PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

FILOGENIA DE DUAS SUBFAMÍLIAS DE CASCUDOS (SILURIFORMES, LORICARIIDAE), USANDO DADOS NUCLEARES, MITOCONDRIAIS E MORFOLÓGICOS

Christian Andreas Cramer
Orientador: Dr. Roberto E. Reis
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TESE DE DOUTORADO
PORTO ALEGRE – RS – BRASIL
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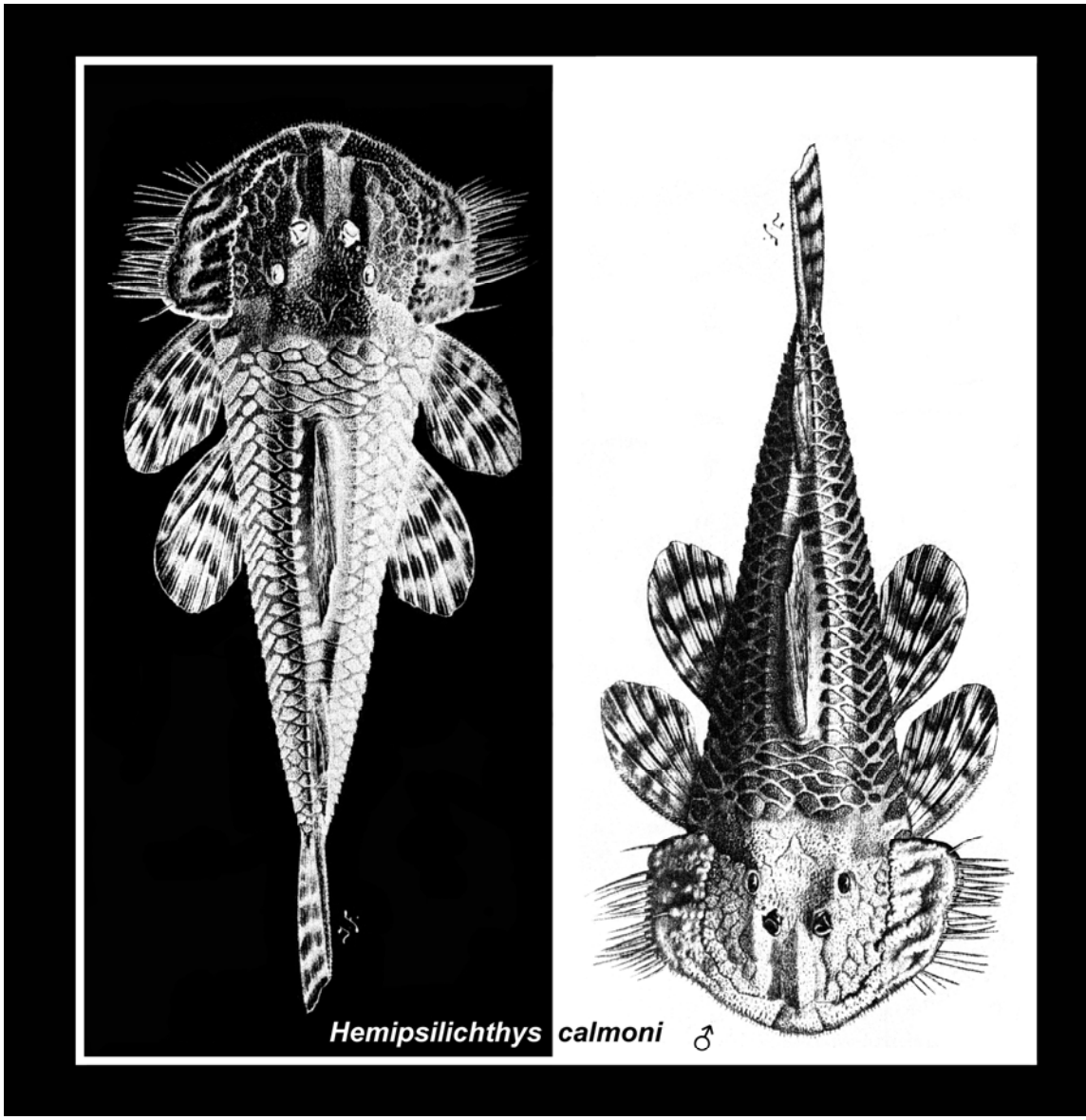
Hemipsilichthys calmoni ♂

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NOTICE

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SUMÁRIO

Agradecimentos ..... **ii**

Resumo ..... **v**

Abstract ..... **vi**

Apresentação **1**

Capítulo I - The phylogenetic relationships of the Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae) as inferred from mitochondrial cytochrome c oxidase I sequences **5**

Capítulo II - Molecular Phylogeny of the Neoplecostominae and Hypoptopomatinae (Siluriformes: Loricariidae) using Multiple Genes **15**

Capítulo III - A Total Evidence Phylogeny of the Neoplecostominae and Hypoptopomatinae catfishes (Siluriformes: Loricariidae) **54**

Conclusões Gerais **98**

Referências **101**

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Resumo

Loricariidae é uma das mais diversas famílias de peixes, atualmente incluindo cerca de 800 espécies reconhecidas. Os loricarídeos são popularmente conhecidos como cascudos ou acaris e têm tamanho de poucos centímetros a mais que um metro. São encontrados somente em água doce, com ampla variação tanto na temperatura, como tipo de ambiente, ou seja, podem ser encontrados desde em córregos frios das montanhas até em lagos da área de inundação do rio Amazonas. A distribuição do grupo é ampla, abrangendo praticamente todas as bacias hidrográficas da América do Sul e parte da América Central, da Argentina até a Costa Rica. Apesar das primeiras espécies terem sido descritas por Linnaeus em 1758 e do trabalho realizado deste então, especialmente nos últimos 15 anos, as relações filogenéticas dos loricarídeos estão apenas parcialmente resolvidas. Os resultados das análises moleculares são conflitantes com os agrupamentos propostos pelas análises morfológicas e um consenso ainda não foi alcançado. Os grupos mais problemáticos são as subfamílias Hypostominae, Hypoptopomatinae e Neoplecostominae. Os estudos morfológicos e moleculares sugerem que Hypoptopomatinae e Neoplecostominae formam um grupo monofilético, porém a monofilia das mesmas ainda é incerta. Por esta razão, o principal objetivo da presente tese é testar a monofilia destas duas subfamílias atráves de uma análise de evidência total. Num primeiro estudo, sequências de um fragmento de 709 pares de bases da primeira subunidade do gene mitocondrial *citocromo c oxidase* (COI) foram usadas. As análises de Máxima Parcimônia (MP) e de Máxima Verossimilhança (ML) incluíram dados de 83 espécies, pertencentes a 29 gêneros, representando cinco subfamílias de Loricariidae. Adicionalmente, oito espécies de quatro famílias próximas foram usadas como grupo externo. Os resultados confirmaram Delturinae como a subfamília mais basal e mostraram a Hypoptopomatinae + Neoplecostominae como grupo irmão de Hypostominae. Corroborando estudos moleculares anteriores, Neoplecostominae é monofilética somente quando incluído o gênero *Pseudotocinclus* (Hypoptopomatinae). Neoplecostominae ficou inserida dentro de Hypoptopomatinae, que assim formou um grupo parafilético. Num segundo estudo, foram adicionados mais táxons e sequências parciais dos genes nucleares *recombination activating gene 1* (RAG1) e *2* (RAG2) e *F-Reticulon 4*, resultando na análise de 136 espécies e 4678 pares de bases. As análises de MP, ML e Bayesianas confirmaram a maioria dos resultados moleculares anteriores, exceto pela polifilia de *Pareiorhaphis*, *Neoplecostomus* e Neoplecostominae. A análise final dos dados constituiu uma análise de evidência total, com o intuito de compreender melhor os conflitos entre as análises morfológicas e moleculares e encontrar, assim, uma solução mais robusta para o grupo. O novo conjunto de dados inclui 207 espécies e sequências concatenadas de COI, RAG1 e RAG2, bem como 472 caracteres morfológicos de estudos anteriores, resultando na maior filogenia de bagres já elaborada. A análise de MP confirmou a monofilia de Hypoptopomatinae e de Neoplecostominae + *Pseudotocinclus*. Dentro de Neoplecostominae, somente o gênero *Pareiorhina* ficou polifilético, provavelmente devido à falta de dados morfológicos. A filogenia do gênero *Pareiorhaphis* revelou um padrão biogeográfico de distribuição previamente desconhecido. Das duas tribos de Hypoptopomatinae, Hypoptopomatini foi recuperada como monofilética, mas Otothyrini se manteve parafilética. O gênero *Parotocinclus* ficou polifilético, formando três clados monofiléticos e parte das espécies espalhada na filogenia. O resultado obtido pela análise de evidência total conseguiu resolver vários conflitos entre as propostas filogenéticas anteriores para Loricariidae. Porém, os grupos não resolvidos foram os que apresentaram a menor quantidade de caracteres, mostrando que, para resolver as relações filogenéticas dentro desta família, é necessária a complementação dos caracteres, assim como a adição de novos táxons.

Abstract

The Loricariidae, or armored catfishes, is one of the most diverse fish families, currently containing nearly 800 recognized species. They are solely freshwater inhabitants, with a distribution from Uruguay and Argentina to Costa Rica. Loricariids occur in every kind of waters, from cool mountain streams to lakes in the Amazon floodplain and reach sizes from a few centimeters up to more than one meter. Though the first species have been described by Linnaeus in 1758 and much effort has been done, especially in the last 15 years, their phylogenetic relationships could be only partly resolved so far. In particular, molecular analyses showed some groupings that conflict with the results from morphological analyses. The most problematic groups are the subfamilies Hypostominae, Hypoptopomatinae, and Neoplecostominae. Both morphologic and molecular studies suggest that the latter two form a monophyletic group, although leaving doubts if they are monophyletic separately. Therefore, the Hypoptopomatinae and the Neoplecostominae were chosen as subjects for the present project. In a first study, sequences from a 709 basepair fragment of the first subunit of the mitochondrial *cytochrome c oxidase* gene (COI) were used. The maximum parsimony (MP) and maximum likelihood (ML) analyses included data from 83 loricariid species from 29 genera representing five loricariid subfamilies. Additionally, eight species from four closely related families were used as outgroup. The results confirmed the Delturinae as the most basal subfamily and showed the Hypoptopomatinae + Neoplecostominae as sister to the Hypostominae. Corroborating previous molecular studies, the Neoplecostominae was monophyletic only when including the hypoptopomatine genus *Pseudotocinclus*. The Hypoptopomatinae formed a paraphyletic group, embracing the Neoplecostominae. In a second study, more taxa as well as partial sequences from the nuclear *recombination activating genes 1* (RAG1) and *2* (RAG2), and the *F-Reticulon 4* gene were included, increasing the data set to a total of 136 species and 4678 basepairs. The MP, ML, and Bayesian analyses confirmed most previous molecular results, but some new polyphyletic taxa were found such as *Pareiorhaphis*, *Neoplecostomus*, and the Neoplecostominae. To try to better understand the conflicts between the morphological and molecular approaches and to reach a more complete solution, a total evidence analysis was undertaken, since this approach had already yielded good solutions for similar problems. A new data set with a total of 207 species and concatenated sequences from COI, RAG1, and RAG2, as well as 472 morphological characters from previous studies was analyzed using MP, resulting in the largest catfish phylogeny done so far. The Hypoptopomatinae and the Neoplecostominae were recovered as monophyletic sister groups, the latter including the genus *Pseudotocinclus*. Inside the Neoplecostominae, only the genus *Pareiorhina* remained polyphyletic, probably because of the lack of morphological data. The phylogeny of the genus *Pareiorhaphis* showed a previously unknown structured biogeographic pattern. From the two hypoptopomatine tribes, Hypoptopomatini was recovered as monophyletic, but Otothyrini remained paraphyletic. Although three monophyletic clades were found for the genus *Parotocinclus*, part of its species remained scattered in the phylogeny. Summarizing, the total evidence analysis was able to resolve several of the previous uncertainties in the loricariid phylogeny, but a further complementation of characters and an expansion of the taxon sampling will be necessary to completely resolve the phylogenetic relationships of this group.

People cannot discover new lands until they have the courage to lose sight of the shore…

André Gide

Apresentação

A ordem Siluriformes é distribuída em todos os continentes, com a exceção da Antártica. Com cerca de 3100 espécies em 36 famílias (Ferraris, 2007), bagres somam aproximadamente 10% de todas as espécies de peixes. Eles são principalmente habitantes de águas doces, com somente duas famílias marinhas, e são mais fortemente representados na América do Sul, com 14 famílias e 64% das espécies (Moyle e Cech, 2000; Rodiles-Hernández et al., 2005). Seis destas famílias formam a superfamília Loricarioidea, um grupo bem documentado com cerca de 1280 espécies ou 41% de todos os bagres: Astroblepidae, Callichthyidae, Loricariidae, Nematogenyidae, Scoloplacidae e Trichomycteridae (Schaefer, 1990). A família Loricariidae, ou cascudos, é uma das mais diversas famílias de peixes, atualmente compreendendo 785 espécies reconhecidas em cerca de 100 gêneros (Eschmeyer e Fricke, 2009). Diferente de outros peixes, cascudos têm a boca modificada em forma de uma ventosa e o corpo é coberto com placas ossificadas. Este grupo mega-diverso ocorre do Uruguai e norte da Argentina à Costa Rica e é encontrado em todos os tipos de águas, de riachos frios nas montanhas com correntezas fortes aos lagos da área de inundação do rio Amazonas, tendo um papel importante na biodiversidade. Ultimamente, loricariídeos foram descobertos introduzidos nos EUA e na Ásia (Chavez et al., 2006; Nico et al., 2009), em alguns lugares em quantidades surpreendentes com consequências ainda desconhecidas para a fauna local.

Apesar de muito trabalho e centenas de publicações deste Linnaeus, ainda existem muitas espécies por serem descobertas e descritas e sua filogenia está somente parcialmente resolvida. A família Loricariidae foi descrita por Rafinesque em 1815 e em seguida, começando em 1831, oito subfamílias foram estabelecidas, das quais seis ainda são reconhecidas (Armbruster, 2004; Reis et al., 2006): Lithogeninae Eigenmann, 1909 (1 gênero, 3 espécies), Delturinae Reis et al., 2006 (2 gêneros, 7 espécies), Neoplecostominae Regan, 1904 (5 gêneros, 39 espécies), Hypoptopomatinae Eigenmann e Eigenmann, 1890 (18 gêneros, 103 espécies), Loricariinae Bonaparte, 1831 (~ 36 gêneros, 222 espécies) e Hypostominae, Kner, 1853 (~ 40 gêneros, 411 espécies). Esta divisão em subfamílias não tem sido estável e ao longo do tempo foram feitas várias mudanças. Ancistrinae e Hypostominae foram descritas em 1853 (Kner, 1853). Descrevendo a Neoplecostominae, Regan (1904) somente incluiu o gênero *Neoplecostomus.* Mais tarde, Gosline (1947) adicionou os gêneros *Canthopomus*, *Corymbophanes*, *Delturus*, *Hemipsilichthys*, *Isbrueckerichthys* (as espécies foram listadas como *Pareiorhaphis* por

que *Isbrueckerichthys* somente foi descrito em 1996), *Kronichthys*, *Pareiorhaphis*, *Pareiorhina*, *Pogonopoma*, *Pogonopomoides* e *Upsilodus*. Isbrücker (1980) reconheceu seis subfamílias, mas listou *Neoplecostomus* como único gênero de Neoplecostominae e transferiu os outros gêneros para Hypostominae.

A primeira filogenia de Loricariidae foi publicada por Howes (1983). Baseado em exames de osteologia e de músculos, ele descreveu a subfamília Chaetostomatinae para os gêneros *Chaetostoma*, *Hemipsilichthys*, *Lasiancistrus* e *Lipopterichthys* e pôs Ancistrinae na sinonímia de Hypostominae. Parecido a esta situação, as filogenias de Schaefer (1986, 1987, 1988) mostraram Hypostominae como parafilética por causa da separação da Ancistrinae; ele não reconheceu a Chaetostomatinae. Mesmo assim, Schaefer decidiu manter o status de subfamília para Ancistrinae, usando a classificação de Isbrücker (1980). O primeiro estudo filogenético com foco em Hypoptopomatinae foi feito por Schaefer (1991), mas somente 16 espécies foram incluídas. Baseado nos seus resultados, ele descreveu as tribos Hypoptopomatini e Otothyrini. Montoya-Burgos et al. (1997, 1998) foram os primeiros a fazer uma análise molecular, usando sequências de nucleotídeos dos genes do rRNA de 12S e 16S. Com exceção de Neoplecostominae, monotípica, Loricariinae foi a única subfamília a ser recuperada como monofilética. Duas descobertas importantes foram feitas neste estudo (Montoya-Burgos et al., 1998): pela primeira vez, *Hemipsilichthys gobio* foi mostrado como táxon basal para todos os outros loricariídeos, e *Pseudotocinclus* (Hypoptopomatinae) foi identificado como grupo irmão de *Pareiorhina* (Neoplecostominae), fazendo uma conexão entre as duas subfamílias. A primeira filogenia ampla para a Loricariidae foi publicada por Armbruster (2004). Ela foi focada em Hypostominae, mas incluiu mais que 120 espécies de todas as subfamílias, usando 215 caracteres morfológicos. Para reter a monofilia da Hypostominae, Armbruster sinonimizou Ancistrinae com Hypostominae e dividiu Hypostominae nas cinco tribos Ancistrini, Corymbophanini, Hypostomini, Rhinelepini e Pterygoplichthini. Ademais, ele encontrou Otothyrini como parafilética e, mesmo sendo um táxon parafilético, devolveu os gêneros *Isbrueckerichthys*, *Kronichthys*, *Pareiorhaphis* e *Pareiorhina* para Neoplecostominae. Adicionalmente, Schaefer confirmou a descoberta de Montoya-Burgos et al. (1998) que *Hemipsilichthys gobio*, junto com *Delturus angulicauda*, forma o grupo-irmão de todos os outros loricariídeos, com exceção de *Lithogenes*. Baseado nestes resultados, Pereira (2005) ressuscitou o gênero *Pareiorhaphis* Miranda Ribeiro, 1918 para a maioria das espécies de *Hemipsilichthys*, deixando somente *H. gobio*, a espécie-tipo, *H. papillatus* e *H. nimius* neste último. No mesmo ano, a filogenia morfológica de Hypoptopomatinae de Gauger e

Buckup (2005), incluindo 31 espécies de quase todos os gêneros da subfamília, mostrou Otothyrini e o gênero *Parotocinclus* como grupos parafiléticos. No ano seguinte, Lehmann (2006) utilizou 169 caracteres morfológicos e um total de 114 espécies, na sua tese de doutorado sobre a filogenia de Hypoptopomatinae. As suas análises filogenéticas recuperaram o gênero *Kronichthys* como grupo mais basal de uma Hypoptopomatinae monofilética. De acordo com os seus resultados, *Pseudotocinclus* não tem nenhuma relação mais próxima com Neoplecostominae, e ele encontrou Otothyrini sendo parafilética. Focados em Hypoptopomatinae e incluindo sequências de DNA de apenas um gene e 44 espécies, Chiachio et al. (2008) encontraram *Pseudotocinclus* fazendo parte de Neoplecostominae, qual foi encontrada dentro de Hypoptopomatinae. A solução dos autores foi elevar Hypoptopomatini e Otothyrini ao nível de subfamília e redefinir Neoplecostominae, resultando em três grupos monofiléticos. Esta mudança nomenclatural deve ser considerada prematura por causa do baixo número de espécies incluídas, do uso de um único gene e das contradições com os estudos recentes, sejam moleculares ou morfológicos. Pouco depois, Pereira (2008) terminou sua tese de doutorado, dedicada à filogenia de Neoplecostominae. Representando a mais completa amostragem de táxons deste grupo e incluindo 303 caracteres morfológicos, este estudo recuperou Hypoptopomatinae e Neoplecostominae como grupos irmãos monofiléticos. Porém, os gêneros *Pareiorhina* e *Kronichthys* foram revelados como táxons irmãos na base de Hypoptopomatinae e, consequentemente, foram incluídos nesta subfamília. Infelizmente, *Pseudotocinclus* não foi incluído na análise de Pereira.

A presente tese reúne os resultados da análise filogenética das duas subfamílias Hypoptopomatinae e Neoplecostominae, usando dados moleculares nucleares e mitocondriais e dados morfológicos. Como todos os estudos anteriores mostram que estas duas subfamílias formam um grupo monofilético, elas foram escolhidas como tópico do presente estudo. Considerando os diferentes resultados apresentados na literatura atual sobre as relações filogenéticas de Neoplecostominae e Hypoptopomatinae, este estudo teve como objetivo ampliar os dados sobre estes grupos, enfocando a relação entre os dados moleculares e as análises morfológicas, constituindo-se, assim, numa análise de evidência total. Foram elaborados três artigos. O primeiro, utilizando sequências parciais da primeira subunidade do gene mitocondrial *citocromo c oxidase* (COI) para 83 táxons foi publicado no Bulletin of Fish Biology (Revista da Sociedade Alemã de Ictiologia), em co-autoria com Ana M. R. Liedke, Sandro L. Bonatto e Roberto E. Reis. Os resultados deste estudo também recuperaram o gênero *Pseudotocinclus* como membro de Neoplecostominae e,

novamente, Neoplecostominae foi encontrada dentro de Hypoptopomatinae. Contrário a resultados anteriores, Hypoptopomatini e Otothyrini não formaram grupos monofiléticos.

