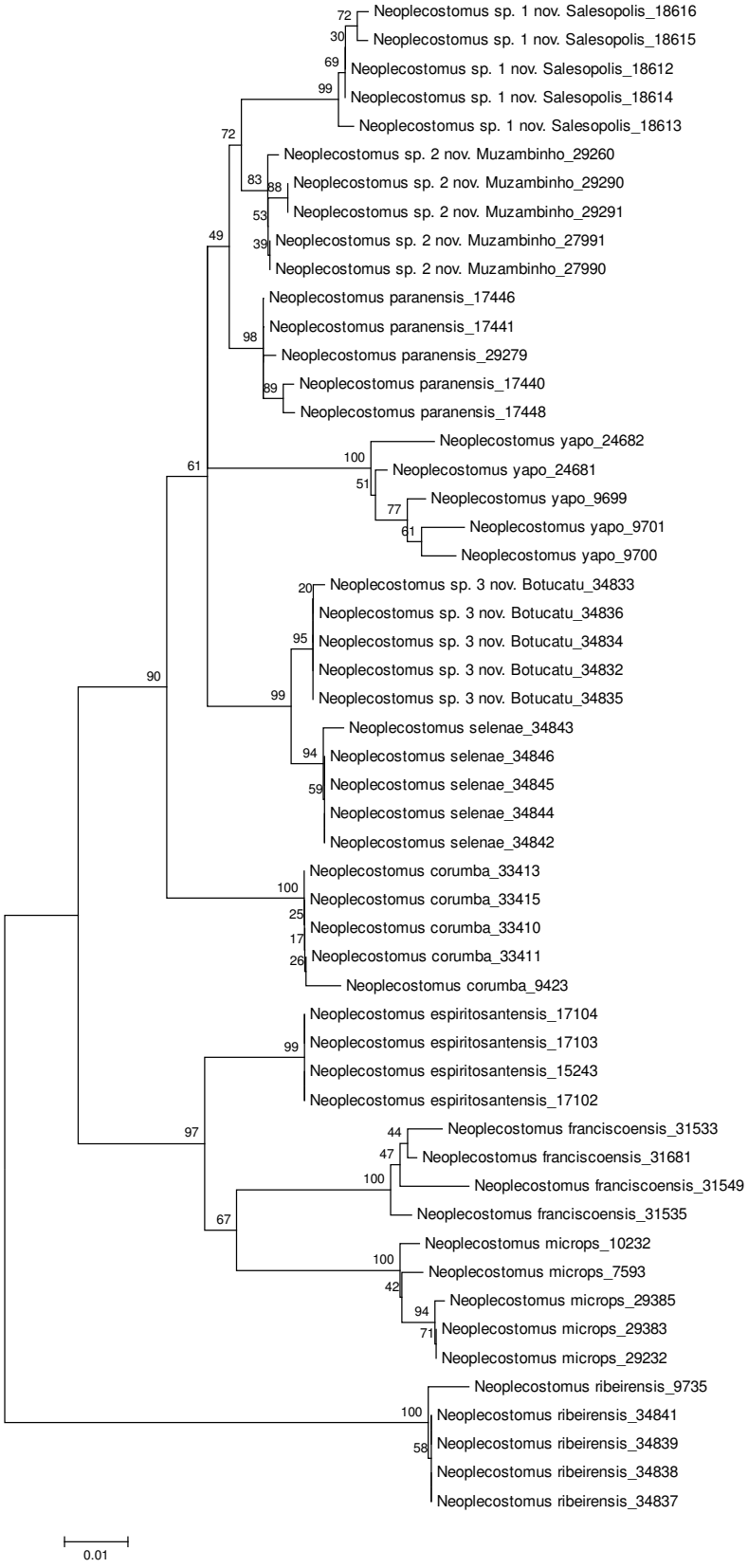


Figure 4. Plylogenetic tree constructed with Neighbor-Joining method with COI gene (591 pb). Numbers above branch are bootstrap values.

Table 2. Morphometric data and counts of *Neoplecostomus sp. nov 1*, *N. sp. nov. 2* and *N. sp. nov 3* from the Upper Rio Paraná Basin. CP=caudal-peduncle, IO=interorbital length, OD=orbital diameter, PDS=predorsal, SL=standard length, ad=adipose-fin, an=anal-fin, cd=caudal-fin, ds=dorsal-fin.