O segundo artigo contém os resultados de análises utilizando sequências parciais de COI e dos genes nucleares *recombination activating gene 1* (RAG1) e *2* (RAG2) e *F-Reticulon 4* para um total de 136 táxons, e será submetido para a revista Molecular Phylogenetics and Evolution, em co-autoria com Sandro L. Bonatto e Roberto E. Reis. Com o objetivo de testar os resultados prévios usando múltiplos genes, os métodos Máxima Parcimônia (MP), Máxima Verossimilhança (ML) e análise Bayesiana foram utilizados. Somente as subfamílias Delturinae e Loricariinae foram encontradas monofiléticas. Outra vez, o gênero *Pseudotocinclus* foi incluído em Neoplecostominae, mas *Pareiorhaphis* foi recuperado como polifilético, inclusive com uma espécie colocada fora de Neoplecostominae. As tribos Hypoptopomatini e Otothyrini se mantiveram não-monofiléticas.

No terceiro artigo, objetivando resolver as contradições entre os dados morfológicos e moleculares, utilizou-se a análise de evidência total, unindo sequências de COI, RAG1 e RAG2 com dados morfológicos das teses de Pablo Lehmann e Edson H. L. Pereira, para 207 táxons. Este artigo será submetido para a revista Systematic Biology, junto com Pablo Lehmann, Edson H. L. Pereira, Sandro L. Bonatto e Roberto E. Reis. A análise de MP recuperou Hypoptopomatinae e Neoplecostominae como grupos irmãos monofiléticos. Quase todos os gêneros de Neoplecostominae resultaram em grupos monofiléticos, com a exceção de *Pareiorhina*, que permanece polifilético. A filogenia do gênero *Pareiorhaphis* mostrou um padrão biogeográfico antes desconhecido. Dentro de Hypoptopomatinae, no entanto, somente Hypoptopomatini foi encontrada como grupo natural, dentro de uma tribo Otothyrini parafilética, e os gêneros *Hisonotus* e *Parotocinclus* foram recuperados como polifiléticos.

Capítulo I

The phylogenetic relationships of the Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae) as inferred from mitochondrial *cytochrome c oxidase I* sequences

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The phylogenetic relationships of the Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae) as inferred from mitochondrial cytochrome c oxidase I sequences

Die Phylogenie der Hypoptopomatinae und Neoplecostominae (Siluriformes: Loricariidae) auf Grundlage von Sequenzen des mitochondrialen Cytochrom-c-Oxidase-I-Gens

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Summary: The phylogenetic relationships of the loricariid subfamilies Neoplecostominae and Hypoptopomatinae are assessed using sequences of the subunit 1 of the cytochrome c oxidase gene (COI), in order to test the contradictory results of previous, mostly morphologic analyses. We obtained an alignment of 709 contiguous nucleotides for 105 sequences of a fragment of COI for 83 species from 29 loricariid genera from representatives of five loricariid subfamilies and for eight outgroup species from four loricarioid families. Both, Maximum Likelihood and Parsimony analyses were conducted. Results show a monophyletic clade composed of Hypoptopomatinae + Neoplecostominae as sister to Hypostominae and this clade sister to Loricariinae. However, neither the Hypoptopomatinae nor the Neoplecostominae are monophyletic groups. Also, the genera *Pareiorhaphis, Hisonotus* and *Parotocinclus* turned out to be polypheletic.

Key words: phylogeny, Loricariidae, Hypoptopomatinae, Neoplecostominae, cytochrome c oxidase, armored catfish

Zusammenfassung: Die phylogenetischen Verwandtschaftsverhältnisse der Harnischwels-Unterfamilien Neoplecostominae und Hypoptopomatinae werden mit Hilfe von Sequenzen der ersten Untereinheit der Cytochrom-c-Oxidase (COI) untersucht, um die widersprüchlichen Ergebnisse früherer, meist morphologischer Arbeiten zu prüfen. Wir erhielten ein Alignment von 709 zusammenhängenden Nukleotiden für 105 Sequenzen eines COI-Fragments für 83 Arten von 29 Gattungen aus fünf Unterfamilien der Harnischwelse sowie acht Außengruppen-Arten aus vier Familien der Loricarioidea. Es wurden Maximum-Parsimony- und Maximum-Likelihood-Analysen durchgeführt. Die Ergebnisse zeigen eine monophyletische Gruppe aus Hypoptopomatinae und Neoplecostominae als Schwestergruppe der Hypostominae, und diese zusammen als Schwestergruppe der Loricariinae. Allerdings sind weder die Hypoptopomatinae noch die Neoplecostominae monophyletische Gruppen. Ebenso stellen sich die Gattungen *Pareiorhaphis, Hisonotus* und *Parotocinclus* als polyphyletisch heraus.

Schlüsselwörter: Phylogenie, Loricariidae, Hypoptopomatinae, Neoplecostominae, Cytochrome-c-Oxidase, Harnischwelse

1. Introduction

With around 2,600 species in 36 families (Ferraris 2007), the order Siluriformes contains about 10% of all fish species worldwide. With the exception of two marine families, they are solely freshwater inhabitants. Catfish are most strongly represented in South America with 14 families and 64% of the species (Moyle & Cech 2000). The armored catfishes (Lorica-

riidae), emphasized here, are endemic to South and Central America. They are widespread and play an important role in biodiversity, as they are one of the most species-rich fish families. Presently approximately 90 genera with about 700 species are recognized. Along with five closely related families, they form the superfamily Loricariodea (Schaefer & Lauder 1986, Schaefer 1990).

The family Loricariidae is divided into six subfamilies (Armbruster 2004, Reis et al. 2006). The subfamily Lithogeneinae with one genus and only two species is the smallest and less known one. The Delturinae contains two genera with seven species. Six genera with 38 species comprise the subfamily Neoplecostominae, and around 94 species in 18 genera are included in the subfamily Hypoptopomatinae. The remaining taxa are dispersed throughout the subfamilies Loricariinae and Hypostominae. Although Linnaeus (1758) described the first loricariid catfish and since then many scientists have been working with this family, their systematics is still insufficiently resolved. So, the monophyly of at least two subfamilies and two tribes (Neoplecostominae and Hypostominae; Ancistrini and Hypostomini) has been rejected by molecular studies (Montoya-Burgos et al. 1998, 2002, Hardman 2005), contrary to morphologic results, and many genera are not adequately defined. Montoya-Burgos et al. (1998) and Armbruster (2004) found the Neoplecostominae and the Hypoptopomatinae to cluster together. The most recent phylogenetic study based on morphology is Lehmann (2006). He found the Neoplecostominae to be a paraphyletic group. The Hypoptopomatinae came out to be monophyletic. Therefore our aim is to test the findings on these two subfamilies using molecular DNA sequence data. Our choice was the cytochrome c oxidase I gene (COI) because it shows a greater range of phylogenetic signal than any other mitochondrial gene (Hebert et al. 2003). Like other protein-coding genes, its third-position nucleotides show a high incidence of base substitutions, leading to a rate of molecular evolution that is about three times higher than that of 12S or 16S rDNA (Knowlton & Weigt 1998). So, the evolution of this gene is rapid enough to allow the separation of not only closely related species, but also phylogeographic groups within a single species (Cox & Hebert 2001, Wares & Cunningham 2001). Because changes in its amino-acid sequence occur more slowly than in any other mitochondrial genes (Lynch & Jarrell 1993), COI is more likely to provide deeper phylogenetic insights than alternatives such as cytochrome b (Simmons & Weller 2001), even if other mitochondrial genes may equally resolve cases of recent divergence.

2. Materials and methods

2.1. Taxon sampling

The specimens and species used in this study are listed in Appendix A. Our aim was to include most of the species of the subfamilies Hypoptopomatinae and Neoplecostominae together with representatives of the other loricariid subfamilies. Unfortunately there was no fresh tissue from *Lithogenes* available. As outgroup taxa we used representatives from the other families of the Loricarioidea but Trichomycteridae (Schaefer & Lauder 1986, Schaefer 1990, de Pinna 1998).

2.2. DNA amplification and sequencing

From total genomic DNA extracted from fresh or ethanol-preserved tissue using the QIAmp tissue kit (Qiagen, Hilden, Germany), we amplified and sequenced a 709 basepair fragment of COI, using the primers LCO1490 and HCO2198 (Hebert et al. 2003). Each PCR was carried out in 20 µl reactions with the following concentrations: 1x Invitrogen PCR buffer (Invitrogen, São Paulo), 1.5-2.5 mM $MgCl_2$, 0.2% Triton, 200 µM of each dNTP, 0.025 U/µl taq polymerase, 0.2 µM of each primer and up to 2 µl of DNA solution.

We used our lab's standard protocol for this primer pair with an initial denaturation step of 1 min at 96 °C followed by 40 cycles of 94 °C for 30 s, annealing at 50 °C for 20 s, 48 °C for 5 s, 46 °C for 5 s, 44 °C for 5 s, 42 °C for 5 s, 40 °C for 20 s and extension at 72 °C for 1 min. This was followed by a final 3 min at 72 °C extension step. Amplification success was evaluated on GelRed™ (BioTium, São Paulo) or ethidium bromide-stained agarose gels (1%) in 0.5% TBE buffer (Sambrook et al. 1989). PCR products were purified using PEG8000, ExoSAP-IT® (USB) or the ilustra™ GFX PCR and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK). Sequencing was done using the DYEnamic™ ET dye terminator kit (GE Healthcare, São Paulo) and a MegaBace1000 sequencer.

Sequences were edited and combined using BioEdit 7.0.1 (Hall 1999).

2.3 Analysis

Sequences were aligned using Clustal X 1.83 (Thompson et al. 1997) using the standard settings. PAUP*4.0b10 (Swofford 2001) was used to analyze the data with respect to the parsimony criterion with the tree-bisection-reconnection (TBR) search algorithm with 1000 replicates in which taxa were added randomly to the starting tree. TNT using its ratchet algorithm (Goloboff et al. 1999) was also used and found the exact same results. All characters were treated as unordered and transformations were assigned equal weight. Nodal support was evaluated with 2000 nonparametric bootstrap pseudoreplicates (Felsenstein 1985) using the TBR search algorithm on a starting tree to which taxa were added randomly. Multiple optimal topologies were summarized through consensus methods.

For the maximum likelihood (ML) analysis we used RAxML-HPC 7 (Stamatakis 2006) with 300 replicates under the GTRGAMMA model. Parameters were estimated for each of four substitution categories over each of three codon positions. Nodal support was evaluated with 1000 nonparametric bootstrap pseudoreplicates.

3. Results

3.1. Data analysis

We obtained an alignment of 709 contiguous nucleotides and 105 sequences of a fragment of COI for 83 species from 29 ingroup genera and for eight outgroup species. No gaps were found. The character matrix contained 278 parsimony-informative characters.

3.2. Parsimony analysis

The MP analysis with PAUP* resulted in 4,400 equally parsimonious trees with a length of 2,827 steps. Their summarized strict consensus tree is shown in fig. 1. TNT found the same consensus tree. Non-parametric bootstrap proportions did not provide evidence of convincing resolution for deeper nodes. The lack of support suggests weak signal overall and probable inaccuracy among deeper nodes recovered by the analysis of these data.

3.3. Maximum likelihood analysis

The best tree found with RAxML has a -ln L-score of -12410.368924 and is shown in fig. 2. Parameter estimates for each of the codon-based models are shown in table 1.

Both, the MP and the ML tree have little bootstrap support for the deep nodes. As the result from the ML analyses shows higher bootstrap confidence intervals and fewer conflicts with the morphologic phylogenies, we will not discuss the MP analysis and will only focus on the ML result.

4. Discussion

Armbruster (2004) found Neoplecostominae and Hypoptopomatinae to be a monophyletic sister group to the Hypostominae + Loricariinae. In our results, they are sister to the Hypostominae and the three subfamilies together are sister to the Loricariinae, corroborating the findings of Lehmann (2006).

The topology of the Hypoptopomatinae found here is nearly inverse to that of Leh-

Fig.1: Summarized strict consensus of 4,400 most parsimonious topologies each of 2,827 steps. Numbers above nodes are bootstrap values recovered by the maximum likelihood analysis for those clades common to both parsimony and likelihood topologies.

Abb. 1: Zusammengefasster strikter Konsensus der 4.400 besten Bäume der Parsimonie-Analyse mit jeweils 2.827 Schritten. Die Zahlen oberhalb der Äste sind Bootstrap-Werte der Likelihood-Analyse für die Gruppen, die sowohl in der Likelihood- als auch in der Parsimony-Analyse gefunden wurden.

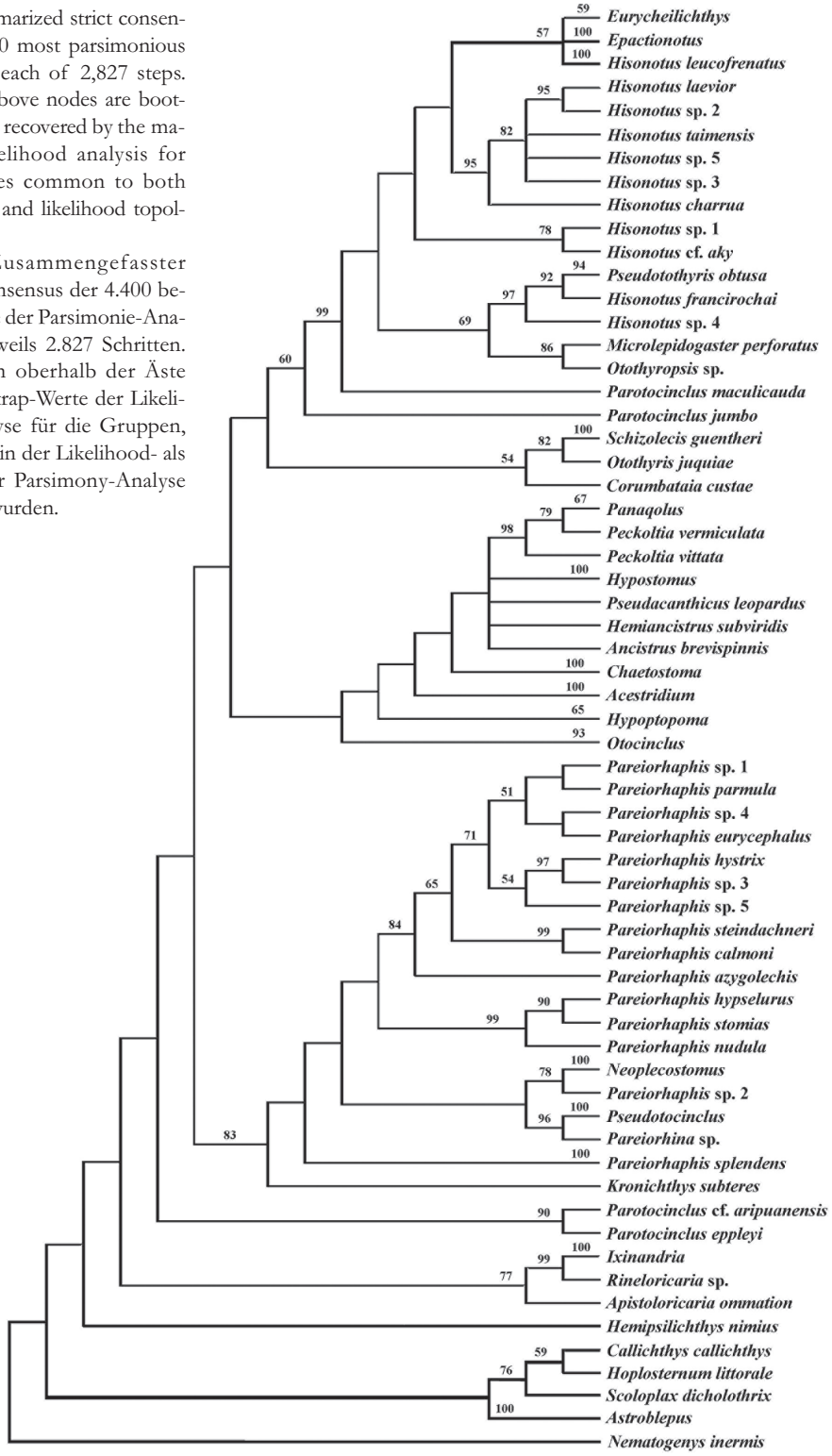

Fig. 2: Best tree from the likelihood analysis (-ln L-12100.250237). Branch lengths are means estimated by RAxML. Parameter estimates are provided in table 1.

Abb. 2: Der beste Baum der Likelihood-Analyse (-ln L -12100,250237). Die Astlängen wurden von RAxML errechnet. Die Parameter-Werte sind in Tabelle 1 gegeben.

Table 1: Model parameters estimated by RAxML during likelihood analysis.
Tabelle 1: Von RAxML während der Likelihood-Analyse berechnete Modell-Parameter

Codon position	1	2	3
P [Adenine]	0.253228	0.157818	0.311361
P [Cytosine]	0.249843	0.278909	0.326364
P [Guanine]	0.309856	0.149425	0.111137
P [Thymine]	0.187074	0.413847	0.251138
A↔C	0.678783	5.048929	0.629409
A↔G	1.984017	6.508516	11.305517
A↔T	1.083618	1.474108	0.525041
C↔G	0.284131	0.772918	0.362623
C↔T	22.954696	2.201741	5.011600
G↔T	1.000000	1.000000	1.000000
α	0.419050	0.621742	2.593663
p-inv	0.296526	0.671512	0.036420
The GTR+ I + G model was estimated for each of the three codon positions independently.			

mann (2006). In his study, *Eurycheilichthys* is the most basal taxon of the Hypoptopomatinae and *Acestridium* the most derived one. Like Lehmann (2006), we found *Acestridium*, *Hypoptopoma* and *Otocinclus* as closely related. *Corumbataia*, *Otothyris* and *Schizolecis* are closely related, as in Lehmann (2006). The genus *Pseudotocinclus* was found to be a sister taxon to *Pareiorhina*, inside the Neoplecostominae, with high bootstrap support. This finding is the same as in Montoya-Burgos et al. (1998) where these two genera and *Hypoptopoma* are closer related with the Neoplecostominae than with the rest of the Hypoptopomatinae. *Pareiorhaphis*, *Parotocinclus* and *Hisonotus* came out as polyphyletic taxa. *Parotocinclus* has already been found to be polyphyletic by Lehmann (2006). No phylogenetic study on *Hisonotus* has been published so far, but Azpelicueta & Almirón (2007) and Britski & Garavello (2003, 2007) state that the genus is insufficiently defined and possibly polyphyletic.

As the present results do not have high bootstrap support, especially on the deeper nodes, there is a need of additional work. To further clarify the relationships of the loricariid subfamilies, a future work should sample additional species to include representatives of all genera of the Hypoptopomatinae and Neoplecostominae, and should also include sequences from nuclear genes. Additionally, both molecular and morphological data should be joined in a total evidence analysis. This procedure already helped to resolve problems in similar cases (Gatesy et al. 2003).

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Appendix: GenBank and depository information for species included in this study

Astroblepidae: *Astroblepus* sp. 1 ANSP 180581 (tag 4805) EU359404. *Astroblepus* sp. 2 ANSP 180605 (tag 4490) EU359405. *Astroblepus* sp. 3 ANSP 180613 (tag 4453) EU359406. *Astroblepus* sp. 4 ANSP 180616 (tag 4436) EU359407. **Callichthyidae**: *Callichthys callichthys* MCP 29384 EU359408. *Hoplosternum littorale* MCP 21196 EU359416. **Loricariidae**: *Acestridium martini* ANSP 182901 (tag V5276) EU359398. *Acestridium martini* ANSP 182901 (tag V5277) EU359399. *Acestridium* sp. 1 MCP 37783 EU359400. *Acestridium* sp. 2 MCP 37785 EU359401. *Ancistrus brevipinnis* MCP 21246 EU359402. *Apistoloricaria ommation* ANSP 182331 (tag P6265) EU359403. *Chaetostoma* sp. 1 ANSP 180446 (tag P4772) EU359409. *Chaetostoma* sp. 2 ANSP 180448 (tag P4814) EU359410. *Corumbataia cuestae* LBP 876 EU371019. *Epactionotus bilineatus* MCP 23679 EU371006. *Epactionotus bilineatus* MCP 26964 EU371008. *Epactionotus bilineatus* MCP 26964 EU371009. *Epactionotus* cf. *gracilis* MCP 35156 EU371007. *Epactionotus gracilis* MCP 23606 EU371005. *Epactionotus itaimbezinho* MCP 23683 EU371004. *Eurycheilichthys limulus* MCP 21270 EU370989. *Eurycheilichthys limulus* MCP 21270 EU370990. *Eurycheilichthys pantherinus* MCP 22373 EU371000. *Eurycheilichthys* sp.1 MCP 21207 EU370995. *Eurycheilichthys* sp. 1 MCP 21207 EU370998. *Eurycheilichthys* sp. 2 MCP 22374 EU370992. *Eurycheilichthys* sp. 2 MCP 22800 EU370994. *Eurycheilichthys* sp. 3 MCP 35049 EU370999. *Eurycheilichthys* sp. 4 MCP 22199 EU370991. *Eurycheilichthys* sp. 5 MCP 22790 EU370993. *Eurycheilichthys* sp. 7 MCP 35071 EU370997. *Eurycheilichthys* sp. 7 MCP 35124 EU370996. *Hemiancistrus subviridis* AUM 42930 (tag P4648) EU359411. *Hemipsilichthys nimius* MCP 30671 EU359412. *Hisonotus* cf. *aky* MCP 40029 EU359413. *Hisonotus charrua* MCP 21644 EU371013. *Hisonotus francirochai* DZJRP 7727 EU359415. *Hisonotus laevior* MCP 23005 EU371015. *Hisonotus leucofrenatus* MCP 31819 EU371001. *Hisonotus leucofrenatus* LBP 873 EU371002. *Hisonotus* sp. 1 MCP 23744 EU371010. *Hisonotus* sp. 2 MCP 25139 EU371014. *Hisonotus* sp. 3 MCP 25159 EU371012. *Hisonotus* sp. 4 LBP 810 EU371018. *Hisonotus* sp. 5 MCP 37682 EU359414. *Hisonotus taimensis* MCP 21375 EU371011. *Hypoptoma bilobatum* MHNG 2588.092 EU370986. *Hypoptopoma* cf. *inexspectatum* ANSP uncat. (tag 5089) EU359417. *Hypoptopoma guianensis* ANSP 180669 (tag T2215) EU359418. *Hypoptopoma gulare* ANSP 178340 (tag 1551) EU359419. *Hypoptopoma inexspectatum* ANSP uncat. (tag 5047) EU359420. *Hypoptopoma steindachneri* ANSP 182723 (tag P6233) EU359421. *Hypostomus boulengeri* NUP uncat. (Z49935) EU359422. *Hypostomus commersonii* MCP 22767 EU359423. *Ixinandria montebelloi* GEN 1713 EU359425. *Ixinandria* sp. MCNI uncat. EU359424. *Ixinandria steinbachi* MCNI 1222 EU359426. *Kronichthys subteres* MCP 31600 EU371021. *Microlepidogaster perforatus* MCP 41912 EU359427. *Neoplecostomus microps* MNR 24005 (#170) EU359429. *Neoplecostomus* sp. MCP 30672 EU359430. *Otocinclus affinis* DZJRP 7610 EU359431. *Otocinclus* cf. *hoppei* MHNG 2613.057 EU370985. *Otocinclus cocama* MCP 34842 EU359432. *Otocinclus flexilis* MCP 25234 EU370983. *Otocinclus flexilis* MCP 41907 EU370984. *Otocinclus vittatus* MCP 35848 EU359433. *Otothyris juquiae* MCP unreg. EU359434. *Otothyropsis* sp. MHNG 2587.011 EU371003. *Panaqolus changae* ANSP 181097 (tag P6218) EU359435. *Panaqolus* sp. ZSM 32728 EU359436. *Pareiorhaphis azygolechis* MCP 41909 EU359437. *Pareiorhaphis calmoni* MCP 41275 EU359438. *Pareiorhaphis eurycephalus* MCP 41458 EU359439. *Pareiorhaphis hypselurus* MCP 21695 EU359440. *Pareiorhaphis hystrix* MCP 22787 EU359441. *Pareiorhaphis nudula* MCP 41906 EU359442. *Pareiorhaphis parmula* MCP 41747 EU359443. *Pareiorhaphis* sp. 1 MCP 22339 EU359444. *Pareiorhaphis* sp. 2 MCP 28683 EU359445.

Pareiorhaphis sp. 3 MCP 40111 EU359446. *Pareiorhaphis* sp. 4 MCP 41296 EU359447. *Pareiorhaphis* sp. 5 MCP 41457 EU359448. *Pareiorhaphis splendens* MCP 22330 EU359449. *Pareiorhaphis splendens* MCP 41263 EU359450. *Pareiorhaphis steindachneri* MCP 41289 EU359451. *Pareiorhaphis stomias* MCP 41910 EU359452. *Pareiorhina* sp. MNRJ 26518 (#281) EU359453. *Parotocinclus* cf. *aripuanensis* MCP unreg. EU359454. *Parotocinclus eppleyi* AUM 43947 (tag V5576) EU359455. *Parotocinclus jumbo* ZSM 32727 EU359456. *Parotocinclus maculicauda* MCP 41911 EU359457. *Peckoltia vermiculata* AUM 39245 (tag V060) EU359458. *Peckoltia vittata* AUM 39248 (tag V114) EU359459. *Pseudacanthicus leopardus* ANSP 179613 (tag 2450) EU359460. *Pseudotocinclus juquiae* LBP 616 EU370988. *Pseudotocinclus tietensis* LBP 696 EU370987. *Pseudotothyris obtusa* MCP 33330 EU371016. *Pseudotothyris obtusa* MCP 33330 EU371017. *Rineloricaria* sp. MCNI 1222 EU359461. *Schizolecis guentheri* MCP 31722 EU359462. *Schizolecis guentheri* MCP 31724 EU371020. **Nematogenyidae**: *Nematogenys inermis* ANSP 180477 (tag 1) EU359428. **Scoloplacidae**: *Scoloplax distolothrix* MCP 40282 EU359463.

Capítulo II

Molecular Phylogeny of the Neoplecostominae and Hypoptopomatinae (Siluriformes: Loricariidae) using Multiple Genes

Molecular Phylogeny of the Neoplecostominae and Hypoptopomatinae (Siluriformes: Loricariidae) using Multiple Genes.

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Abstract

A phylogenetic analysis of nearly all genera of the loricariid subfamilies Neoplecostominae and Hypoptopomatinae is provided based on fragments of the subunit 1 of the *cytochrome* c *oxidase gene* (COI), the *recombination activating genes* 1 (RAG1) & 2 (RAG2), and the *F-reticulon 4 gene* in order to test the contradictory results of previous analyses. We obtained an alignment of 4678 contiguous nucleotides for 136 species from 50 loricariid genera from representatives of five loricariid subfamilies plus ten outgroup species from five loricarioid families, resulting in the largest phylogeny of the Loricariidae published so far. Our results from Maximum Likelihood, Maximum Parsimony, and Bayesian analyses show a monophyletic clade composed by the Hypoptopomatinae + Neoplecostominae as sister to the Hypostominae and this clade sister to the Loricariinae. Delturinae is the sister-group of the above clade. However, neither the Hypoptopomatinae nor the Neoplecostominae were recovered as monophyletic groups. Previously hypothesized monophyly of the Hypoptopomatini and Otothyrini could not be confirmed. Furthermore, the genera *Pareiorhaphis*, *Pareiorhina*, *Hisonotus* and *Parotocinclus* were recovered as polyphyletic.

Keywords: Loricariidae, Neoplecostominae, Hypoptopomatinae, phylogeny, armored catfish, COI, RAG1, RAG2

Introduction

With around 3100 species in 36 families (Ferraris, 2007), the order Siluriformes contains about 10% of all fish species worldwide. With the exception of two marine families, they are solely freshwater inhabitants. Catfish are most strongly represented in South America with 14 families and 64% of the species (Moyle and Cech, 2000; Rodiles-Hernández et al., 2005). Loricariidae (armored catfishes) is the largest catfish family, with approximately 100 genera and 785 species currently recognized (Eschmeyer and Fricke, 2009). Armored catfishes are endemic to South and Central America, but recently there are more and more records of introduced species from North America and Asia. As loricariids inhabit all kinds of waters from small and cool mountain streams to large and warm rivers, they play an important role in biodiversity. Along with five closely related families, they form the superfamily Loricarioidea (Schaefer and Lauder, 1986; Schaefer, 1990). The family Loricariidae is divided into six subfamilies (Armbruster, 2004; Reis et al., 2006): Lithogeninae (1 genus, 3 species), Delturinae (2 genera, 7 species), Neoplecostominae (5 genera, 39 species), Hypoptopomatinae (18 genera, 103 species), Loricariinae (~ 36 genera, 222 species), and Hypostominae (~ 40 genera, 411 species). However, the classification of genera in subfamilies has not been stable. The Neoplecostominae was modified several times (for a good overview see Reis et al., 2006). The monophyly of at least two subfamilies and two tribes (Neoplecostominae and Hypoptopomatinae; Ancistrini and Hypostomini) has been rejected by molecular studies (Montoya-Burgos et al., 1998, 2002; Hardman, 2005; Cramer et al., 2008), contrary to morphologic results.

Based on morphological characters, Schaefer (1997, 1998) considered the Hypoptopomatinae to be monophyletic, consisting of the two monophyletic tribes Hypoptopomatini and Otothyrini. Montoya-Burgos et al. (1998), using molecular data, were the first to find the Neoplecostominae and the Hypoptopomatinae closely connected, with

Pseudotocinclus being the sister group of *Pareiorhina* (Fig. 1). Cramer et al. (2008) came to similar results, with the Neoplecostominae as a paraphyletic group inside the Hypoptopomatinae (Fig. 2). In contrast to this, based on morphology, Armbruster (2004) found the Hypoptopomatinae to be a monophyletic group inside the Neoplecostominae (Fig. 3). Recently, Chiachio et al. (2008) made an attempt to solve these problems including *Pseudotocinclus* in the Neoplecostominae and elevating the Otothyrini to subfamily level. Unfortunately, however, this does not seem to be the ultimate solution as well as it contradicts the most recent morphological analysis (Lehmann, 2006), our former results (Cramer et al., 2008), and the findings presented here.

As there are strong contradictions between the morphological phylogenies and the molecular studies published so far (the most recent ones based on a single gene each; both with relatively few species), our aim is to join data from mitochondrial and nuclear sequences and to include the maximum number of species available. Despite the differences between previous results, both morphological and molecular studies found the Hypoptopomatinae + Neoplecostominae to form a monophyletic group. Therefore these two subfamilies were chosen to be the main focus of our study presented here.

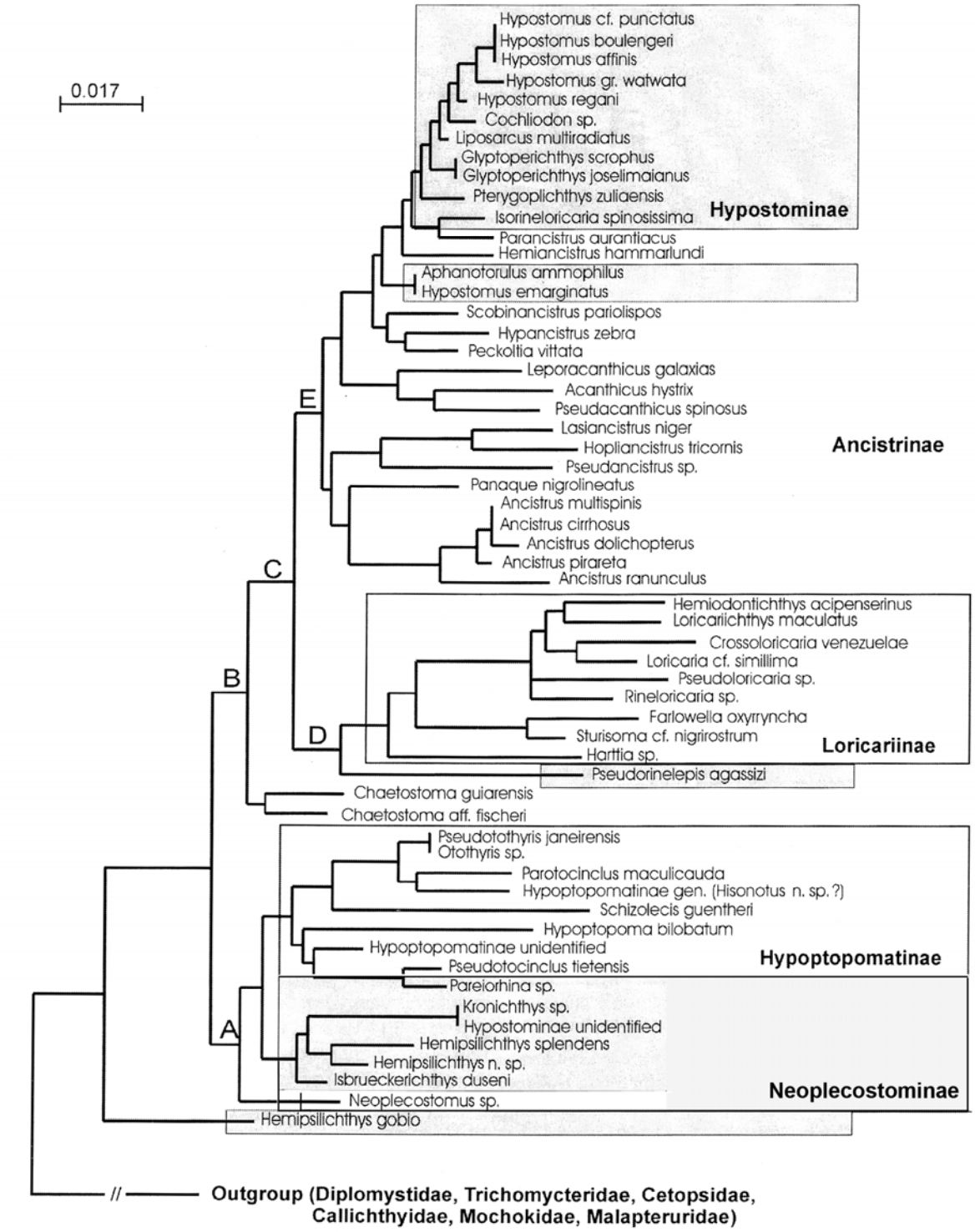

Fig. 1 Phylogeny from Montoya-Burgos et al. (1998: modified from Fig. 3) based on sequence data from mitochondrial 12S and 16S.

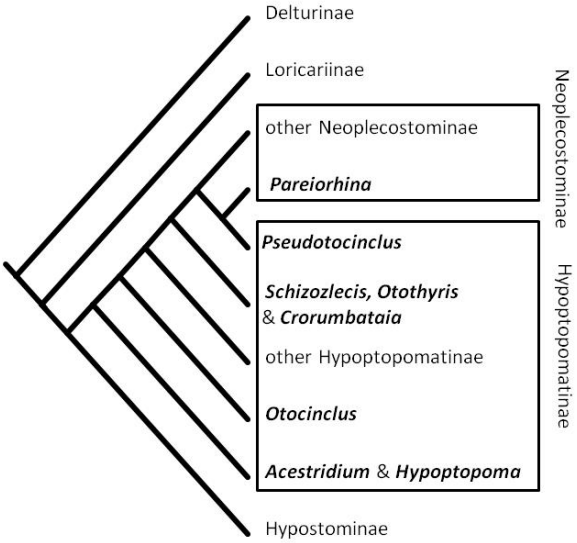

Fig. 2 Phylogenetic interrelationships of the Loricariidae, modified from Cramer et al. (2008)

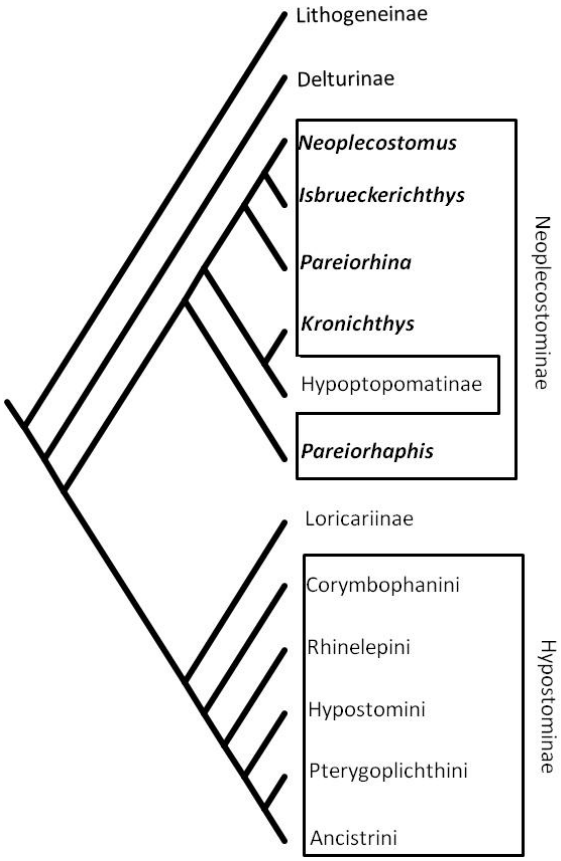

Fig. 3 Phylogenetic interrelationships of the Loricariidae modified from Armbruster (2004)

2. Material and methods:

2.1. Taxon sampling and marker selection

The specimens and species used in this study are listed in Appendix A. Our aim was to include all genera and as many species as possible of the subfamilies Hypoptopomatinae and Neoplecostominae together with representatives of the other loricariid subfamilies. Unfortunately there was no fresh tissue from *Niobichthys* or from *Lithogenes* available. As outgroup taxa we used representatives from the other five families of the Loricarioidea (Schaefer and Lauder, 1986; Schaefer, 1990; de Pinna, 1998).

Four genes were used in these analyses.

The closely linked nuclear *recombination activating genes* (RAG1 and RAG2) are present in all jawed vertebrates and code for components of the recombinase involved in V (D) J recombination of T-receptor and immunoglobulin genes (Bartl et al., 1994; Bernstein et al., 1996; Peixoto et al., 2000). Genes with immunological functions should provide an estimate of evolution largely uncorrelated with morphologic adaptations. These genes are usually highly conservative and underlay very little evolutionary pressure for adaptations on the environment (Hoofer et al., 2003). RAG1 and RAG2 have shown to be useful to reconstruct deep phylogenetic relationships in a series of studies (e.g. Sullivan et al., 2000, 2006; Lovejoy and Collette, 2001; Hardman and Page, 2003; Hardman, 2004; Lavoué and Sullivan, 2004; López et al., 2004; Calcagnotto et al., 2005).

The *cytochrome c oxidase I gene* (COI) shows a greater range of phylogenetic signal than any other mitochondrial gene (Hebert et al., 2003). Like other protein-coding genes, its third-position nucleotides show a high incidence of base substitutions, leading to a rate of molecular evolution that is about three times higher than that of 12S or 16S rDNA (Knowlton and Weigt, 1998). So, the evolution of this gene is rapid enough to allow the separation of not only closely related species, but also phylogeographic groups within a single species (Cox and Hebert, 2001; Wares and Cunningham, 2001).

Additional sequences from the nuclear *F-Reticulon 4* gene were available for the group we are studying from Chiachio et al. (2008). Thirty of their 53 sequences matched taxa we used and were included in our analyses.

2.2. DNA amplification and sequencing

Total genomic DNA was extracted from ethanol-preserved tissue using the QIAmp tissue kit (Qiagen, Hilden, Germany). PCR were carried out in 20 µl reactions. Primers are shown in Table 1. If necessary, a nested PCR was done using internal primers we designed. We amplified a 690 bp fragment of the subunit 1 of the *cytochrome c oxidase* using the following PCR conditions: 1x Invitrogen PCR buffer (Invitrogen, São Paulo), 1.5-2.5 mM $MgCl_2$, 0.2% Triton X-100, 200 µM of each dNTP, 0.025 U/µl Platinum® *Taq* polymerase (Invitrogen, São Paulo), 0.2 µM of each primer, and up to 2 µl of DNA solution.

We used our lab's standard protocol for this primer pair with an initial denaturation step of 1 min at 96 °C followed by 40 cycles of 94 °C for 30 s, annealing at 50 °C for 20 s, 48 °C for 5 s, 46 °C for 5 s, 44 °C for 5 s, 42 °C for 5 s, 40 °C for 20 s, and extension at 72 °C for 1 min. This was followed by a final 3 min at 72 °C extension step.

For the *recombination activating gene 1* we amplified a 983 bp fragment using the PCR conditions as described above, but 1.5 mM $MgCl_2$, 0.05 U/µl Platinum® *Taq* polymerase (Invitrogen, São Paulo), and 0.5 µM of forward and reverse primer, usually without Triton X-100.

The following thermocycler conditions were used (S. Hoegg, personal communication): an initial denaturation step of 5 min at 94 °C followed by 10 cycles of 94 °C for 30 s, annealing at 52 to 57 °C (each +0.5 °C for the first 10 cycles) for 40 s, extension at 72 °C for 4 min, followed by 25 cycles at 94 °C for 30 s, 55 °C for 40 s, 72 °C for 4 min, and a final extension at 72 °C for 5 min.

For the *recombination activating gene 2* we amplified a 961 bp fragment using the PCR conditions as described for RAG1, but 3.0 mM $MgCl_2$, 0.04 U/µl Platinum® *Taq* (Invitrogen, São Paulo), and 0.4 µM of forward and reverse primer. We used an initial denaturation step of 1min at 94 °C followed by 35 cycles of 94 °C for 30 s, annealing at

59 °C (51 °C for the nested PCR) for 30 s, and extension at 72 °C for 2 min. This was followed by a final 10 min at 72 °C extension step (Sullivan et al., 2006). Sometimes we used additionally 0.1 mg/µl BSA, 1M Betaine, 0.3% Trealose, and/or 0.2% Triton X-100.

Amplification success was evaluated on GelRed™ (BioTium, São Paulo) or ethidium bromide-stained agarose gels (Sambrook, 1989). PCR products were purified using PEG8000, ExoSAP-IT® (USB) or the ilustra™ GFX PCR and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK). In case of multiple PCR products, an extraction from the gel was done using the ilustra™ GFX PCR and Gel Band Purification Kit. Sequencing was done using the DYEnamic™ ET dye terminator kit (GE Healthcare, São Paulo) read in a MegaBace1000 sequencer. Chromatograms were visualized, edited and assembled using BioEdit 7.0.1 (Hall, 1999). Sequence alignments for each partition were done using Clustal X 1.83 (Thompson et al., 1997) with the standard settings and concatenated in a single alignment.