Character	*Neoplecostomus sp. nov. 1* n = 20					*Neoplecostomus sp. nov. 2* n = 32					*Neoplecostomus sp. nov. 3* n = 46				
	Holotype	Low	High	Mean/ Mode	SD	Holotype	Low	High	Mean/ Mode	SD	Holotype	Low	High	Mean/ Mode	SD
Standard length	109.9	53.8	109.9	86.3	16.77	73.1	51.9	85.5	66.1	6.59	98.6	56.2	102.2	78.5	11.47
Percents of SL															
Predorsal length	42.6	42.3	46.8	44.0	1.13	44.9	41.6	47.7	44.5	1.51	40.9	39.6	44.0	41.9	0.96
Head length	30.8	30.4	34.3	31.8	1.00	32.7	30.9	34.3	32.0	0.93	27.6	27.6	31.8	29.5	0.92
Head width	26.9	26.1	28.9	27.1	0.82	28.0	25.8	29.9	27.6	0.92	26.1	25.0	28.4	26.4	0.72
Cleithral width	25.8	25.2	29.6	26.6	0.91	27.9	25.6	30.0	27.4	0.95	25.8	25.0	27.9	26.0	0.61
Occipital-dorsal distance	12.4	12.1	14.9	13.3	0.89	12.6	11.7	15.1	13.3	0.76	13.2	11.3	15.0	13.2	0.91
Thoracic length	13.6	12.4	15.4	13.6	0.81	14.2	11.0	16.5	13.8	1.13	14.6	12.8	16.3	14.1	0.90
Interdorsal length	18.4	16.3	20.8	18.3	1.28	17.2	14.8	19.5	17.6	1.20	21.8	18.3	23.6	20.2	1.36
CP length	29.6	27.2	32.8	29.4	1.18	25.8	25.8	31.6	28.3	1.33	30.6	28.2	33.2	30.0	1.10
CP depth	7.2	6.2	7.8	6.8	0.42	7.3	5.7	8.0	6.8	0.56	6.9	6.1	8.3	6.8	0.50
Body depth	15.6	12.2	17.9	15.5	1.70	16.0	13.3	19.1	16.1	1.42	17.9	14.7	20.1	17.4	1.52
Preanal length	64.0	58.8	70.7	63.2	2.18	65.2	58.7	66.8	63.3	1.95	61.4	58.4	63.5	61.0	1.13
Percents of head length															
Head width	87.2	82.1	89.6	85.5	2.20	85.6	82.5	89.8	86.3	1.94	94.4	85.0	95.6	89.5	2.81
Head depth	50.3	41.8	54.2	49.4	3.36	50.7	42.7	56.8	49.2	3.25	60.0	54.2	63.9	57.7	2.67
Snout length	67.5	61.8	69.7	66.9	1.78	64.7	60.3	67.7	64.2	1.67	68.9	61.2	68.9	65.6	1.56
OD	7.4	6.7	10.8	8.2	1.06	10.1	8.3	11.4	9.8	0.74	9.4	8.0	10.6	9.5	0.65
Interorbital width	31.7	29.9	35.2	32.3	1.44	32.1	29.5	34.6	31.9	1.11	36.1	31.6	36.1	34.0	1.17
Mandibullary width	19.1	15.7	23.4	18.2	1.67	14.9	13.4	19.9	16.4	1.58	15.0	12.1	17.8	14.3	1.37
Other percents															
Snout length/OD	11.0	9.8	16.1	12.3	1.82	15.7	12.9	18.5	15.2	1.31	13.6	12.2	16.6	14.5	1.14
IO/OD	23.5	20.4	31.1	25.4	2.91	31.6	27.0	35.7	30.6	2.52	26.0	23.2	32.0	27.9	2.14
IO/mandibullary width	60.0	45.9	66.5	56.6	5.13	46.4	43.6	59.9	51.5	5.02	41.6	34.7	50.5	42.2	4.20
PDS length/first ds ray length	44.2	40.6	48.7	43.8	2.06	47.9	41.7	53.8	47.6	2.94	45.5	38.7	48.2	44.0	2.45
CP length/CP depth	24.3	20.8	26.1	23.3	1.56	28.5	19.8	29.0	24.1	2.25	22.7	19.8	25.9	22.5	1.48
Pelvic-fin length/CP depth	29.0	25.8	33.9	29.1	1.81	28.2	22.9	34.5	27.6	2.98	28.3	24.9	33.9	28.0	2.40
Lower cd spine/CP depth	30.5	26.3	33.9	30.3	2.37	32.1	23.0	32.1	28.1	2.32	30.8	26.0	36.3	29.6	2.72
Counts															
Lateral-line plates	32	31	36	32	1.47	28	26	30	28	1.11	31	27	34	30	1.56
Predorsal plates	8	6	9	8	0.80	5	4	6	5	0.45	7	5	8	7	0.71
Plates of dorsal-fin base	6	6	7	6	0.23	6	5	7	6	0.40	5	5	7	6	0.53
Plates between ds and cd	19	19	23	19	1.15	18	16	22	19	1.39	20	17	21	18	0.80
Plates between ad and cd	8	7	9	8	0.61	7	7	9	8	0.57	9	8	10	9	0.67
Plates between an and cd	15	14	18	16	1.11	12	11	15	12	1.01	14	12	16	14	0.75
Premaxillary teeth	23	18	42	18	6.40	32	21	76	33	17.49	15	14	29	17	3.72
Dentary teeth	19	12	41	17	8.19	27	19	77	29	17.91	12	11	23	12	3.05

Table 3. Genetic distance between *Neoplecostomus* species and between samples of the same species (main diagonal).

		1	2	3	4	5	6	7	8	9	10	11
1	*Neoplecostomus sp. nov. 1*	0.004										
2	*Neoplecostomus sp. nov. 2*	0.023	0.003									
3	*Neoplecostomus sp. nov. 3*	0.038	0.025	0.001								
4	*Neoplecostomus paranensis*	0.028	0.014	0.029	0.004							
5	*Neoplecostomus corumba*	0.048	0.038	0.038	0.042	0.002						
6	*Neoplecostomus yapo*	0.057	0.044	0.050	0.041	0.065	0.015					
7	*Neoplecostomus selenae*	0.040	0.026	0.010	0.031	0.044	0.049	0.001				
8	*Neoplecostomus microps*	0.090	0.083	0.087	0.079	0.083	0.106	0.090	0.006			
9	*Neoplecostomus espiritosantensis*	0.072	0.065	0.069	0.059	0.062	0.080	0.071	0.053	0.000		
10	*Neoplecostomus franciscoensis*	0.092	0.085	0.088	0.077	0.086	0.091	0.090	0.059	0.046	0.012	
11	*Neoplecostomus ribeirensis*	0.109	0.097	0.107	0.101	0.113	0.118	0.107	0.112	0.111	0.127	0.003

The results found by morphological and molecular tools were congruent, thus both analyses were efficient to distinguish species of the genus *Neoplecostomus*. In molecular analysis we used the COI gene that has been used to distinguish species, common used on barcode studies (Hebert 2003). But we believe that three aspects are important to distinguish species using molecular and morphological tools: the formation of different clusters in COI gene dendrogram, the genetic divergence higher or similar than the mean of the described species from the genus *Neoplecostomus* and morphological aspects.

The formation of different clusters show us that the populations are structured and do not reproduce with each other or the transference of gene between the members of different populations are low. In our view this is the most important aspect because reproductive isolation is first part of the speciation process.

The genetic divergence is a variable aspect to analyze, because different groups have different evolutionary rates. Then the value of genetic distance which is used to make the break and discriminate two samples as two species is arbitrary. But is important to analyze this aspect and consider this like a complementary one in genetic analyze to help us understand the evolution of the genus *Neoplecostomus*.

All specimens sequenced formed monophyletic clusters at species level that were divergent from the other species (Figure 4). This isolation can happen because *Neoplecostomus* species live in very specific habitats, in headwater streams, were the water run fast, with the bottom covered by sand and rocks, 1 meter depth, and where the water has a great amount of oxygen (Langeani 1990). In Upper Rio Paraná Basin a lot of new species is being discovered, others were just published (Zawadzki et al. 2008).

The species *Neoplecostomus ribeirensis* has the higher genetic divergence for all others analyzed species (average between 11%). That value is higher than the distance between different genera found in different works about barcode (Hebert 2003; Ward 2005, 2008). On the other hand, the genetic distance among the samples of *Neoplecostomus ribeirensis* is very low (0.3%), even considering that they came from two distant geographic locations in Rio Ribeira de Iguape Basin, one from Rio Betari, Iporanga, São Paulo and the other from Rio Água Doce, Tapiraí, São Paulo.

In the group formed by *Neoplecostomus yapo* the intraspecific genetic divergence average is 1.5% and between this species the others species of the upper rio Paraná Basin is 5.1%, the highest in this basin. This can be explained because this

species has the highest distribution sampled and is possible that have more than one species in this group. The genetic distance found between members from *Neoplecostomus yapo* could be enough to distinguish different species from this group. But, until now, none morphological characters were found to break this population in different species.

Neoplecostomus paranensis has as type locality the municipality of Cajuru, in São Paulo state. In our analysis we included samples from Cajuru and a sample from Caldas, in Minas Gerais state. These samples stayed together with a low genetic divergence (average of 0.4%), suggesting that those samples represents two populations of the same species. Morphologically those samples are contiguous. They have an adipose fin moderately developed and sometimes out.

Neoplecostomus paranensis (see Figure 1) formed a sister group with *N. sp. nov. 1* plus *N. sp. nov. 2*. The last one is from Rio Grande basin, the same hydrographic basin that *Neoplecostomus paranensis*. This can explain the low genetic divergence found between then (1.4%). But morphologically the two species are different. *Neoplecostomus sp. nov. 2* has a developed adipose fin. *Neoplecostomus paranensis* has a moderately adipose fin and sometimes even do not have an adipose fin. *Neoplecostomus sp. nov. 1* has a genetic distance of 2.8% with *N. paranensis* and 2.3% with *N. sp. nov. 2*. Those values can be explained because the species of *N. tietensis* is from Rio Tietê Basin and *N. paranensis* and *N. sp. nov. 2* are from Rio Grande Basin.

Neoplecostomus selenae and *N. sp. nov. 3* have 1.0% of genetic divergence each other. The two samples are from the same hydrographic basin, Rio Paranapanema Basin. It suggests that those groups are the same species. However in morphological analysis those species are different. *Neoplecostomus selenae* is a species with a great number of odontodes found in the snout and with the standard length lower than all others described species of the genus.