Table 1 Primers used

Gene fragment	Primer sequence	Source
LCO1490	5‘-GGT CAA CAA ATC ATA AAG ATA TTG G-3‘	Hebert et al. (2003)
HCO2198	5‘-TAA ACT TCA GGG TGA CCA AAA AAT CA-3‘	Hebert et al. (2003)
RAG1-L3a	5’-GCR TTN CCA ATG TCA CAR TG-3’	Martin (1999)
RAG1-MFL1	5’-AGC TGC AGY CAR TAY CAY AAR ATG TA-3’	Martin (1999)
RAG2-MHF1	5’-TGy TAT CTC CCA CCT CTG CGy TAC C-3’	Sullivan et al. (2006)
RAG2-MHR1	5’- TCA TCC TCC TCA TCk TCC TCw TTG TA-3’	Sullivan et al. (2006)
Internal primers		
COI-CMF	5‘-GCT AGC CTG TTA ATT CG-3‘	This study
COI-CMR	5‘-AAA GTG GTG TTH AAG TTT CG-3‘	This study
RAG1-CCF	5‘-TGG ACG TCG ATC TTT CAA CCC-3‘	This study
RAG1-CCR	5‘-CTA ATG TGG GCT GTG TCT CCA T-3‘	This study
RAG2-MCF	5‘-CCG TAC ACC CAA TGA-3‘	This study
RAG2-MCR	5‘-AAA TTC AGT AGA TTC TTG ACT GC-3‘	This study

2.3. Phylogenetic analyses

Maximum likelihood (ML) analyses were done using RAxML 7.0.3 (Stamatakis, 2006) with 1000 replicates under the GTRGAMMA model. The model parameters were estimated for the following seven partitions: each of the three codon positions for COI, RAG1 and RAG2 each, the two introns, and the two exons of the F-Reticulon fragment. Nodal support was evaluated with 2000 nonparametric bootstrap pseudoreplicates.

Bayesian analyses were done using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The same partitions as under ML were used. Appropriate substitution models for each of the partitions were estimated with the Akaike Information Criterion (AIC) as implemented in MrModeltest 2.3 (Nylander, 2004). Some of the Bayesian analyses were run on the Bioportal TITAN server (Oslo University).

Maximum Parsimony (MP) analyses were done using the new technology search as implemented in TNT 1.1 (Goloboff et al., 2008). We executed 1000 sequential ratchets of 200 iterations each, followed by sectorial searches and tree fusion. For nodal support, the Bremer support (decay index) was calculated using the converse constraint method as implemented in TNT 1.1. 2000 nonparametric bootstrap pseudoreplicates were also calculated.

To test our topologies, we made six more MP analyses using constraints to enforce the monophyly of the subfamilies Hypostominae and Neoplecostominae, and the genera *Neoplecostomus*, *Pareiorhaphis*, and *Pareiorhina*. The resulting alternative topologies were evaluated using the Kishino and Hasegawa test (KH) (Kishino and Hasegawa, 1989) as implemented in PAUP 4b10 (Swofford, 2001).

3. Results and Discussion

The present study is the largest loricariid phylogeny in number of species published so far. We included representatives from five of the six loricariid subfamilies. The Lithogeninae could not be included because of the poor quality of the DNA extracted from formalin fixed tissue. The Hypoptopomatinae and the Neoplecostominae were included with taxa from all described genera but *Niobichthys*. The latter is a monotypic genus only known from one location in the Neblina Mountains in Venezuela. It was not possible to get tissue from this taxon. The Neoplecostominae is represented by most of its described

species plus some new taxa, lacking only four species of *Pareiorhaphis* (two of them without confirmed locations and only known from few specimens) and five of *Neoplecostomus*.

3.1. Sequence statistics

We were able to sequence fragments of up to 690 bp for COI, 983 bp for RAG1, and 961 bp for RAG2. Additional sequences were taken from GenBank (Cramer et al. (2008) and Rodriguez et al. (2008): COI; Chiachio et al. (2008): F-Reticulon 4). In our analysis we included sequences of 161 specimens from 146 species and 57 genera, most of them represented by sequences of the three genes we amplified (species and GenBank accession numbers in Appendix A). 136 species from 50 genera are loricariids, 103 species from 30 genera are from the subfamilies Neoplecostominae and Hypoptopomatinae. *Nematogenys inermis* is the most basal taxon of the super family Loricarioidea according to Schaefer and Lauder (1986) and was used to root the trees.

Together with the F-Reticulon sequences, we obtained a total alignment length of 4678 bp. Out of these, 2258 (48.2%) were variable and 1588 (33.9%) were parsimony-informative. For the separate partitions these values were: COI: 1st codon position: 81 (35.2%) and 57 (24.7%), 2nd codon position: 25 (10.9%) and 8 (3.5%), 3rd codon position: 224 (97.4%) and 221 (96.1%), RAG2: 616 (64.1%) and 455 (47.3%), RAG1: 462 (47.0%) and 348 (35.4%), F-Reticulon: intronic positions: 724 (58.0%) and 424 (33.9%), and exonic positions: 134 (15.1%) and 80 (9.0%).

3.2. Phylogenetic analyses

The ML analyses resulted in one best tree (-ln L 51854.558340) that is shown in Fig. 4. Most clades received high bootstrap values. To test our best tree, we ran another ML analysis taking one of the most parsimonious trees as starting tree. This analysis resulted in the same topology as the previous best tree.

For the Bayesian analyses, the best models of sequence evolution for the data partitions found according to the AIC criterion were: SYM + G + I for the 1st codon position in COI, F81 + I for the 2nd codon position in COI, GTR + G + I for the 3rd codon position in COI and RAG2, HKY + G + I for RAG1 and the F-Reticulon exonic positions, and GTR + G for the F-Reticulon intronic positions. Two runs of four chains each (three heated, one cold) were run simultaneously with tree space sampled every 1000th generation. Convergence between chains occurred after 10 million generations (standard deviation of split frequency < 0.01). A graphical analysis of the evolution of the likelihood scores showed that the stationary phase was reached after 600,000 generations. Therefore, the first 2 million generations (20%) were discarded as burn-in. The remaining trees were used to calculate the consensus tree (Fig. 5; Appendix B). There are only few minor differences between the best tree from the ML analyses and the consensus from the Bayesian analysis, with one exception, positions changed only one node. The only striking difference is that the Bayesian analysis did not resolve *Lampiella gibbosa* as sister to the genus *Otocinclus*, but as sister to *Hisonotus leucofrenatus.* To test this result, we performed additional MP and ML analyses (not shown here) for only the COI, RAG1, and RAG2 sequences, without F-Reticulon. Analyzing each gene separately or the three together led to very similar topologies, always revealing *Lampiella gibbosa* as sister to *Hisonotus leucofrenatus*.

The MP analyses resulted in 358 best trees with 9363 steps (CI: 0.36; HI: 0.64; RI: 0.69; RC: 0.25). Their strict consensus is shown in Fig. 6 (Appendix C).

Besides the lower resolution of the consensus of MP trees, there are only five minor differences between the results from the MP and the ML analyses, all with little support.

ML shows *Delturus carinotus* as sister taxon of the genus *Hemipsilichthys*, MP revealed it as sister taxon to *Hemipsilichthys nimius*. In the ML analysis, *Rhinelepis aspera* is sister to all loricariids but the Delturinae, in the MP analyses the Delturinae and the Loricariinae are basal to *Rhinelepis*. MP resolved *Pareiorhaphis hypselurus*, *P. nudula*, and *P. stomias* as sister group to *Isbrueckerichthys*, whereas ML puts them as sister group to the remaining *Pareiorhaphis*. ML shows *Pareiorhaphis parmula* as sister of *P. azygolechis*, *P.* sp. 1, and *P.* sp.4, MP resolved *P. azygolechis* as sister of the other named species. The only taxon whose position differed more than one node is *Parotocinclus jumbo*. MP showed it as sister to *Lampiella gibbosa* on the base of the genus *Otocinclus*, whereas ML placed it basal to the clade containing the majority of the Otothyrini.

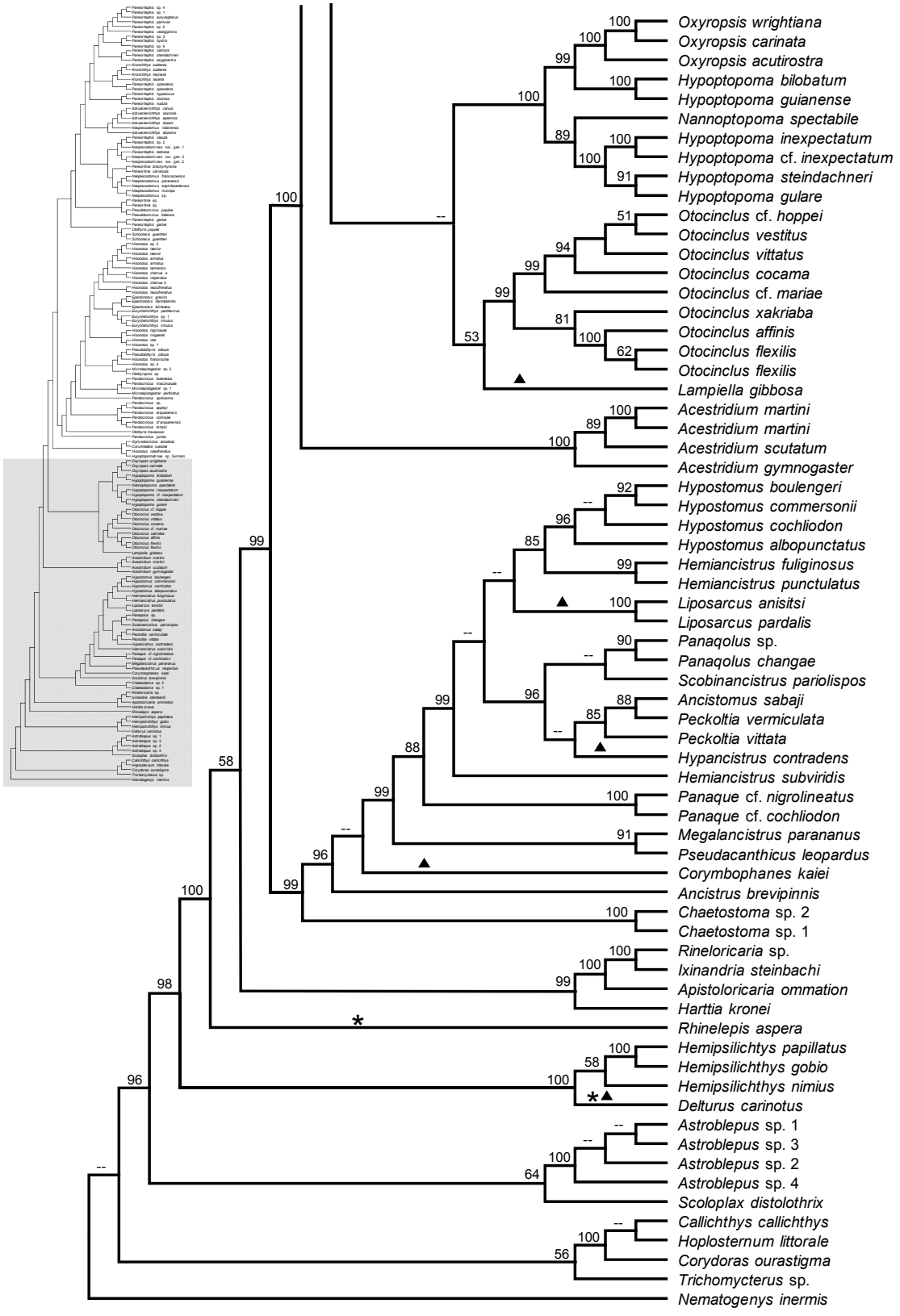

Fig. 4a Relationships of the taxa within the Delturinae, Loricariinae, Hypostominae and Hypoptopomatini based on the Maximum Likelihood analysis. This is part of the best tree (-lnL 51854.558340), remainder of the tree is in Fig. 4b and c. Likelihood bootstrap proportions shown on the branches (≥ 50). Branches marked with * differ in their position from the MP analysis; branches marked with ▲ differ in their position from the Bayesian analysis.

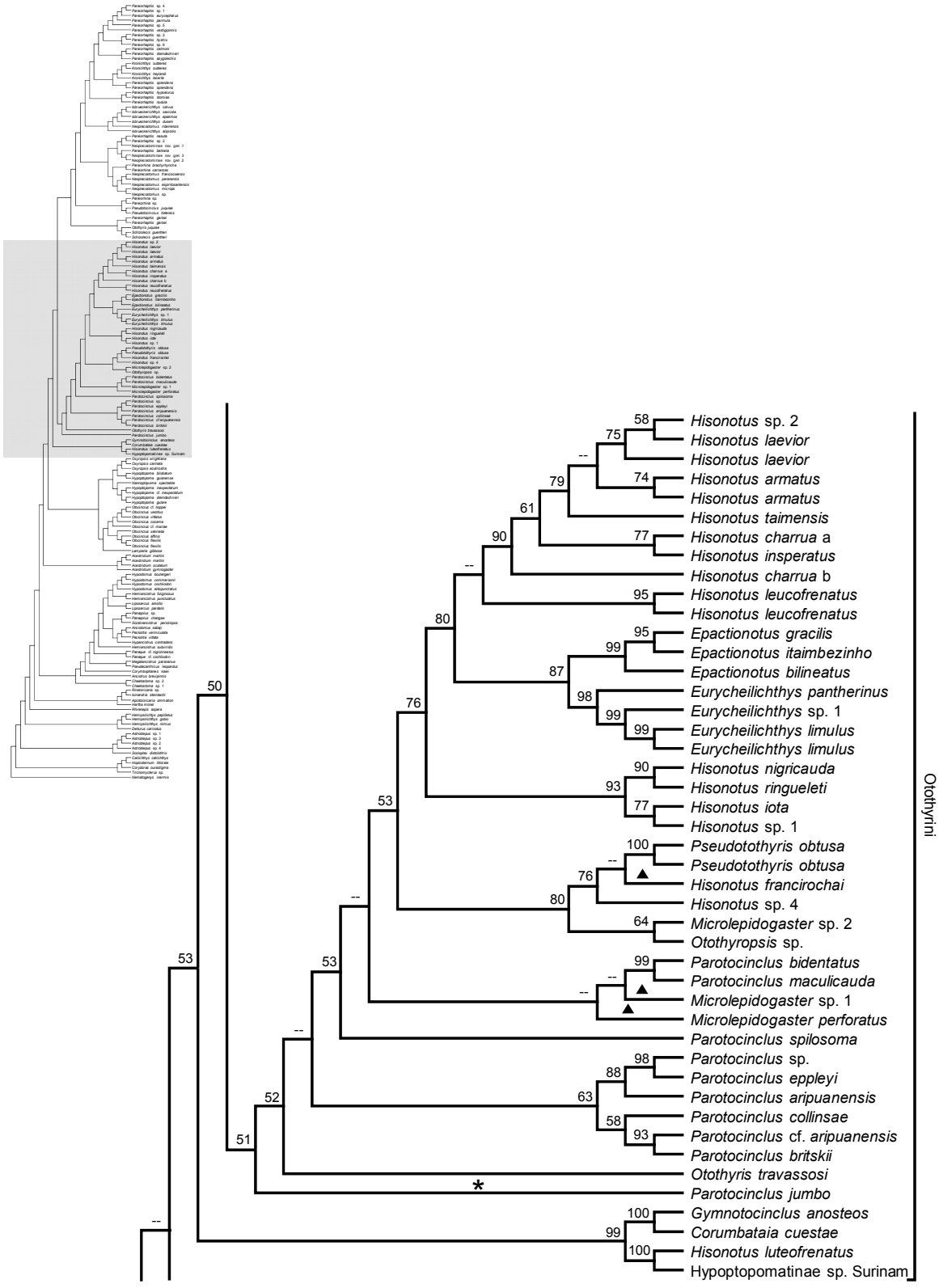

Fig. 4b Relationships of the taxa within the Otothyrini (without *Otothyris*, *Schizolecis*, and *Pseudotocinclus*) based on the Maximum Likelihood analysis. This is part of the best tree (-lnL 51854.558340), remainder of the tree is in Fig. 4a and c. Likelihood bootstrap proportions shown on the branches (≥ 50). Branches marked with * differ in their position from the MP analysis; branches marked with ▲ differ in their position from the Bayesian analysis.

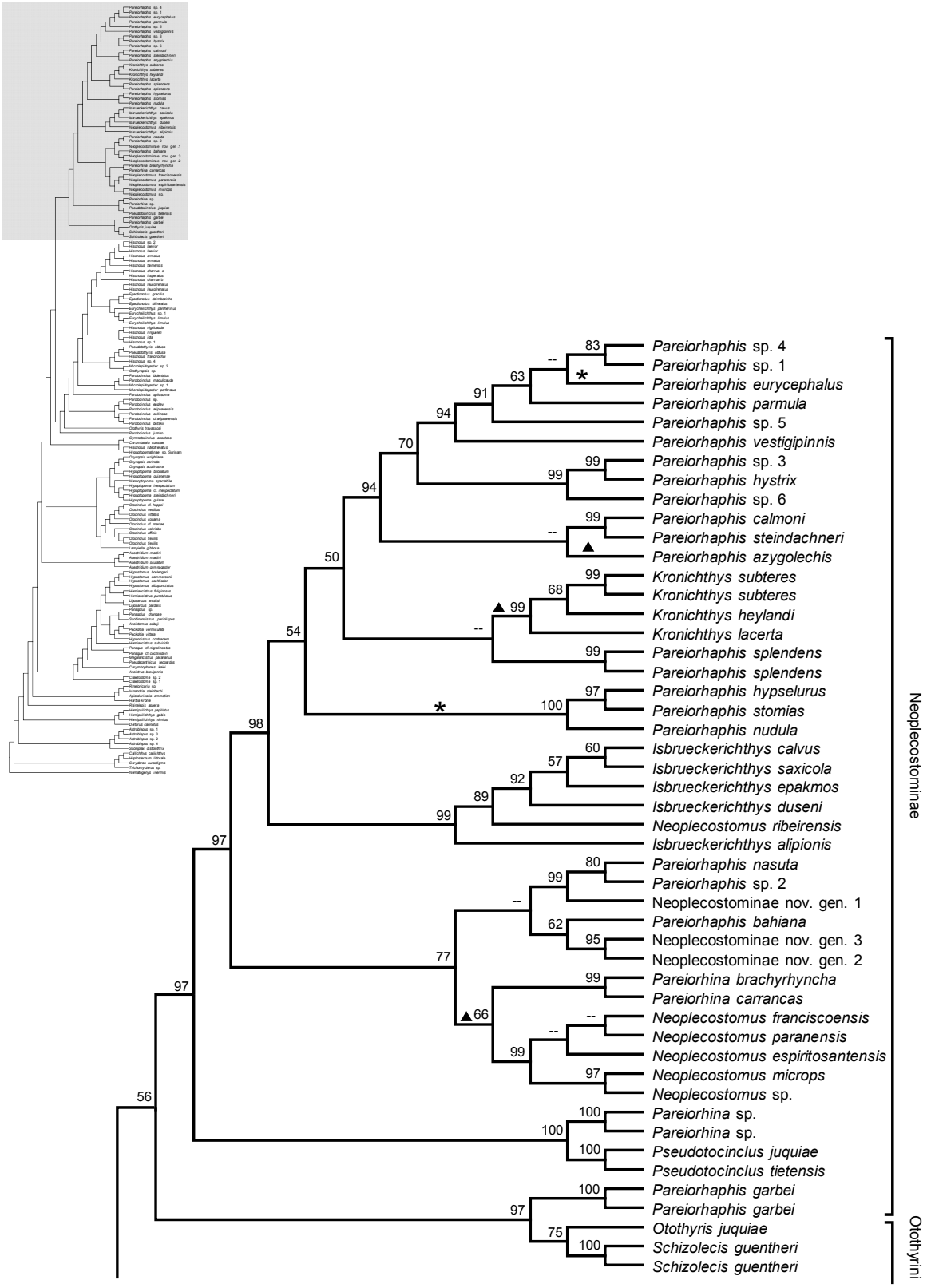

Fig. 4c Relationships of the taxa within the Neoplecostominae plus *Otothyris*, *Schizolecis*, and *Pseudotocinclus* based on the Maximum Likelihood analysis. This is part of the best tree (-lnL 51854.558340), remainder of the tree is in Fig. 4a and b. Likelihood bootstrap proportions shown on the branches (≥ 50). Branches marked with * differ in their position from the MP analysis; branches marked with ▲ differ in their position from the Bayesian analysis.

3.3. Phylogenetic relationships

The few differences between the results of MP, ML, and the Bayesian analyses are low support resolutions and are not unacceptable in morphological ground. We therefore will use the ML tree for further discussion because of its better resolution than the consensus from MP and its similarity to the consensus from the Bayesian analyses. Branch lengths from the ML tree are similar to the ones from the Bayesian analysis, so they are only shown once (Fig. 5).

Below we discuss the results of the ML analyses starting from the base of the tree and climbing upwards.