The species from the Rio São Francisco Basin, Rio Jucu Basin and Rio Paraiba do Sul Basin have a genetic divergent higher that the mean of the species from upper Rio Paraná Basin. It happens because the speciation of those species is older than the speciation of the species from upper Rio Paraná Basin. The fragmentation between those basins is older than the isolation of this species in the basin of Upper Rio Paraná. In this basin the mean of the genetic divergent is 3.3%. Then most of the time species higher than those values found in this basin can be new species. The number is high when you compare the disjunction of specific species of the group of upper Rio Paraná basin, because we are comparing species from different localities in a great portion in

this basin. When we look by the mean in other basins the number is low because we are comparing samples from the same species and most of the time from the same geographic position.

References

Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Research **22:**4692-4693.

Alves AL, Oliveira C, Foresti F (2005) Comparative cytogenetic analysis of eleven species of subfamilies Neoplecostominae and Hypostominae (Siluriformes: Loricariidae). Genetica **124:**127-136.

Armbruster JW (2004) Phylogenetic relationships of the sucker-mouth armored catfishes (Loricariidae) with particular emphasis on the Hypostominae and the Ancistrinae. Zoological Journal of the Linnean Society **141:**1-80.

Britski HA, Langeani F (1988) *Pimelodus paranaensis*, sp. n., um novo Pimelodidae (Pisces, Siluriformes) do alto Paraná, Brasil. Revista Brasileira de Zoologia **5:**409–417.

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

Cramer CA, Liedke AMR Bonatto SL, Reis RE (2008) The phylogenetic relationship of the Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae) as inferred from mitochondrial cytochrome c oxidase I sequences. Bulletin of fish Biology **9:**51-59.

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

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

Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B **270:**313-322.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution **16:**111-120.

Langeani F (1990) Revisão do gênero *Neoplecostomus*, com a descrição de quatro espécies novas do sudeste brasileiro (Ostariophysi, Siluriformes, Loricariidae). Comunicações do Museu de Ciências da PUCRS, Série Zoologia **3:**3-31.

Philippsen JS, Renesto E, Gealh AM, Artoni RF, Shibatta OA, Zawadzki CH (2009) Genetic variability in four samples of *Neoplecostomus yapo* (Teleostei: Loricariidae) from the rio Paranapanema basin, Brazil. Neotropical Ichthyology **7:**25-30.

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

Souza Braga FM, Gomiero LM, Souza UP (2008) Aspectos da reprodução e alimentação de *Neoplecostomus microps* (Loricariidae, Neoplecostominae) na microbacia do Ribeirão Grande, serra da Mantiqueira oriental (Estado de São Paulo). Acta Scientiarum Biological Sciences **30:**455-463.

Swofford DL, Olsen GJ, Waddell PJ, Hillis DM (1996) Phylogenetic inference. Pp. 407-514. *In*: Hillis, D. M., B. K. Mable & C. Moritz (Eds.), Molecular Systematics. Sinauer, Sunderland, 655pp.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Molecular Biology and Evolution **24:**1596-1599.

Taylor WR, Van Dyke GC (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium **9:**1007-109.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society, Series B **360:**1847-1857.

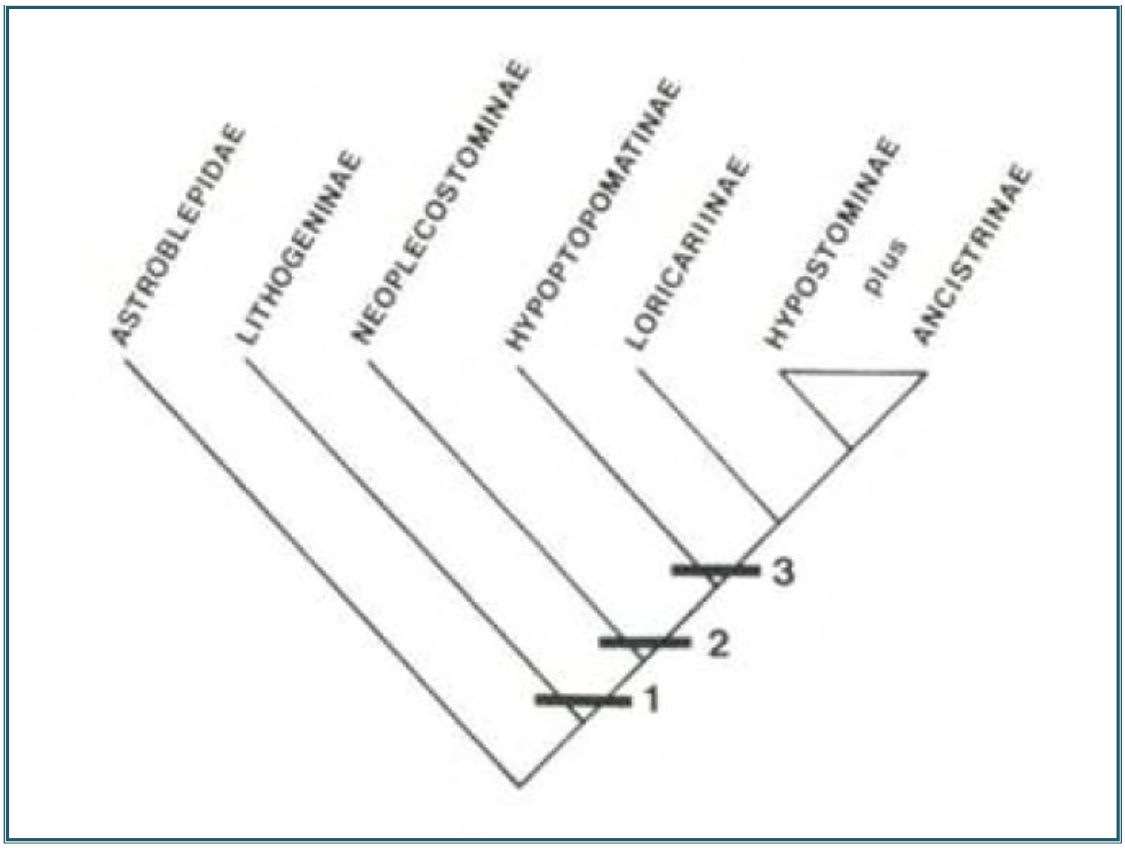
Ward RD, Holmes BH, Yearsleydna GK (2008) Barcoding reveals a likely second species of Asian sea bass (barramundi) (*Lates calcarifer*). Journal of Fish Biology **72:**458-463.

Xia X, Xie Z (2001) DAMBE: Data analysis in molecular biology and evolution. Journal of Heredity **92:**371-373.

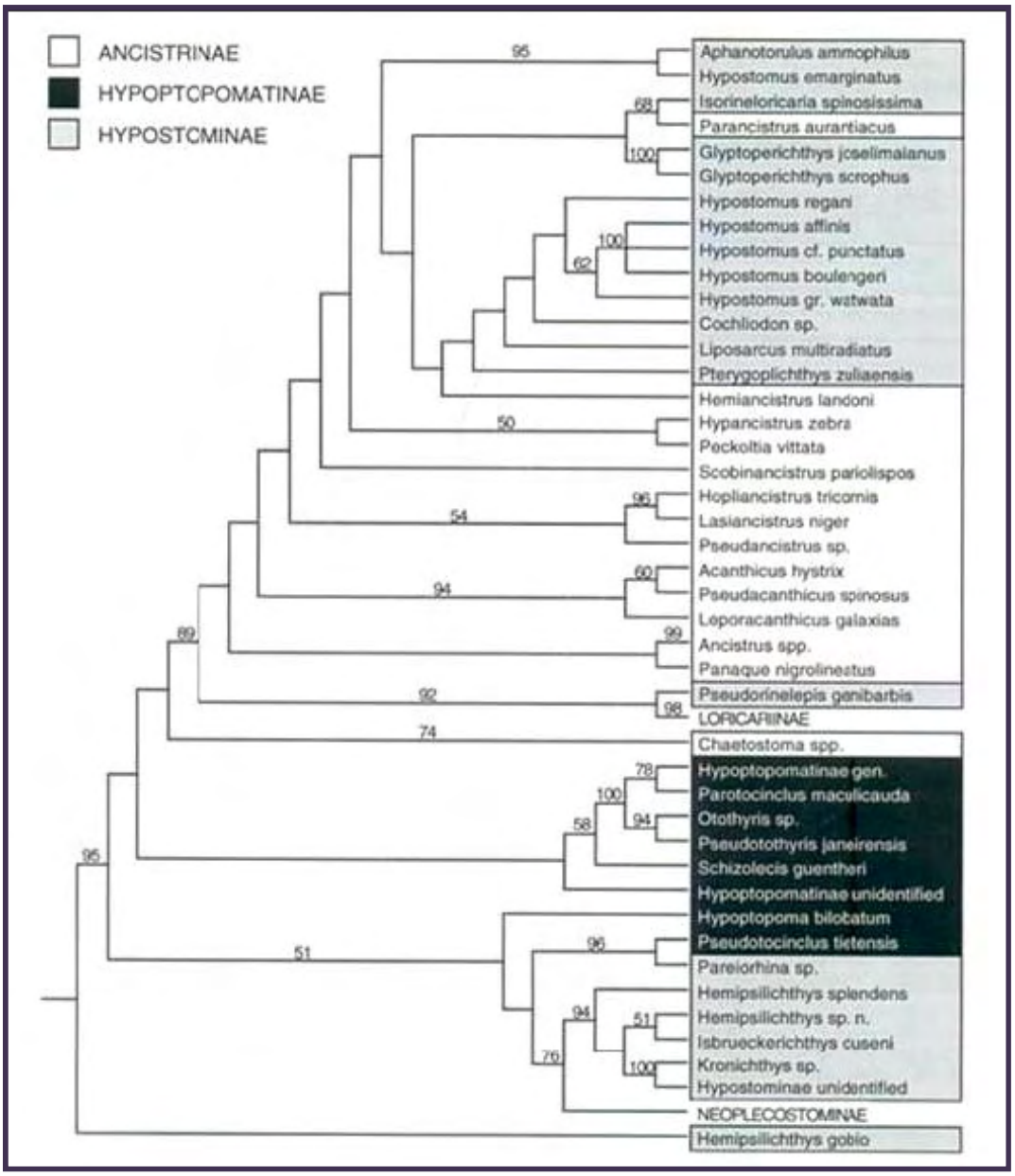
Zawadzki CH, Alves AL, Renesto E, Oliveira C (2004) Biochemical evidence of a possible new species of *Neoplecostomus* (Teleostei: Loricariidae) from the upper Rio Paraná basin, Brazil. Biochemical Systematics and Ecology **32:**573-582.