Inside the outgroup, an interesting finding is the long branch of *Astroblepus* sp. 4. The COI and RAG1 sequences from the four *Astroblepus* are relatively similar compared with the other species from the genus (0.876-0.965 and 0.981 to 0.991, respectively). The same is true for the RAG2 sequences from species 1 to 3 (0.992 to 0.997), only the one from species 4 is significantly different from its congeners (0.716 to 0.722), but the translation to amino acids resulted in very similar sequences (0.956 to 0.972 vs. 0.984 to 1 between species 1 to 3 and 0.995/0.985 to 1 in COI/RAG1).

The Loricariidae forms a monophyletic group. Without the presence of taxa from the Lithogeninae, the Delturinae is the most basal loricariid clade, as Reis et al. (2006) already suggested based on morphological results. A somewhat strange finding is that *Rhinelepis* is located clearly separated from the other hypostomine taxa. Most other studies placed members of the Rhinelepini as relatively basal taxa within the Hypostominae (e.g. Schaefer, 1986; Armbruster, 2004), though Montoya-Burgos et al. (1998) revealed *Pseudorinelepis* (Rhinelepini) as sister taxon of the Loricariinae. *Rhinelepis* differs from all other Hypostominae in some aspects. Besides its somewhat archaic appearance, they are

the only loricariids which do spawning migration and which produce millions of eggs (up to 180,000; vs. dozens or a few hundred in other loricariids) that are spread freely in the current (Suzuki et al., 2000). *Rhinelepis* are the only hypostomins without parental care of eggs or fries.

An alternative topology with an enforced monophyly of the Hypostominae resulted in seven additional steps and showed *Rhinelepis* as sister of the remaining hypostomine taxa. This constrained topology could not be statistically refused by the KH test (p=0.6283).

Inside the Hypostominae, *Chaetostoma* was placed as the most basal taxon and not *Corymbophanes* as in Armbruster (2004). Also, *Panaqolus* and *Scobinancistrus* are clearly separated from *Panaque*, suggesting that they are not synonyms or subgenera as proposed by Armbruster (2004). Neither the Ancistrini nor the Hypostomini seem to form monophyletic groups.

But, as the Hypostominae is not the focus of this article, we included only few taxa and further studies have to analyze this subfamily with more details.

Concordant with former studies (e.g. Armbruster, 2004; Cramer et al., 2008), we found the Hypoptopomatinae + Neoplecostominae to form a monophyletic clade, but our data did not recover the Hypoptopomatini, Otothyrini (sensu Schaefer, 1991), or the Neoplecostominae as natural groups. Some of the most parsimonious trees even show the Hypoptopomatini as monophyletic like found by Lehmann (2006) and Chiachio et al. (2008), but with low support.

The results from the complete dataset using ML and MP place *Lampiella gibbosa* as sister to the genus *Otocinclus*. Analyzing only the sequences from COI, RAG1 and RAG2, this species is positioned as sister to *Hisonotus leucofrenatus*, getting high bootstrap values. The Bayesian analyses of the complete dataset come to the same result. Therefore,

it still cannot be resolved if *Lampiella* really is a distinct genus or if it is a synonym of *Otocinclus* as proposed by Schaefer (2003). *Hypoptopoma* forms a paraphyletic group including the genera *Nannoptopoma* and *Oxyropsis*. If *Nannoptopoma* (as proposed by Chiachio et al. [2008]) and potentially *Oxyropsis* should be treated as synonyms of *Hypoptopoma*, or if a new genus should be established for part of the *Hypoptopoma* species, should be investigated by a future study including more species and morphological data.

Corumbataia, *Gymnotocinclus*, *Hisonotus luteofrenatus*, and Hypoptopomatinae sp. Surinam (probably the same species as shown in Le Bail et al. [2000 p. 262] and Schaefer [1998: taxon 3]) form a group basal to the remaining Otothyrini + Neoplecostominae, with *Corumbataia* being sister to *Gymnotocinclus*. As already predicted by Schaefer (Le Bail et al., 2000: p. 262), *Hisonotus luteofrenatus* (which belongs to the same undescribed genus, in preparation by Buckup, Britto, and Reis) and Hypoptopomatinae sp. Surinam also are sister taxa.

The next bigger clade is formed by the Neoplecostominae + *Pseudotocinclus*, *Schizolecis*, and *Otothyris juquiae*. The two latter taxa, together with *Pareiorhaphis garbei*, form the basal group of this clade. Thus, *Otothyris* turns out to be a polyphyletic taxon. Lehmann (2006) also found *Otothyris* and *Schizolecis* to be sister taxa, but there is no morphological evidence for a closer relation between *Pareiorhaphis garbei* and these two genera (Pereira, 2008). *Pareiorhaphis garbei* shows characteristics that are exclusive for the Neoplecostominae (e.g. presence of an accessory process on the crest of the muscle *levator arcus palatine* and a branched canal on the *canal plate*) or for the genus *Pareiorhaphis* (e.g. *canal plate* strongly articulated with the pre-operculum in mature males) (Pereira, 2008). Our additional constrained MP analyses enforced monophyly for

the genus *Pareiorhaphis* and the Neoplecostominae. The first analysis resulted in significantly worse trees (p=0.0001), by KH test, with 46 additional steps. The second analysis generated best trees with six additional steps, showing *Pareiorhaphis garbei* as sister to the remaining neoplecostomines. This result could not be rejected by the KH test (p=0.4796).

Inside the Neoplecostominae, *Pseudotocinclus* and part of the genus *Pareiorhina* form the most basal group. That corroborates the results of Montoya-Burgos et al. (1998), Cramer et al. (2008), and Chiachio et al. (2008), but this is the first time that *Pareiorhina* is recovered as polyphyletic.

An enforced monophyly for *Pareiorhina* caused 35 additional steps and showed all *Pareiorhina* as sister of the genus *Pseudotocinclus*. The KH test refused the constrained topology as significantly worse (p=0.0012).

Our results place *Neoplecostomus ribeirensis* inside the genus *Isbrueckerichthys*. There is no morphological corroboration for this finding as *N. ribeirensis* shares the morphological synapomorphies of the genus *Neoplecostomus* (dorsal profile of the unbranched pectoral spine with accentuated curvature; leteropterygium with small expansion in the distal portion; lower lip with papillae forming conspicuous series localized posterior of the dentary) and does not show the synapomorphy of the genus *Isbrueckerichthys* (crest of the *levator arcus palatine* with dorsal direction) (Pereira, 2008). To exclude the possibility of a misidentification or contamination, we reexamined the voucher specimen, repeated the DNA extraction and sequenced the three fragments again, with the very same result. The remaining species of *Neoplecostomus* form a well supported monophyletic group, as expected from morphologic data (Pereira, 2008). A constrained topology with an enforced monophyly for the genus Neoplecostomus resulted in significantly worse trees (p=0.0140) with 28 additional steps.

Contrary to the results of Pereira (2008), we found *Pareiorhaphis* to be polyphyletic. *Pareiorhaphis bahiana* and the new genera Neoplecostominae nov. gen. 2 & 3 form a biogeographical clade. The three taxa are from coastal rivers in Bahia state. Neoplecostominae nov. gen. 1, *Pareiorhaphis nasuta*, and *P.* sp. 2 form another geographical clade from the rio Doce basin. Being sister clades, the two groups form a third biogeographical clade from the rio São Francisco drainage. In agreement with Pereira, (2008) we found *P. hypselurus*, *P. nudula*, and *P. stomias* as well as *P. calmoni* and *P. steindachneri* to form two groups with strong support. Our constraint forcing the genus *Pareiorhaphis* (without *P. garbei*) as monophyletic resulted in significantly worse trees (p=0.0222) with 28 additional steps.

The remaining taxa of the Otothyrini (sensu Schaefer, 1991) form a poorly supported group. Concordant with Gauger and Buckup (2005) and Lehmann (2006), *Parotocinclus* turned out to be polyphyletic. *Parotocinclus aripuanensis*, *P. britskii*, *P. collinsae*, *P. eppleyi* and an undescribed species form a geographical group from the Guyana shield. *Parotocinclus bidentatus* and *P. maculicauda* are well supported sister taxa. *Hisonotus* is another genus that is highly polyphyletic, needing a morphological revision.

Striking in our results is that the “Hypoptopomatini” was recovered as the most basal hypoptopomatine taxon and *Eurycheilichthys* one of the most derived. This is the extreme opposite of what the morphologic data of Lehmann (2006) revealed.

At present, our molecular data suggest that Hypoptopomatini, Otothyrini, and Neoplecostominae are not monophyletic. To further investigate this group with respect of the differences between molecular and morphologic evidences we suggest a combined analysis of these data, an approach that has been successful in similar cases

(e.g. Gatesy et al., 2003; Mattern and McLennan, 2004), together with a continuing expansion of the species sampling.

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Appendix A: Species, depository information and GenBank accession numbers (COI, RAG2, RAG1, F-Reticulon) included in this study. Sequences marked with * were taken from GenBank; O: no sequence available.

Astroblepidae: *Astroblepus* sp. 1 ANSP 180581 (tag 4805) EU359404*, GQ225437, O, O. *Astroblepus* sp. 2 ANSP 180605 (tag 4490) EU359405*, GQ225438, GQ214567, O. *Astroblepus* sp. 3 ANSP 180613 (tag 4453) EU359406*, GQ225439, GQ214568, O. *Astroblepus* sp. 4 ANSP 180616 (tag 4436) EU359407*, GQ225440, GQ214569, O. **Callichthyidae:** *Callichthys callichthys* MCP 29384 EU359408*, GQ225443, GQ214572, O. *Corydoras ourastigma* MCP 28799 GQ225387, GQ225447, GQ214575, O. *Hoplosternum littorale* MCP 21196 EU359416*, GQ225475, GQ214599, O. **Loricariidae:** *Acestridium martini* ANSP 182901 (tag V5276) EU359398*, GQ225430, GQ214560, O. *Acestridium martini* ANSP 182901 (tag V5277) EU359399*, GQ225431, GQ214561, O. *Acestridium* sp. 1 MCP 37783 EU359400*, GQ225432, GQ214562, O. *Acestridium* sp. 2 MCP 37785 EU359401*, GQ225433, GQ214563, O. *Ancistomus sabaji* AUM 35537 (tag T2031) O, GQ225434, GQ214564, O. *Ancistrus brevispinnis* MCP 21246 EU359402*, GQ225435, GQ214565, O. *Apistoloricaria ommation* ANSP 182331 (tag P6265) EU359403*, GQ225436, GQ214566, O. *Chaetostoma* sp. 1 ANSP 180446 (tag P4772) EU359409*, GQ225444, GQ214573, O. *Chaetostoma* sp. 2 ANSP 180448 (tag P4814) EU359410*, GQ225445, GQ214574, O. *Corumbataia cuestae* LBP 876 EU371019*, GQ225446, O, EU817521*. *Corymbophanes kaiei* AUM 28163 (tag MH217) O, GQ225448, GQ214576, O. *Delturus carinotus* MCP no number (P 22) GQ225388, GQ225449, GQ214577, O. *Epactionotus bilineatus* MCP 26964 EU371008*, GQ225450, O, EU817553*. *Epactionotus gracilis* MCP 23606 EU371005*, GQ225451, GQ214578, O. *Epactionotus itaimbezinho* MCP 23683 EU371004*, GQ225452, O, O. *Eurycheilichthys limulus* MCP 21270 EU370989*, GQ225453, GQ214579, O. *Eurycheilichthys limulus* MCP 21270 EU370990*, O, O, O. *Eurycheilichthys pantherinus* MCP 22373 EU371000*, GQ225454, GQ214580, O. *Eurycheilichthys* sp. 1 MCP 21207 EU370995*, O, O, EU817529*. *Gymnotocinclus anosteos* UFRGS no number O, GQ225456, O, O. *Harttia kronei* MCP 31596 GQ225390, O, GQ214582, O. *Hemiancistrus fuliginosus* MCP 37566 O, GQ225457, GQ214583, O. *Hemiancistrus punctulatus* MCP 21248 O, GQ225458, GQ214584, O.

Hemiancistrus subviridis AUM 42930 (tag P4648) EU359411*, GQ225459, GQ214585, O. *Hemipsilichthys gobio* MCP 42452 GQ225391, GQ225460, GQ214586, EU817547*. *Hemipsilichthys nimius* MCP 30671 EU359412*, GQ225461, GQ214587, O. *Hemipsilichtys papillatus* MCP 43954 GQ225392, GQ225462, GQ214588, O. *Hisonotus armatus* MCP 25159 EU371012*, O, O, O. *Hisonotus armatus* MCP 37682 EU359414*, GQ225463, GQ214589, O. *Hisonotus charrua* a MCP 21644 EU371013*, O, O, EU817523*. *Hisonotus charrua* b UFRGS 7185 GQ225393, GQ225465, GQ214590, O. *Hisonotus francirochai* DZJRP 7727 EU359415*, GQ225466, GQ214591, EU817518*. *Hisonotus insperatus* O, GQ225467, O, EU817524*. *Hisonotus iota* MCP 40029 EU359413*, GQ225464, O, O. *Hisonotus laevior* MCP 23005 EU371015*, GQ225469, GQ214593, O. *Hisonotus laevior* MCP 37684 O, GQ225468, GQ214592, O. *Hisonotus leucofrenatus* MCP 31819 EU371001*, GQ225470, GQ214594, EU817530*. *Hisonotus leucofrenatus* LBP 873 EU371002*, GQ225471, GQ214595, O. *Hisonotus luteofrenatus* MCP 32668 O, GQ225483, O, O. *Hisonotus nigricauda* UFRGS 9120 GQ225394, GQ225472, GQ214596, EU817522*. *Hisonotus ringueleti* UFRGS 9119 GQ225395, GQ225473, GQ214597, EU817531*. *Hisonotus* sp. 1 MCP 23744 EU371010*, O, O, O. *Hisonotus* sp. 2 MCP 25139 EU371014*, O, O, O. *Hisonotus* sp. 4 LBP 810 EU371018*, O, O, O. *Hisonotus taimensis* MCP 21375 EU371011*, GQ225474, GQ214598, O. *Hypancistrus contradens* AUM 39241 O, GQ225476, GQ214600, O. *Hypoptoma bilobatum* MHNG 2588.092 EU370986*, GQ225477, O, O. *Hypoptopoma* cf. *inexspectatum* ANSP uncat. (tag 5089) EU359417*, GQ225478, GQ214601, O. *Hypoptopoma guianensis* ANSP 180669 (tag T2215) EU359418*, GQ225479, GQ214602, O. *Hypoptopoma gulare* ANSP 178340 (tag 1551) EU359419*, GQ225480, GQ214603, EU817541*. *Hypoptopoma inexspectatum* ANSP uncat. (tag 5047) EU359420*, GQ225481, GQ214604, EU817555*. *Hypoptopoma steindachneri* ANSP 182723 (tag P6233) EU359421*, GQ225482, GQ214605, O. Hypoptopomatinae sp. Surinam MCP 43953 GQ225396, GQ225484, O, O. *Hypostomus albopunctatus* MCP 37990 O, GQ225485, GQ214606, O. *Hypostomus boulengeri* NUP uncat. (Z49935) EU359422*, GQ225486, GQ214607, EU817560*. *Hypostomus cochliodon* NUP uncat. Z64017 GQ225397, GQ225487, GQ214608, O. *Hypostomus commersonii* MCP 22767 EU359423*, GQ225488, GQ214609, O. *Isbrueckerichthys alipionis* MCP no number GQ225398,

GQ225489, GQ214610, EU817566*. *Isbrueckerichthys calvus* MZUEL 4949 GQ225399, GQ225490, GQ214611, O. *Isbrueckerichthys duseni* MCP 42421 GQ225400, GQ225491, GQ214612, EU817548*. *Isbrueckerichthys epakmos* MCP 42436 GQ225401, GQ225492, GQ214613, O. *Isbrueckerichthys saxicola* MCP 43154 O, GQ225493, GQ214614, O. *Ixinandria steinbachi* EU359426*, GQ225494, GQ214615, O. *Kronichthys heylandi* MCP 31574 GQ225402, GQ225495, GQ214616, O. *Kronichthys lacerta* MCP no number GQ225403, GQ225496, GQ214617, O. *Kronichthys subteres* MCP 42443 GQ225404, GQ225497, GQ214618, O. *Kronichthys subteres* MCP 31600 EU371021*, GQ225498, GQ214619, O. *Lampiella gibbosa* MCP 43883 GQ225405, GQ225499, GQ214620, EU817545*. *Liposarcus anisitsi* MCP 37992 O, GQ225500, O, O. *Liposarcus pardalis* ANSP 178396 tag: 1559 O, GQ225501, O, O. *Megalancistrus parananus* MCP 37991 O, GQ225503, GQ214621, O. *Microlepidogaster perforatus* MNRJ 31886 O, GQ225505, GQ214623, O. *Microlepidogaster* sp. 1 MCP 41913 GQ225407, GQ225506, GQ214624, O. *Microlepidogaster* sp. 2 MCP 41912 EU359427*, GQ225504, GQ214622, O. *Nannoptopoma spectabile* MCP 43952 GQ225408, GQ225507, GQ214625, O. Neoplecostominae nov. gen. 1 MCP 42693 GQ225389, GQ225455, GQ214581, O. Neoplecostominae nov. gen. 2 MCP 42460 GQ225386, GQ225441, GQ214570, O. Neoplecostominae nov. gen. 3 MCP no number O, GQ225442, GQ214571, O. *Neoplecostomus espiritosantensis* MNRJ 22457 O, GQ225509, GQ214627, O. *Neoplecostomus franciscoensis* MCP 42428 GQ225409, GQ225510, GQ214628, O. *Neoplecostomus microps* MNRJ 24005 (#170) EU359429*, GQ225511, GQ214629, EU817568*. *Neoplecostomus paranensis* MNRJ 23974 O, GQ225512, O, O. *Neoplecostomus ribeirensis* MCP 42480 GQ225410, GQ225513, GQ214630, O. *Neoplecostomus* sp. MCP 30672 EU359430*, GQ225514, GQ214631, O. *Otocinclus affinis* DZJRP 7610 EU359431*, GQ225515, GQ214632, O. *Otocinclus* cf. *hoppei* MHNG 2613.057 EU370985*, O, O, O. *Otocinclus* cf. *mariae* MHNG no number (SU07-350) GQ225411, GQ225516, GQ214633, EU817558*. *Otocinclus cocama* MCP 34842 EU359432*, GQ225517, GQ214634, O. *Otocinclus flexilis* MCP 25234 EU370983*, GQ225518, GQ214635, EU817546*. *Otocinclus flexilis* MCP 41907 EU370984*, O, GQ214636, O. *Otocinclus vestitus* INHS 54583 GQ225412, GQ225519, GQ214637, O. *Otocinclus vittatus* MCP 35848 EU359433*, GQ225520,

GQ214638, EU817544*. *Otocinclus xakriaba* ANSP 180689 (tag: SAS93-13B no.4) GQ225413, GQ225521, GQ214639, O. *Otothyris juquiae* MCP 43983 EU359434*, GQ225522, GQ214640, O. *Otothyris travassosi* MNRJ 22947 (#645) O, GQ225523, O, EU817526*. *Otothyropsis* sp. MHNG 2587.011 EU371003*, GQ225524, GQ214641, O. *Oxyropsis acutirostra* ANSP 180816 (tag: 4015) GQ225414, GQ225525, GQ214642, EU817542*. *Oxyropsis carinata* INHS 52488 GQ225415, GQ225526, O, O. *Oxyropsis wrightiana* UF 126342 GQ225416, GQ225527, O, O. *Panaqolus changae* ANSP 181097 (tag P6218) EU359435*, GQ225528, GQ214643, O. *Panaqolus* sp. ZSM 32728 EU359436*, GQ225529, AY552031, O. *Panaque* cf. *cochliodon* O, GQ225530, AY552039, O. *Panaque* cf. *nigrolineatus* GQ225417, GQ225531, AY896736, O. *Pareiorhaphis azygolechis* MCP 41909 EU359437*, GQ225532, GQ214644, EU817564*. *Pareiorhaphis bahiana* MCP no number GQ225418, GQ225533, GQ214645, O. *Pareiorhaphis calmoni* MCP 41275 EU359438*, GQ225534, GQ214646, O. *Pareiorhaphis eurycephalus* MCP 41458 EU359439*, GQ225536, GQ214648, O. *Pareiorhaphis garbei* MCP 43598 O, GQ225537, GQ214649, O. *Pareiorhaphis garbei* MCP 43597 O, GQ225538, GQ214650, O. *Pareiorhaphis hypselurus* MCP 21695 EU359440*, GQ225539, GQ214651, O. *Pareiorhaphis hystrix* MCP 22787 EU359441*, GQ225540, GQ214652, O. *Pareiorhaphis nasuta* MCP 37176 GQ225420, GQ225541, GQ214653, O. *Pareiorhaphis nudula* MCP 41906 EU359442*, GQ225542, GQ214654, O. *Pareiorhaphis parmula* MCP 41747 EU359443*, GQ225543, GQ214655, O. *Pareiorhaphis* sp. 1 MCP 22339 EU359444*, GQ225546, GQ214658, O. *Pareiorhaphis* sp. 2 MCP 28683 EU359445*, GQ225547, GQ214659, O. *Pareiorhaphis* sp. 3 MCP 40111 EU359446*, GQ225548, GQ214660, O. *Pareiorhaphis* sp. 4 MCP 41296 EU359447*, GQ225544, GQ214656, O. *Pareiorhaphis* sp. 5 MCP 41457 EU359448*, GQ225545, GQ214657, O. *Pareiorhaphis* sp. 6 MCP 41460 GQ225419, GQ225535, GQ214647, O. *Pareiorhaphis splendens* MCP 22330 EU359449*, GQ225549, GQ214661, EU817565*. *Pareiorhaphis splendens* MCP 41263 EU359450*, GQ225550, GQ214662, O. *Pareiorhaphis steindachneri* MCP 41289 EU359451*, GQ225551, GQ214663, O. *Pareiorhaphis stomias* MCP 41910 EU359452*, GQ225552, GQ214664, O. *Pareiorhaphis vestigipinnis* MCP 43034 GQ225421, GQ225553, GQ214665, O. *Pareiorhina brachyrhyncha* MCP 42434 GQ225422, GQ225554, GQ214666, O. *Pareiorhina carrancas* MCP 36915