Zawadzki CH, Pavanelli CS, Langeani F (2008) *Neoplecostomus* (Teleostei: Loricariidae) from the upper Rio Paraná basin, Brazil, with description of three new species. Zootaxa **1757:**31-48.

Anexos



Cladograma da hipótese das relações de Loricariidae proposta por Schaefer (1987).



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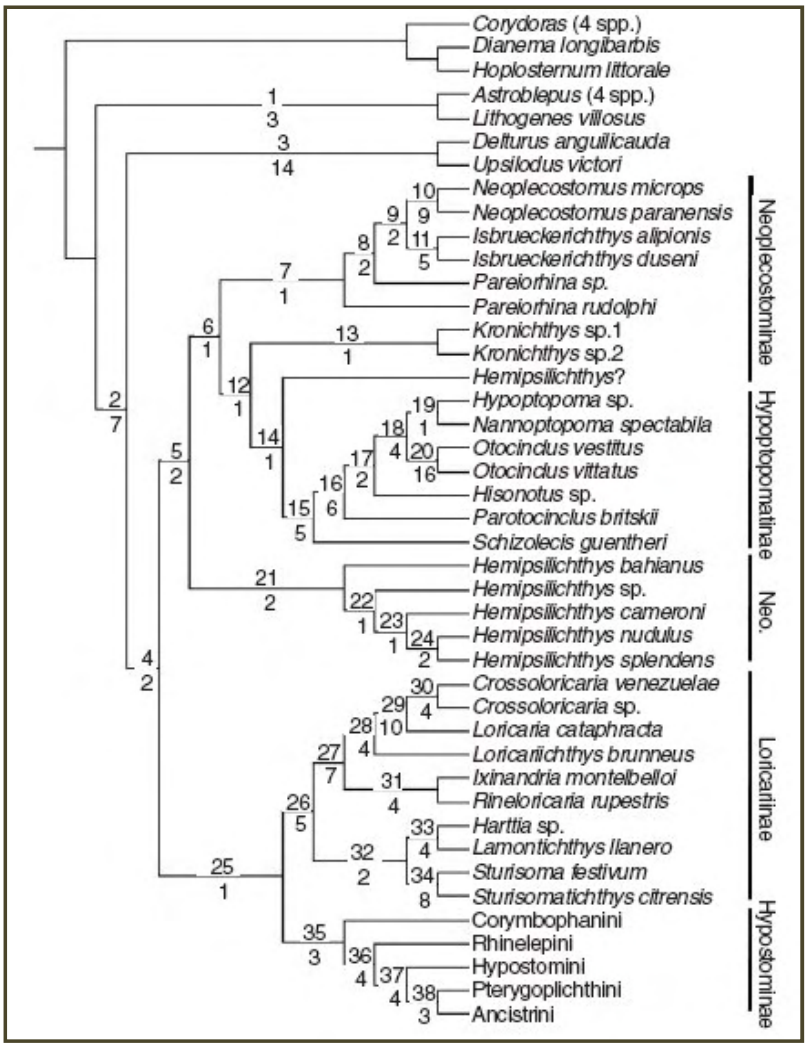
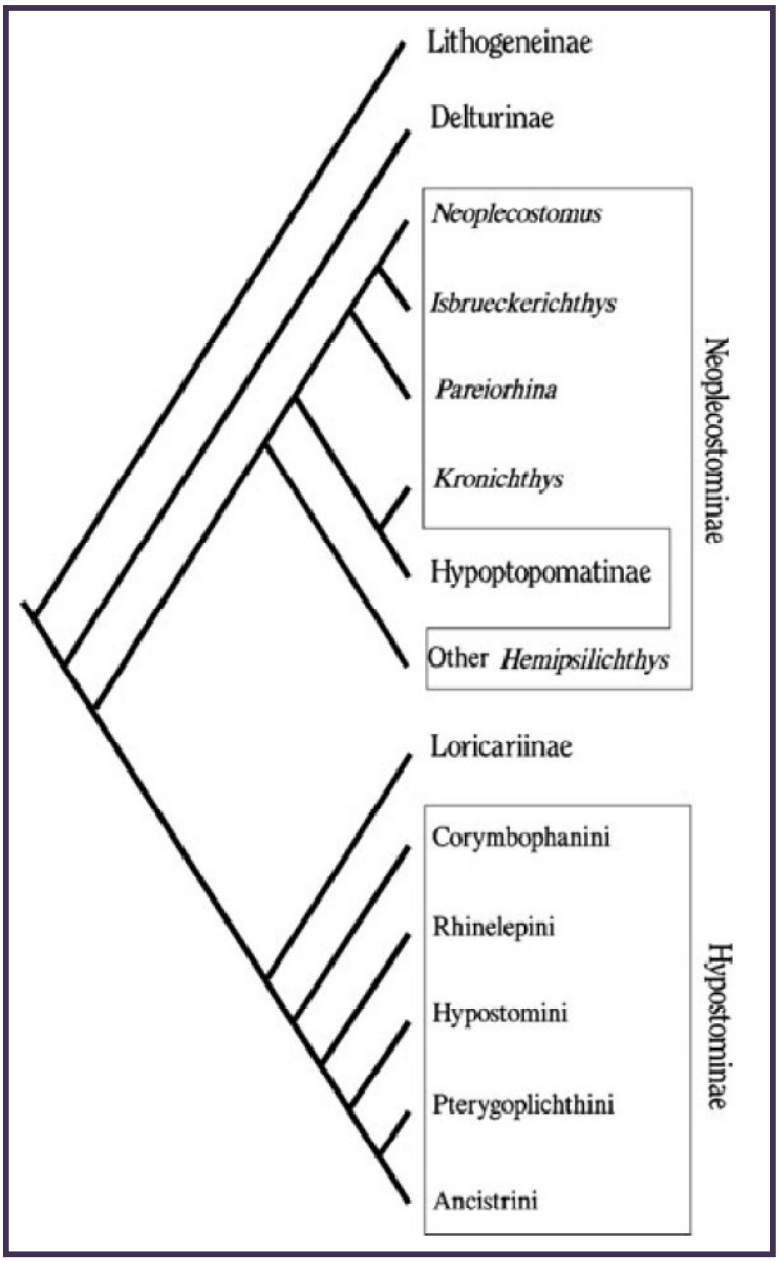
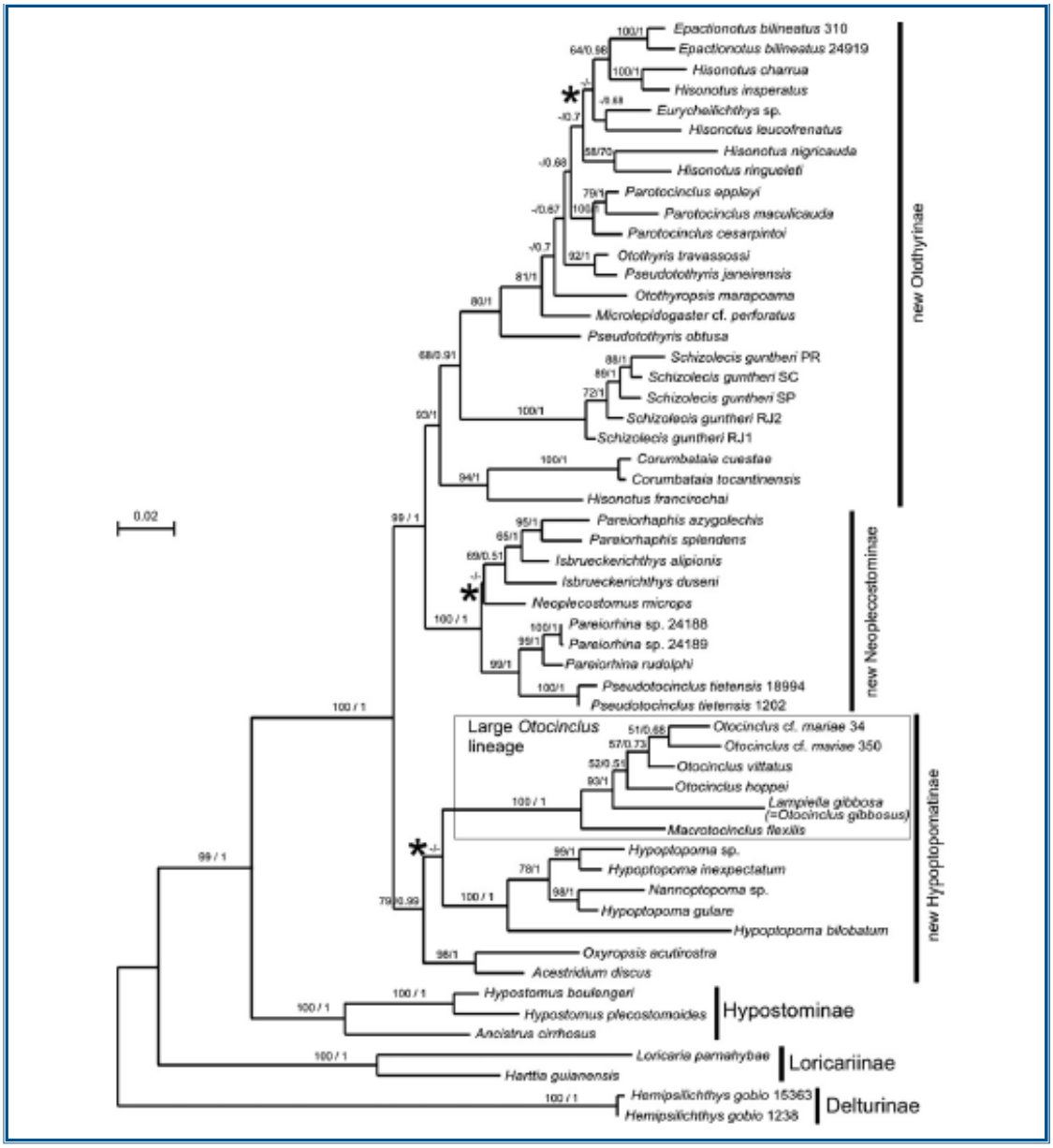


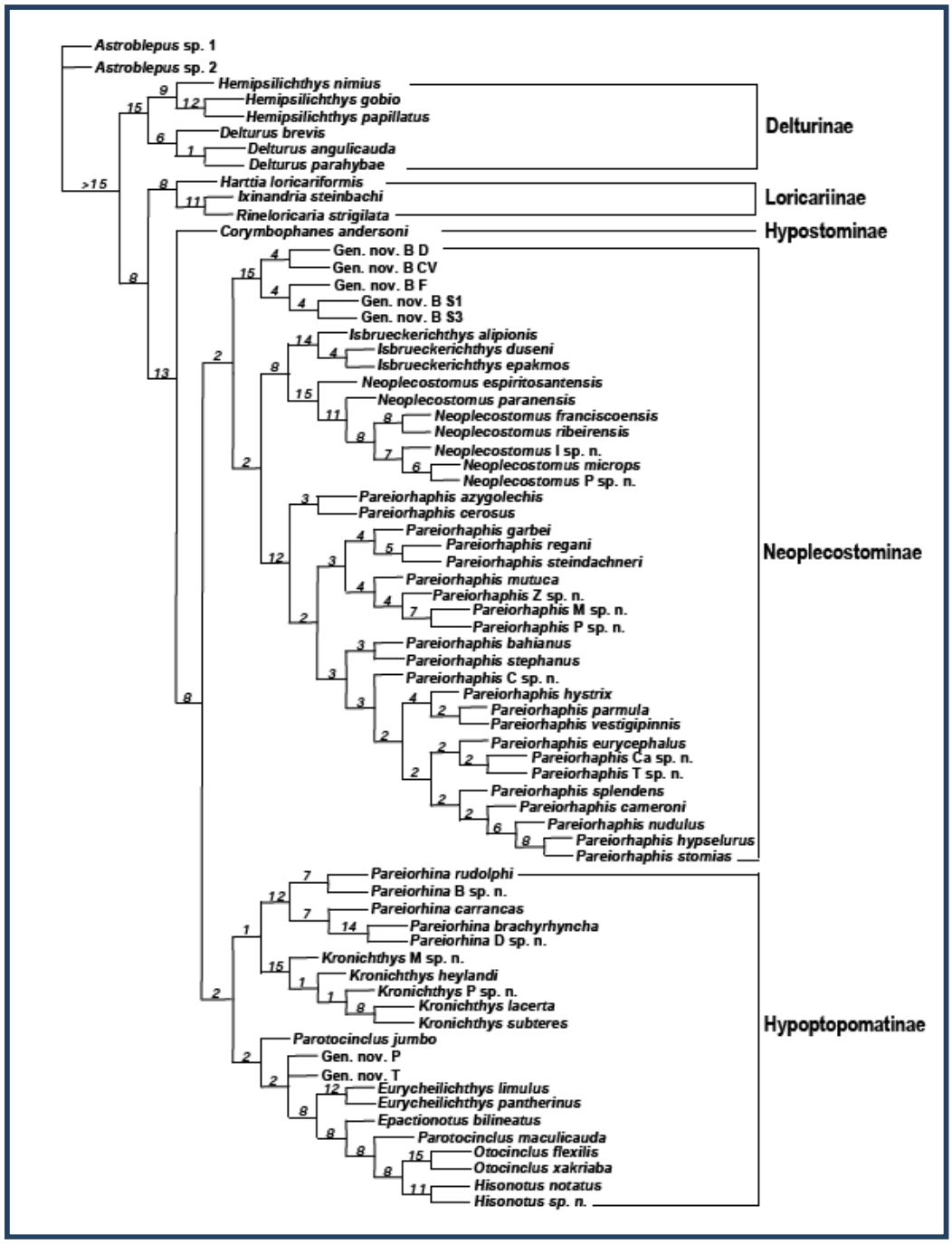
Cladograma da hipótese das relações de Loricariidae proposta por Armbruster (2004).



Phylogenetic interrelationships of the Loricariidae modified from Armbruster (2004). (Reis et al. 2006)



Maximum likelihood tree of the new Hypoptopomatinae (former Hypoptopomatini), new Otothyrinae (former Otothyrini) and new Neoplecostominae, based on the sequences of the partial F-reticulon 4 gene. (Chiachio et al. 2008)



Cladograma de consenso estrito da análise filogenética de Neoplecostominae a partir de dois cladogramas fundamentais maximamente parcimoniosos com 2171 passos cada. (Pereira 2008)