GQ225423, GQ225555, GQ214667, O. *Pareiorhina* sp. MNRJ 26518 (#281) EU359453*, GQ225556, GQ214668, O. *Pareiorhina* sp. MNRJ 26529 (#282) GQ225424, GQ225557, GQ214669, O. *Parotocinclus aripuanensis* MCP 35884 O, GQ225558, GQ214670, O. *Parotocinclus bidentatus* MCP 42430 GQ225425, GQ225559, GQ214671, O. *Parotocinclus britskii* ANSP 179131 (tag: 2112) GQ225426, O, O, O. *Parotocinclus* cf. *aripuanensis* MCP 43950 EU359454*, GQ225560, O, O. *Parotocinclus collinsae* ANSP 179140 (tag: 2268) O, GQ225561, GQ214672, O. *Parotocinclus eppleyi* AUM 43947 (tag V5576) EU359455*, O, GQ214673, EU817528*. *Parotocinclus jumbo* ZSM 32727 EU359456*, GQ225562, AY552048, O. *Parotocinclus maculicauda* MCP 41911 EU359457*, GQ225563, GQ214674, EU817527*. *Parotocinclus* sp. MCP 35875 O, GQ225564, GQ214675, O. *Parotocinclus spilosoma* MCP 43951 GQ225427, O, O, O. *Peckoltia lineola* AUM 39245 (tag V060) EU359458*, GQ225565, GQ214676, O. *Peckoltia vittata* AUM 39248 (tag V114) EU359459*, GQ225566, GQ214677, O. *Pseudacanthicus leopardus* ANSP 179613 (tag 2450) EU359460*, GQ225567, GQ214678, O. *Pseudotocinclus juquiae* LBP 616 EU370988*, GQ225568, O, O. *Pseudotocinclus tietensis* LBP 696 EU370987*, GQ225569, GQ214679, EU817519*. *Pseudotothyris obtusa* MCP 33330 EU371016*, GQ225570, GQ214680, EU817525*. *Pseudotothyris obtusa* MCP 33330 EU371017*, GQ225571, GQ214681, O. *Rhinelepis aspera* NUP uncat. (PR108) GQ225428, GQ225572, GQ214682, O. *Rineloriciaria* sp. MCNI 1224 EU359461*, GQ225573, GQ214683, O. *Schizolecis guentheri* MCP 31722 EU359462*, GQ225574, O, EU817536*. *Schizolecis guentheri* MCP 31724 EU371020*, GQ225575, GQ214684, EU817539*. *Scobinancistrus pariolispos* ANSP 177883 GQ225429, GQ225576, GQ214685, O. **Nematogenyidae:** *Nematogenys inermis* ANSP 180477 (tag 1) EU359428*, GQ225508, GQ214626, O. **Scoloplacidae:** *Scoloplax distolothrix* MCP 40282 EU359463*, DQ492323*, GQ214686, O. **Trichomycteridae:** *Trichomycterus* sp. MCP 41292 O, GQ225577, DQ492431*, O.

Appendix B: Majority rule consensus of the Bayesian analyses

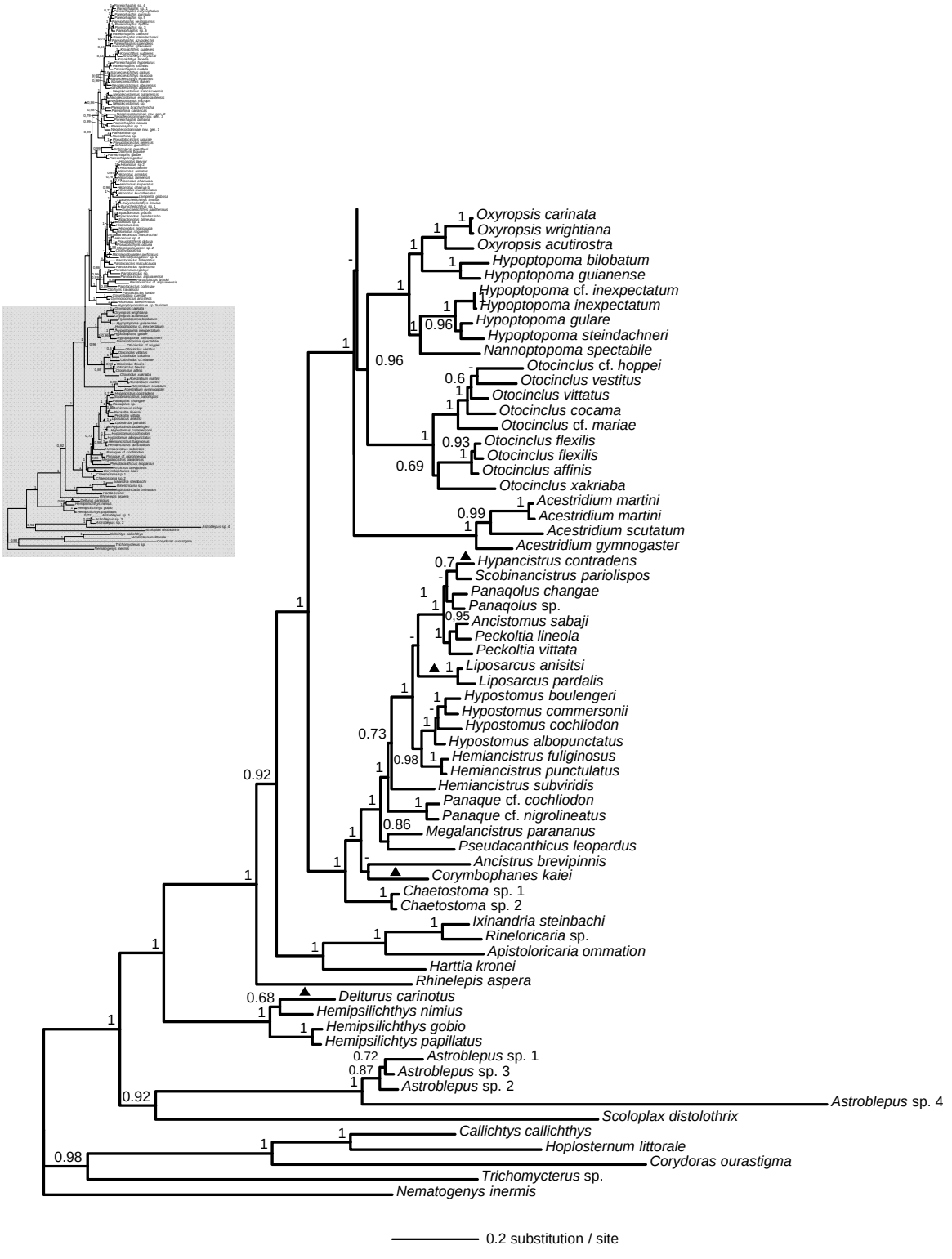

Fig. 5a Majority rule consensus of the Bayesian analyses, remainder of the tree is in Fig. 5b. Branch lengths are drawn proportional to the amount of change. Branches marked with ▲ differ in their position from the best ML tree. Bayesian posterior probabilities of clades marked on branches (≥ 0.50).

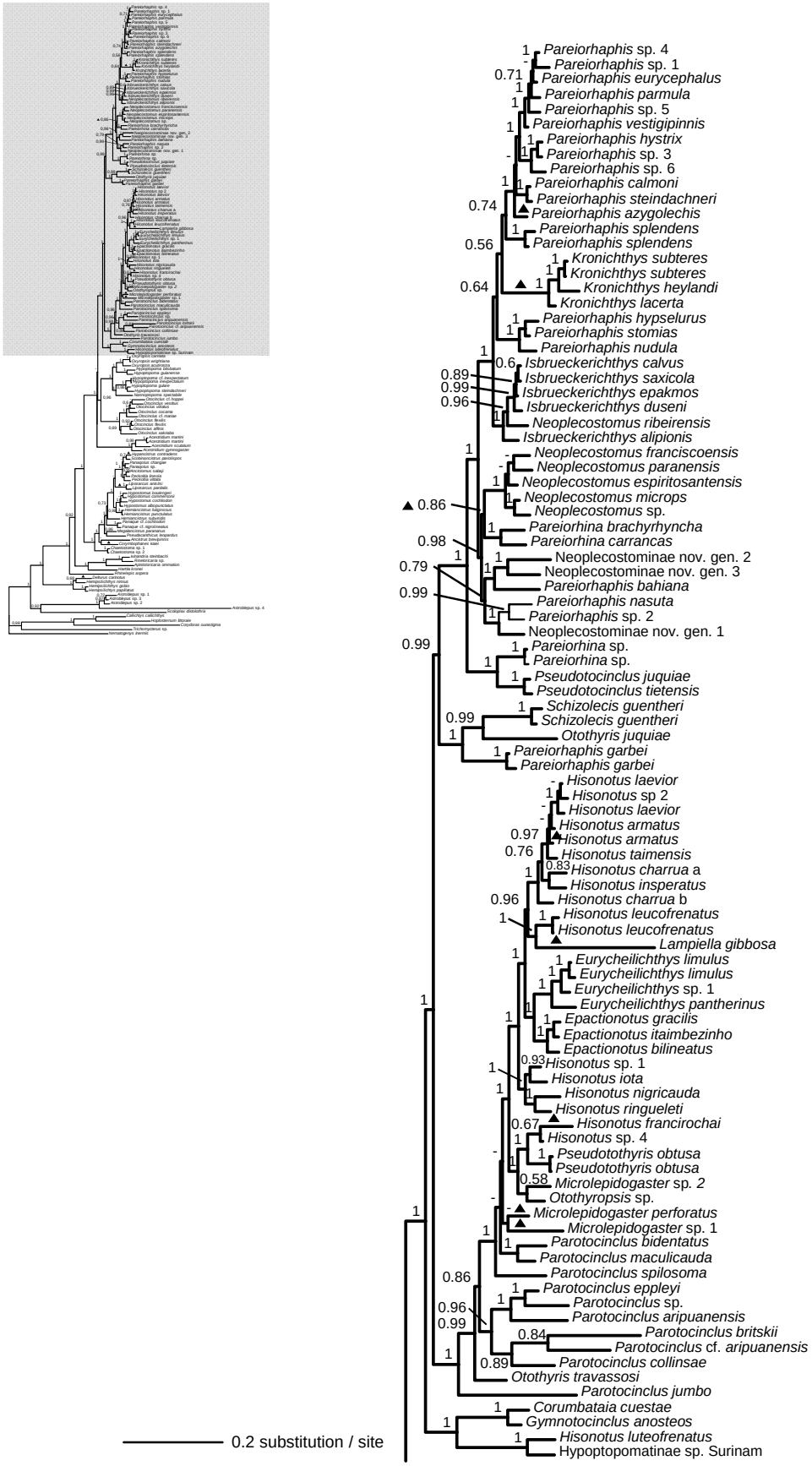

Fig. 5b Majority rule consensus of the Bayesian analyses, remainder of the tree is in Fig. 5a. Branch lengths are drawn proportional to the amount of change. Branches marked with ▲ differ in their position from the best ML tree. Bayesian posterior probabilities of clades marked on branches (≥ 0.50).

Appendix C: Results of the MP analyses

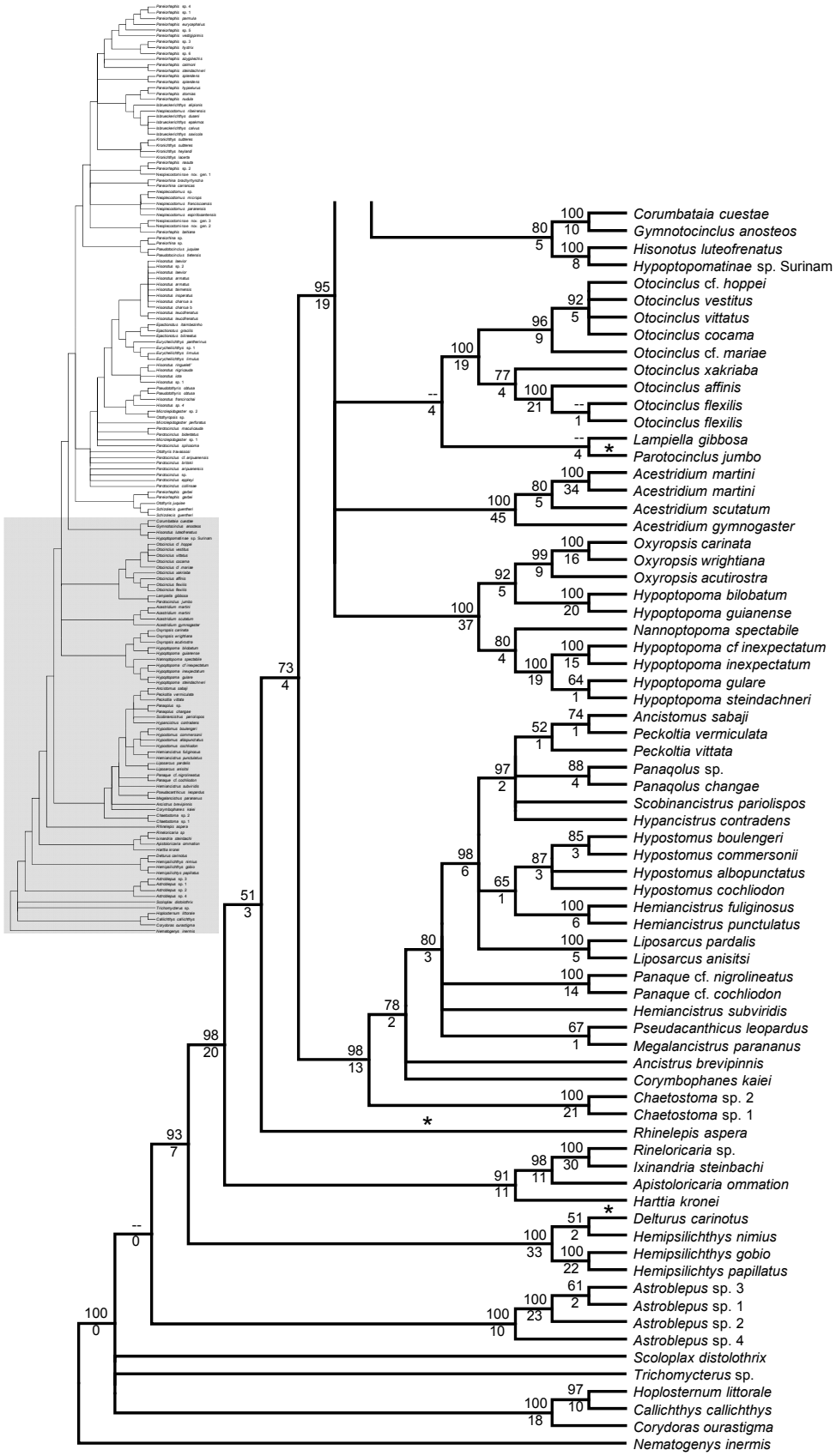

Fig. 6a Result of the MP analysis. Basal part of the strict consensus of 358 most parsimonious trees with 9363 steps, CI: 0.36, remainder of the tree is in Fig. 6b. Branches marked with * differ in their position from the best ML tree. Parsimony bootstrap proportions (≥ 50) are shown above branches, decay (Bremer) indices are shown below.

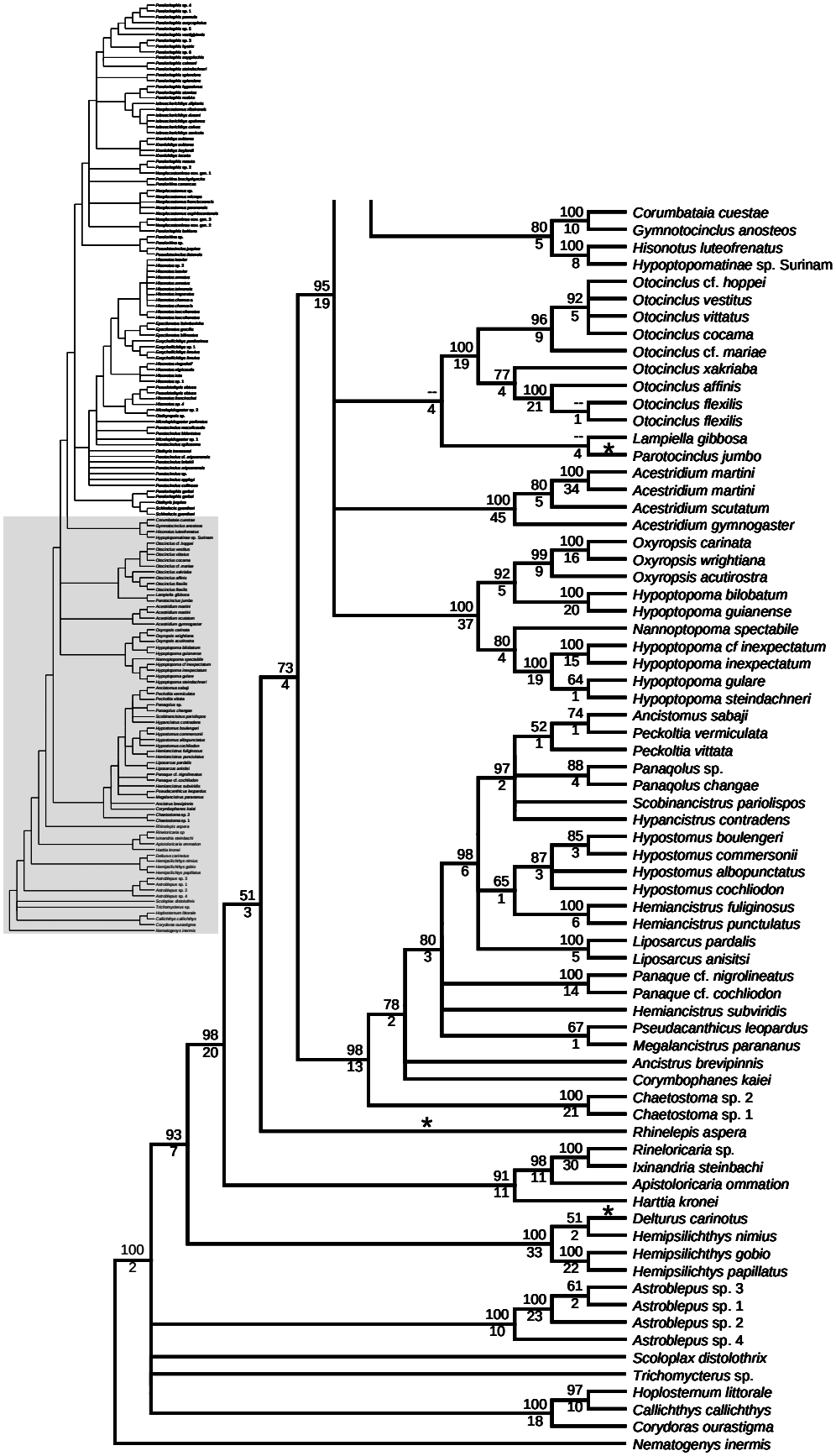

Fig. 6b Result of the MP analysis. Upper part of the strict consensus of 358 most parsimonious trees with 9363 steps, CI: 0.36, remainder of the tree is in Fig. 6a. Branches marked with * differ in their position from the best ML tree. Parsimony bootstrap proportions (≥ 50) are shown above branches, decay (Bremer) indices are shown below.

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Capítulo III

A Total Evidence Phylogeny of the Neoplecostominae and Hypoptopomatinae catfishes (Siluriformes: Loricariidae)

A Total Evidence Phylogeny of the Neoplecostominae and Hypoptopomatinae catfishes (Siluriformes: Loricariidae).

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Abstract

A total evidence phylogeny of all genera of the loricariid subfamilies Neoplecostominae and Hypoptopomatinae is presented combining 472 morphological characters and 2634 basepairs from fragments of three genes, both nuclear and mitochondrial. We obtained data for 207 species from 53 loricariid genera with representatives of five loricariid subfamilies and ten outgroup species from five loricarioid families, resulting in the largest catfish phylogeny published so far. Maximum Parsimony analyses were conducted in order to seek a solution for the contradictory results of previous solely morphological or molecular analyses. Our results recovered the Hypoptopomatinae and the Neoplecostominae as monophyletic sister groups and revealed a biogeographic distribution pattern for the genus *Pareiorhaphis*. Concordant with previous publications, the genus *Pseudotocinclus* was placed into the Neoplecostominae and the Delturinae was confirmed as the most basal loricariid subfamily except of *Lithogenes*. Inside the Hypoptopomatinae, the tribe Hypoptopomatini formed a natural group, but the previously hypothesized monophyly of the Otothyrini could not be confirmed. Furthermore, the genera *Hisonotus*, *Pareiorhina* and *Parotocinclus* were recovered as polyphyletic, though three monophyletic groups could be separated for the latter.

Keywords: Loricariidae, Hypoptopomatinae, Neoplecostominae, phylogeny, total evidence, COI, RAG1, RAG2

The order Siluriformes is distributed on all continents but the Antarctic. With around 3100 species in 36 families (Ferraris 2007), catfishes sum about 10% of all fish species. They are mainly freshwater inhabitants, with only two marine families, and are most strongly represented in South America with 14 families and 64% of the species (Moyle and Cech 2000; Rodiles-Hernández et al. 2005). Six of these families form the superfamily Loricarioidea, a well documented clade with about 1280 species or 41% of all catfishes: Astroblepidae, Callichthyidae, Loricariidae, Nematogenyidae, Scoloplacidae, and Trichomycteridae (Schaefer and Lauder 1986; Schaefer 1990). The family Loricariidae is one of the most diverse fish families, currently containing 785 recognized species distributed in around 100 genera (Eschmeyer and Fricke 2009). Unlike most other catfishes, they possess a mouth that is modified into a sucking disk and are armor-plated, giving them the common name “armored catfishes”. This mega diverse group is spread from Uruguay and northern Argentina to the Costa Rica and its species inhabit every kind of water, from cool fast running hill streams to the lakes in the Amazon floodplain. Ultimately they even have been found introduced in Asia and North America (Chavez et al. 2006; Page and Robins 2006; Nico et al. 2009), sometimes in astonishing quantities with yet unknown consequences for the native fauna. Loricariid size ranges from few centimeters (e.g. *Nannoptopoma*) up to more than one meter (e.g. *Acanthicus*) (Evers and Seidel 2005). In spite of much effort and hundreds of publications since Linnaeus, there still are many species to be discovered and described and their phylogeny is only partly resolved. The family Loricariidae was described by Rafinesque in 1815 and subsequently, beginning from 1831, eight subfamilies have been established, of which six are still recognized (Armbruster 2004; Reis et al. 2006): Lithogeninae Eigenmann, 1909 (1 genus, 3 species), Delturinae Reis et al., 2006 (2 genera, 7 species), Neoplecostominae Regan, 1904 (5 genera, 39 species), Hypoptopomatinae Eigenmann and Eigenmann, 1890 (18 genera, 103 species), Loricariinae Bonaparte, 1831 (~ 36 genera, 222 species), and Hypostominae, Kner, 1853 (~ 40 genera, 411 species). This division into

subfamilies has not been stable, and along the time, several changes have been made. The Ancistrinae and the Hypostominae were described in 1853 (Kner 1853). When erecting the Neoplecostominae, Regan (1904) only included the genus *Neoplecostomus*. Later, Gosline (1947) added the genera *Canthopomus*, *Corymbophanes*, *Delturus*, *Hemipsilichthys*, *Isbrueckerichthys* (the species were listed as *Pareiorhaphis* since *Isbrueckerichthys* was only described in 1996), *Kronichthys*, *Pareiorhaphis*, *Pareiorhina*, *Pogonopoma*, *Pogonopomoides*, and *Upsilodus*. Isbrücker (1980) recognized six subfamilies and listed only the genus *Neoplecostomus* for the Neoplecostominae, transferring the other genera to the Hypostominae.

The first phylogeny for the Loricariidae was published by Howes (1983). Based on his examinations of osteology and muscles he described the subfamily Chaetostomatinae for the genera *Chaetostoma*, *Hemipsilichthys*, *Lasiancistrus*, and *Lipopterichthys* and put the Ancistrinae in the synonymy of the Hypostominae (Fig. 1). This division did not last a long time as Schaefer (1986, 1987, 1988) did not recognize the Chaetostomatinae. Though his phylogenies show the Hypostominae as paraphyletic because of the separation of the Ancistrinae (Fig. 2), Schaefer decided to maintain the subfamily status of the Ancistrinae, using Isbrücker's (1980) classification. The first phylogenetic examination with focus on the Hypoptopomatinae was done by Schaefer (1991), but only 16 species were included. Based on his results, he described the tribes Hypoptopomatini and Otothyrini. Montoya-Burgos et al. (1997, 1998) made the first molecular analyses of the family, using 12S and 16S rRNA nucleotide sequences. Besides the monotypic Neoplecostominae, the Loricariinae was the only subfamily that was recovered as monophyletic. Two important discoveries were made in that study (Montoya-Burgos et al. 1998): for the first time, *Hemipsilichthys gobio* was shown as a taxon basal to all other loricariids and *Pseudotocinclus* (Hypoptopomatinae) was placed as sister of *Pareiorhina* (Neoplecostominae), making a connection between the two subfamilies (Fig. 3). The first ample loricariid phylogeny was published by Armbruster

(2004). It was focused on the Hypostominae, but included more than 120 species from all subfamilies using 215 morphological characters. In order to retain the monophyly of the Hypostominae, Armbruster synonymized the Ancistrinae under the Hypostominae and split the Hypostominae into the five tribes Ancistrini, Corymbophanini, Hypostomini, Rhinelepini, and Pterygoplichthini. Furthermore, he found the Otothyrini as paraphyletic and, even being a paraphyletic taxon, he returned the genera *Isbrueckerichthys*, *Kronichthys*, *Pareiorhaphis*, and *Pareiorhina* to the Neoplecostominae (Fig. 4). Additionally, he confirmed the findings of Montoya-Burgos et al. (1998) that *Hemipsilichthys gobio*, together with *Delturus angulicauda*, is a sister group to all other loricariids except *Lithogenes* Eigenmann, 1909. Based on this result, Pereira (2005) resurrected the genus *Pareiorhaphis* Miranda Ribeiro, 1918 for most of the species of *Hemipsilichthys*, remaining only *H. gobio*, *H. papillatus*, and *H. nimius* in the latter. In the same year, a morphologic phylogeny of the Hypoptopomatinae (Gauger and Buckup 2005), including 31 species from nearly all genera of this subfamily, showed the Otothyrini and the genus *Parotocinclus* as paraphyletic groups. In the following year, Lehmann (2006) used 169 characters and a total of 114 species, mainly hypoptopomatines, in his doctoral thesis. His phylogenetic analyses recovered the genus *Kronichthys* as the most basal of a monophyletic Hypoptopomatinae. According to his results, *Pseudotocinclus* did not have any closer relation to the Neoplecostominae, and again, he found the Otothyrini to be paraphyletic (Fig. 5). Within a few months, three studies dedicated to the phylogeny of the Hypoptopomatinae and the Neoplecostominae appeared in 2008. Two of them (Chiachio et al. 2008; Cramer et al. 2008) were based on sequences of a single gene (F-Reticulon 4 and COI respectively). Focused on the Hypoptopomatinae and including only 44 species, Chiachio et al. (2008) found *Pseudotocinclus* to be part of the Neoplecostominae and the latter was found inside the Hypoptopomatinae. Their solution was to elevate the Hypoptopomatini and the Otothyrini to subfamily rank and to redefine the Neoplecostominae, resulting in three monophyletic groups (Fig. 6). This act must be considered somewhat

premature because of the very low number of species included, the use of sequences of a single gene, and the contradictions to the most recent studies, both molecular and morphologic. Their results also conflict with the findings of Cramer et al. (2008) based on sequences for 83 loricariid species with focus on the Hypoptopomatinae and the Neoplecostominae. These also found the genus *Pseudotocinclus* as a member of the Neoplecostominae and the latter amidst the Hypoptopomatinae, but neither the Hypoptopomatini nor the Otothyrini could be resolved as monophyletic (Fig. 7). The third study concluded that year was a doctoral thesis dedicated to the Neoplecostominae (Pereira 2008). Representing the most complete taxon sampling of this group and using 303 morphological characters, it revealed the Neoplecostominae and the Hypoptopomatinae as monophyletic sister groups. *Pareiorhina* and *Kronichthys* were resolved as sister taxa on the base of the Hypoptopomatinae and thus were included in the latter (Fig. 8). Unfortunately, no *Pseudotocinclus* was included in that thesis. The most recent study treating the phylogeny of loricariid catfishes (Cramer et al. this volume) used sequences from four genes, both nuclear and mitochondrial, for 136 ingroup species, 103 of them representing 30 genera of the Hypoptopomatinae and Neoplecostominae. Solely the Delturinae and the Loricariinae were found as monophyletic. *Pseudotocinclus* was again recovered inside the Neoplecostominae, and *Pareiorhaphis* ended up as polyphyletic, with one species even being placed outside the Neoplecostominae. Once more, the Neoplecostominae caused a paraphyly of the Hypoptopomatinae and the Otothyrini, and not even the Hypoptopomatini could be resolved as monophyletic (Fig. 9).

In summary, much effort has been done, but neither morphological nor molecular data alone have been able to resolve the phylogenetic relationships inside the Loricariidae, rather resulting in contradictory topologies. In similar cases, a total evidence analysis has been successful for a variety of groups (e.g. Eernisse and Kluge 1993 (Amniotes); Gatesy et al. 2003 (Crocodylians); Mattern and McLennan 2004 (Gasterosteidae [Gasterosteiformes]);

Pretti et al. 2009 (Acestrorhynchus [Characiformes])). When combined, both molecular and morphological characters contribute positively to the analysis (Baker et al. 1998). As morphological characters usually exhibit higher consistency, they continue to be useful in phylogenetic studies despite the overwhelming number of available molecular data (Baker et al. 1998). Therefore, the aim of the present study was to try this approach to find a well supported solution for the Loricariidae, taking advantage of the large quantity of data already available.

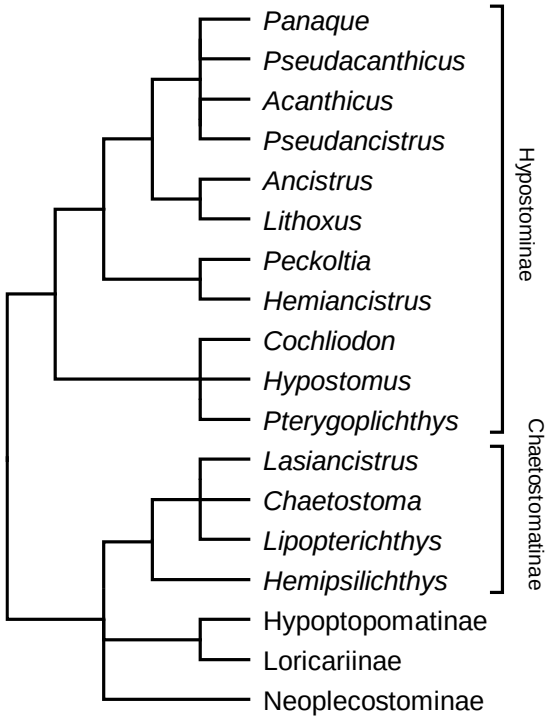

Fig. 1 Phylogeny from Howes (1983) based on osteology and myology.

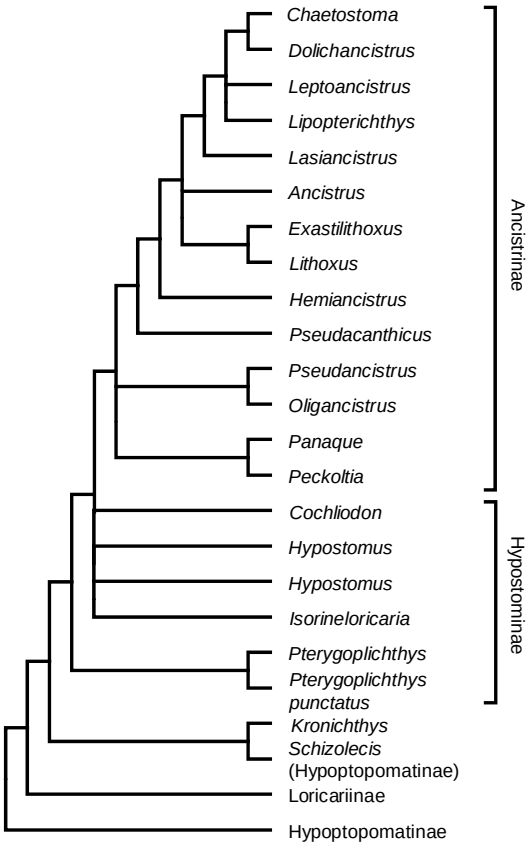

Fig. 2 Phylogeny from Schaefer (1986) based on osteology. Some names have been changed to update taxonomy and to correct misidentifications following Armbruster (2004).

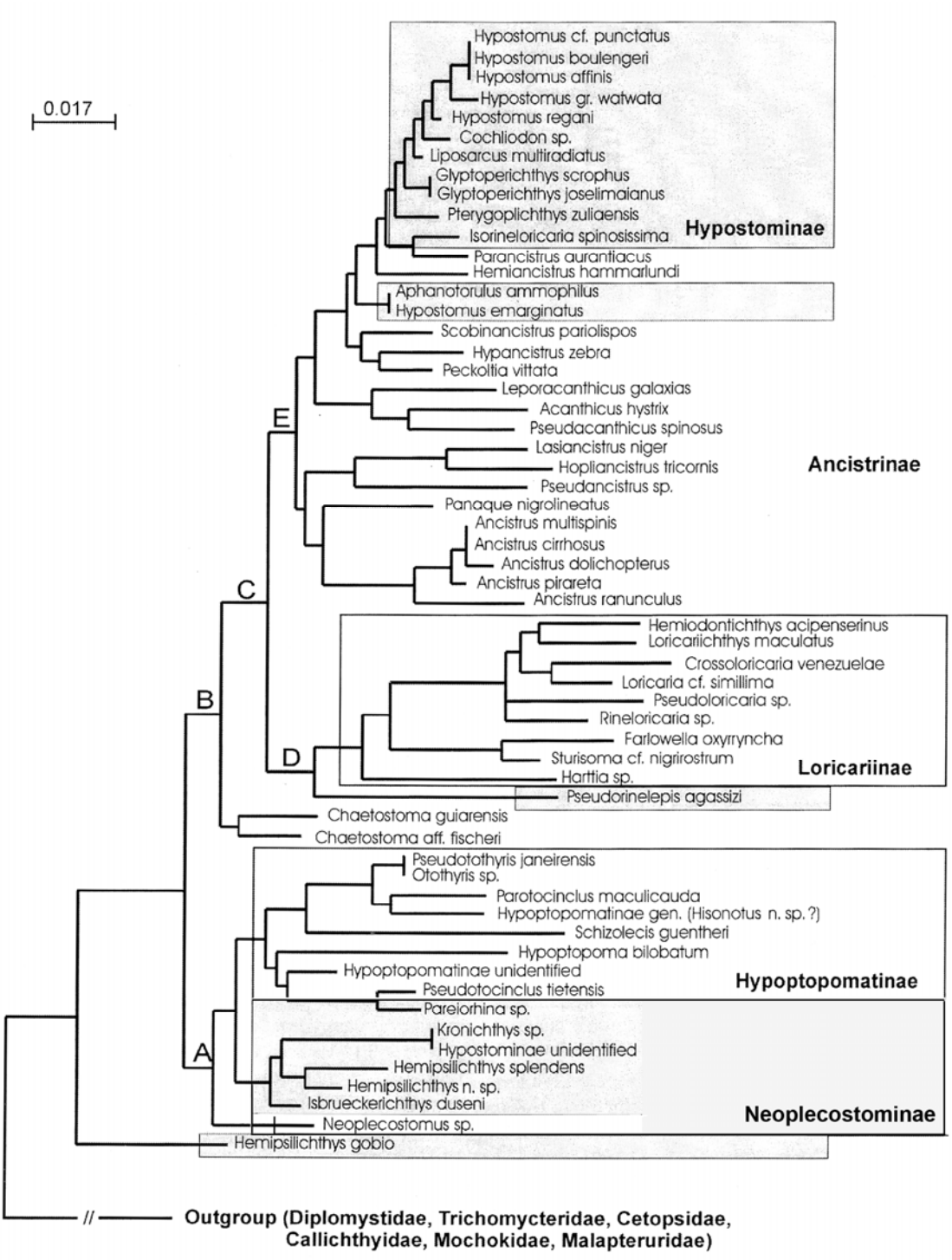

Fig. 3 Phylogeny from Montoya-Burgos et al. (1998: modified from Fig. 3) based on sequence data from mitochondrial 12S and 16S.

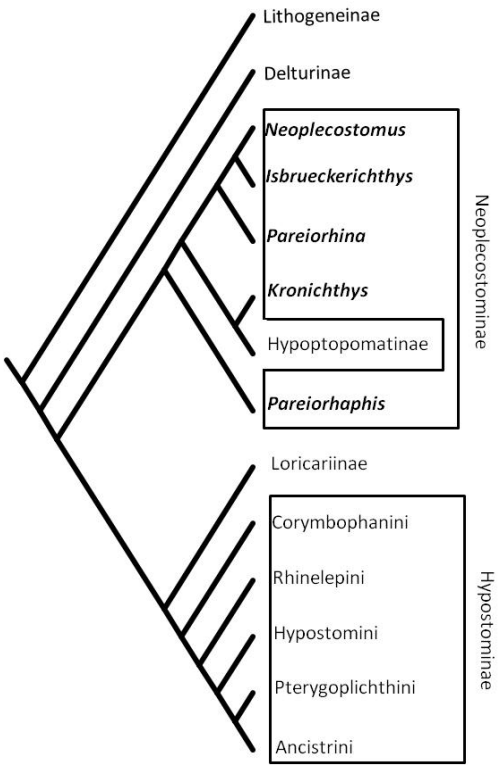

Fig. 4 Phylogenetic interrelationships of the Loricariidae based on osteology modified from Armbruster (2004) .

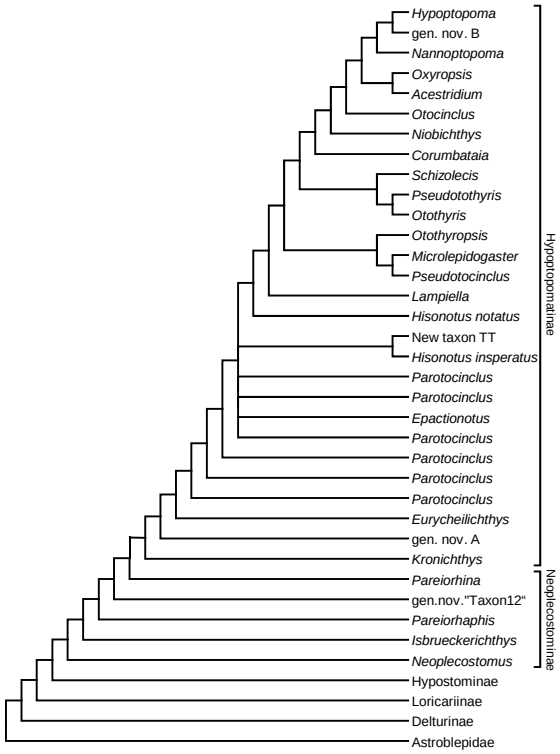

Fig. 5 Phylogeny from Lehmann (2006) based on morphology (simplified from Fig. 91).

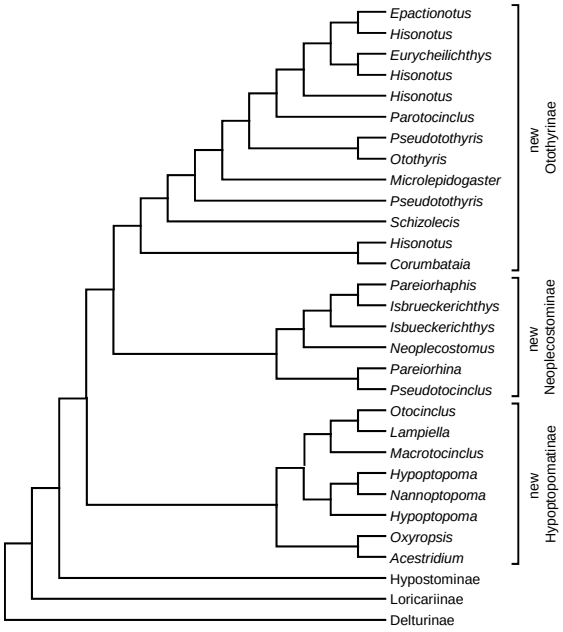

Fig. 6 Phylogeny from Chiachio et al. (2008) based on sequence data from nuclear F-Reticulon 4 (modified from Fig. 2).

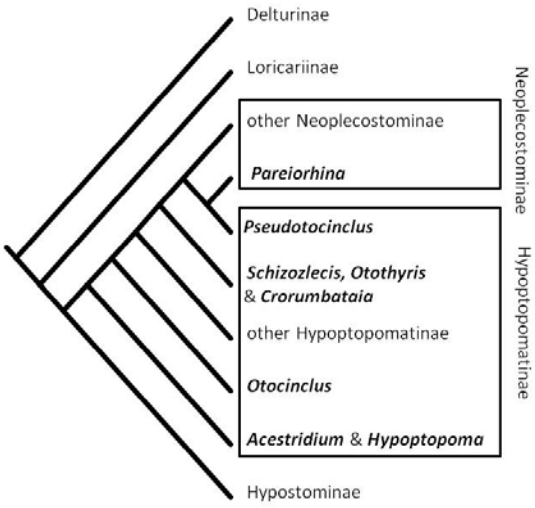

Fig. 7 Phylogenetic interrelationships of the Loricariidae based on sequence data from mitochondrial COI, modified from Cramer et al. (2008).

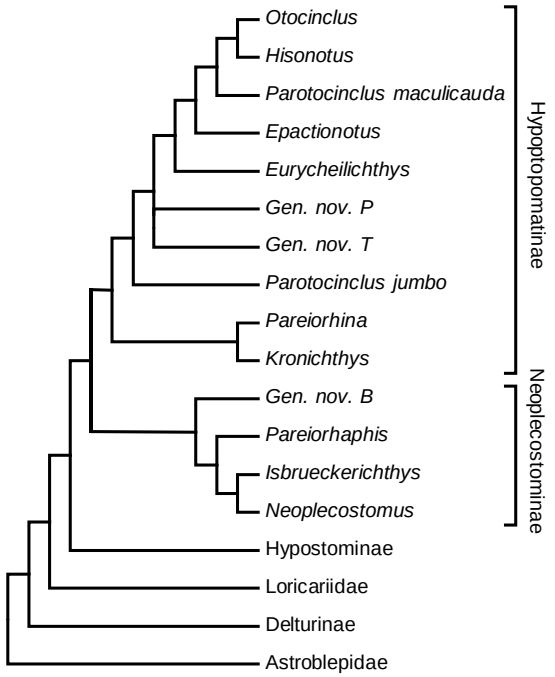

Fig. 8 Phylogeny from Pereira (2008) based on morphologic characters (simplified from Fig. 82).

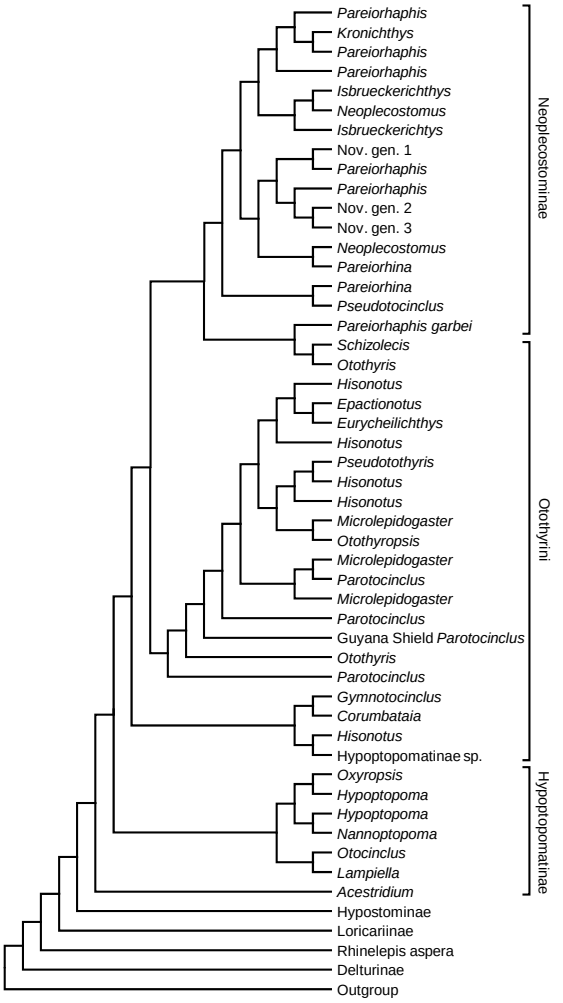

Fig. 9 Phylogenetic interrelationships of the Loricariidae based on sequence data from mitochondrial COI and nuclear RAG1 and RAG2. Simplified from Cramer et al. (this volume: Fig. 4).

MATERIAL AND METHODS:

The nucleotide alignment from Cramer et al. (this volume) with all sequences for the genes *cytochrome c oxidase I* (COI), *recombination activating gene 1* (RAG1), and *recombination activating gene 2* (RAG2) was taken. Additional sequences for the COI fragment were used from Cramer et al. (2008) and are listed in the Appendix 1. Morphological data are mostly osteological and were taken from Lehmann (2006: 169 characters for 114 species; focused on the Hypoptopomatinae) and Pereira (2008: 303 characters for 71 species; focused on the Neoplecostominae). These data were concatenated in one matrix and treated as independent data set. Collection numbers of vouchers and GenBank accession numbers are listed in the cited studies. Taxa that received different denominations by the different authors are listed in Table 1.

Whenever possible, only data from the same species were joined. Five exceptions were made as there were no data available from the same species (see Table 2). If molecular data were available for more than one specimen from the same species and there were no doubts about the validity and the identification of the species, the same morphological data were assigned for both individuals. The kind of data available for each terminal is specified in Fig. 10 and Table 3. Our resulting matrix consisted of a total of 3106 characters (2634 molecular and 472 morphological; 1561 of them informative for parsimony) for 235 terminals.

Table 1 Taxa where denomination differed in earlier studies

Denomination previously used by Cramer et al. (2008)[1], Cramer et al. (this volume)[2], Lehmann (2006)[3], or Pereira (2008)[4]	Denomination used here
Acestridium sp. 1[1,3]; *Acestridium scutatum*[2]	*Acestridium scutatum*
Acestridium sp. 2[1,3]; *Acestridium gymnogaster*[2]	*Acestridium gymnogaster*
Eurycheilichthys sp. 1[1]; *Eurycheilichthys* sp. n. „Pin“[3]	*Eurycheilichthys* sp. 1
Eurycheilichthys sp. 2[1]; *Eurycheilichthys* sp. n. „Neo“[3]	*Eurycheilichthys* sp. 2
Eurycheilichthys sp. 3[1]; *Eurycheilichthys* sp. n. „Lis”[3]	*Eurycheilichthys* sp. 3
Eurycheilichthys sp. 4[1]; *Eurycheilichthys* sp. n. „Taq“[3]	*Eurycheilichthys* sp. 4
Eurycheilichthys sp. 7[1]; *Eurycheilichthys* sp. n. „Pir“[3]	*Eurycheilichthys* sp. 7
Gymnotocinclus anosteus[2]; Gen. nov. T[4]	*Gymnotocinclus anosteus*
Hisonotus cf. *aky*[1]	*Hisonotus iota*
Hisonotus sp. 3[1]; *Hisonotus armatus*[2]	*Hisonotus armatus*
Hisonotus sp. 5[1]; *Hisonotus armatus*[2]; Hisonotus sp. n.[4]	*Hisonotus armatus*
Neoplecostominae nov. gen. 1[2]; Gen. nov. A[3]; Gen. nov. P[4]	Neoplecostominae nov. gen. 1
Neoplecostominae nov. gen. 2[2]; Gen. nov. unnamed “Taxon 12”[3]; Gen. nov. B[4]	Neoplecostominae nov. gen. 2
Neoplecostomus sp.[1,2]; *Neoplecostomus* P sp. n.[4]	*Neoplecostomus* sp. P
New taxon TT sp. “Torp. BDX”[3]; *Hisonotus luteofrenatus*[2]	*Hisonotus luteofrenatus*
New taxon TT sp. “Torp. QPT”[3]	*Hisonotus chromodontus*
New taxon TT sp. “Torp. QPX”[3]	*Hisonotus chromodontus*
Pareiorhaphis nasuta[2]; *Pareiorhaphis* M sp. n.[4]	*Pareiorhaphis nasuta*
Pareiorhaphis sp. 2[1,2]; *Pareiorhaphis* P sp. n.[4]	*Pareiorhaphis* sp. 2
Pareiorhaphis sp. 5[1,2]; *Pareiorhaphis* T sp. n.[4]	*Pareiorhaphis* sp. 5
Pareiorhaphis sp. 6[2]; *Pareiorhaphis* Ca sp. n.[4]	*Pareiorhaphis* sp. 6
Pareiorhina sp. n.[3]; *Pareiorhina* sp. B[4]	*Pareiorhina* sp. B
Parotocinclus sp.[2]; *Parotocinclus* sp. 15[3]	*Parotocinclus* sp. 15

Table 2 Cases where data from different species where combined

Species of the molecular data	Species of the morphological data	Denomination used in the text and figures
Ancistrus brevipinnis	*Ancistrus reisi*	*Ancistrus*
Astroblepus sp. 1	*Astroblepus* sp. "transandino" = *Astroblepus* sp. 2	*Astroblepus* 1
Astroblepus sp. 2	*Astroblepus* sp. "cisandino" = *Astroblepus* sp. 1	*Astroblepus* 2
Chaetostoma sp. 1	*Chaetostoma leucomelas*	*Chaetostoma*
Corymbophanes kaiei	*Corymbophanes andersoni*	*Corymbophanes*
Harttia kronei	*Harttia loricariformis*	*Harttia*
Rineloricaria sp.	*Rineloricaria strigilata*	*Rineloricaria*

Phylogenetic Analyses

Maximum Parsimony (MP) analyses were performed using the new technologies as implemented in TNT 1.1 (Goloboff et al. 2008). We executed the driven search option that chooses the parameters for the sectorial search, the ratched, and the tree fusion search algorithms, followed by tree bisection and reconnection (TBR). For nodal support, the Bremer support (decay index; Bremer 1994) was calculated using negative constraints as implemented in TNT 1.1. Also, 2000 nonparametric bootstrap pseudoreplicates were calculated. All characters were run unweighted and unordered.

As the strict consensus of all MP trees contained large polytomies, using the "prunn =4" command in TNT, eight taxa were detected that jump along different positions in alternative trees, causing multiple nodal collapses in the consensus (see Table 3). These taxa were excluded from the strict consensus.

To test our topology, we made another maximum parsimony analysis, using constraints to enforce the monophyly of the genus *Pareiorhina*. The Kishino Hasegawa test (Kishino and Hasegawa 1989) as implemented in PAUP* 4b10 (Swofford 2001) was used to evaluate the alterative constrained topologies.

To see if it was possible to resolve the relationships inside the genus *Pareiorhaphis* including the species *P. cerosus* and *P. regani* that have been excluded from the strict consensus in Figure 10, we made an additional analysis including all *Pareiorhaphis* plus four more species from the Neoplecostominae.

As the three data sets were treated as independent in spite of the fact that Pereira (2008) used various characters from Lehmann (2006), two additional analyses were done to examine the influence of the duplicated characters. The first analysis included the sequence data and morphological data only from Lehmann (2006), and the second one the sequence data and morphological data only from Pereira (2008). The analyses were done as described above. Again, the taxa from Table 3 were excluded from the strict consensus.

RESULTS AND DISCUSSION

The MP analyses resulted in 150 shortest trees of 11135 steps (CI: 0.28, HI: 0.72, RI: 0.71, and RC: 0.20). The strict consensus of these trees contained large polytomies. TNT detected eight taxa that caused multiple nodal collapses in the consensus (see Table 3). The clades where the polytomies were caused were numbered and are also given in Table 3. As the strict consensus where these taxa were excluded shows a significantly better resolution, it is used here for our further discussion (Fig. 10).

The separate MP analysis of the genus *Pareiorhaphis* resulted in three best trees with 1628 steps (CI: 0.50, HI: 0.50, RI: 0.51 and RC: 0.26; 426 characters were informative for parsimony). Their strict consensus contains only two trichotomies and is shown in Figure 11.

The additional analyses including only one of the morphological data sets resulted in 216 best trees with 8810 steps (only from Lehmann [2006]; Fig. 13, Appendix2) and 95 best trees with 8826 steps (only from Pereira [2008]; Fig. 14, Appendix 3) respectively.

Table 3 Taxa that jump along different positions in the trees and that were excluded from the consensus.

Taxon	Clade where the polytomy was caused	Data available for this taxon
Hisonotus notatus	VI	Morphological from Lehmann (2006) and Pereira (2008)
Hypoptopomatinae sp. Surinam	III	Molecular (COI, RAG2)
Microlepidogaster sp. 1	V	Molecular (COI, RAG1, RAG2)
New taxon 22 "Microhypostomus"	I	Morphological from Lehmann (2006)
Parotocinclus bidentatus	V	Molecular (COI, RAG1, RAG2)
Parotocinclus cristatus	IV	Morphological from Lehmann (2006)
Pareiorhaphis cerosus	II	Morphological from Pereira (2008)
Pareiorhaphis regani	II	Morphological from Pereira (2008)

Here we present the largest catfish phylogeny in number of species published so far and simultaneously the first phylogeny for the Loricariidae using total evidence. Our results include representatives from all loricarioid families and 207 species from the Loricariidae. Inside the Loricariidae, we were able to include species from five of the six subfamilies. Solely *Lithogenes* was not available because of its geographical restriction and the resulting rarity in zoological collections. The 58 species (34 described + 24 undescribed) of the Neoplecostominae include all known genera and nearly all described species, only five species of *Neoplecostomus* lacking. The 111 species (71 described + 40 undescribed) of the Hypoptopomatinae cover all described genera and most species of this subfamily.

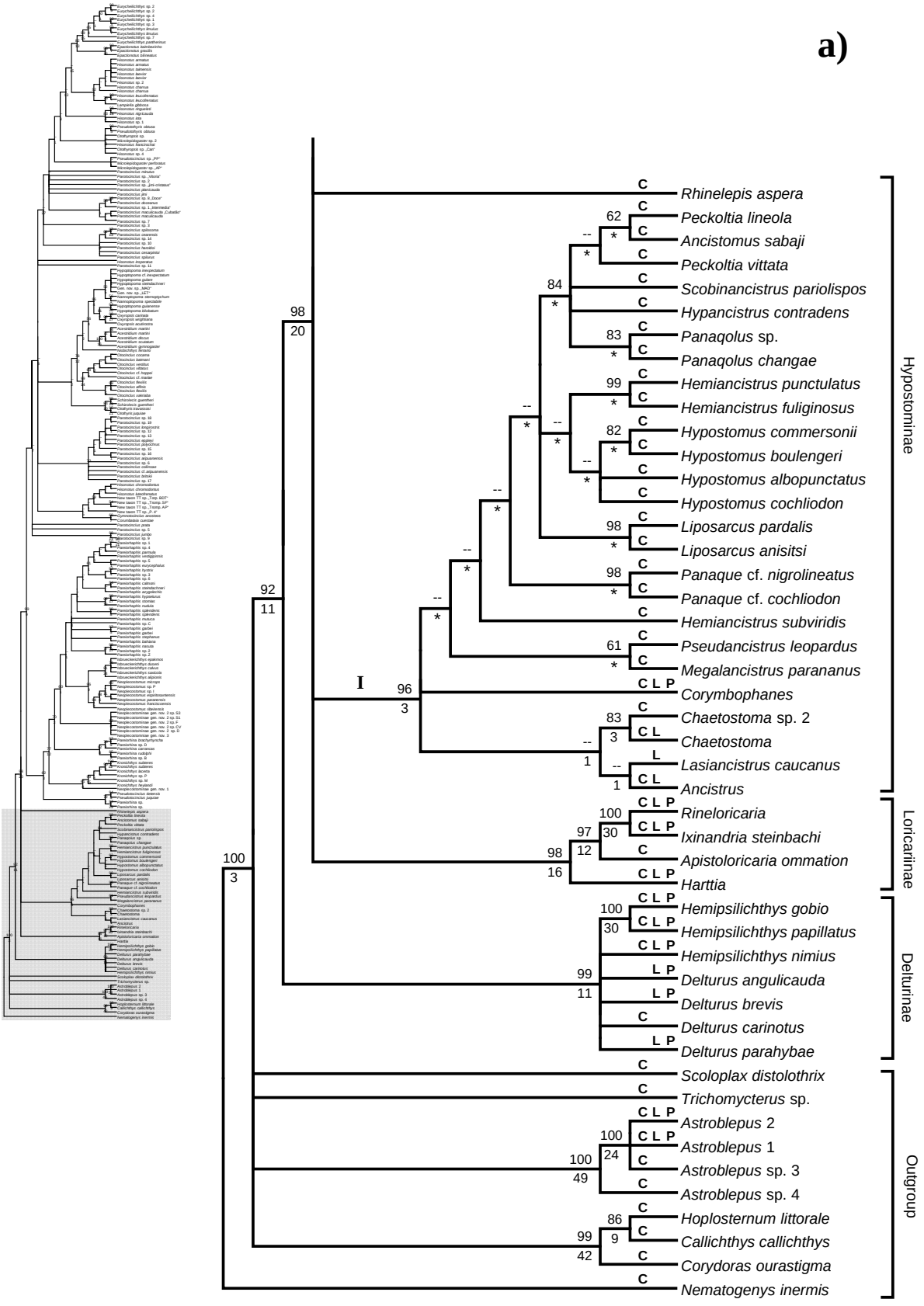

Fig. 10a First part of the strict consensus of the 150 most parsimonious trees (11135 steps; CI: 0.28) with the jumping taxa (Table 3) excluded. Numbers above branches are values from 2000 bootstrap replicates, numbers below are Bremer support (clades marked with * were only resolved after the exclusion of the taxa from Table 3). Remainder of the tree in Fig. 10b-d. Roman numbers name clades. C, L, and P specify the kind of data available for each terminal: C = molecular, L = morphological from Lehmann 2006, and P = morphological from Pereira 2008.

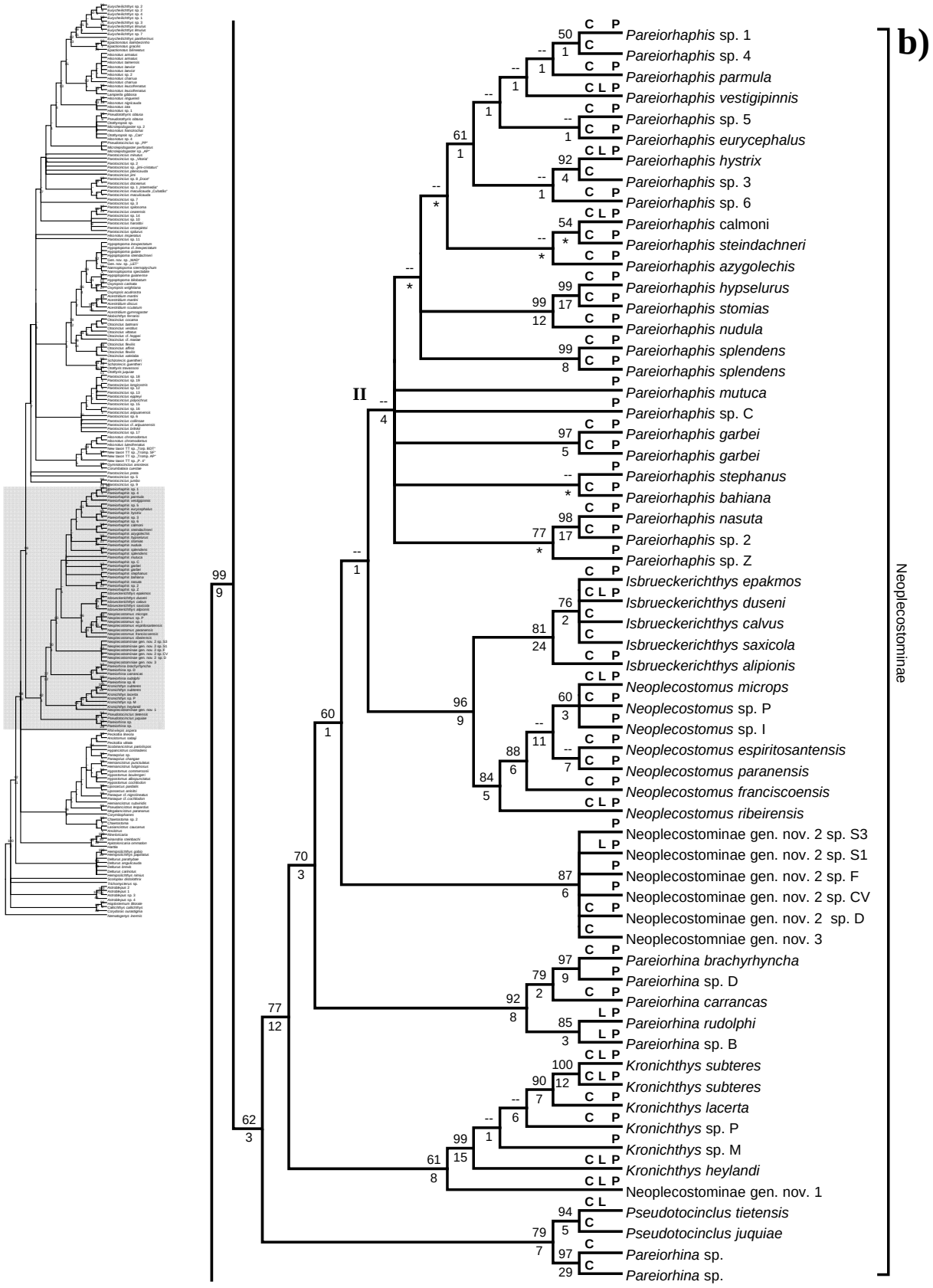

Fig. 10b Second part of the strict consensus of the 150 most parsimonious trees (11135 steps; CI: 0.28) with the jumping taxa (Table 3) excluded. Numbers above branches are values from 2000 bootstrap replicates, numbers below are Bremer support (clades marked with * were only resolved after the exclusion of the taxa from Table 3). Remainder of the tree in Fig. 10a, c, and d. Roman numbers name clades. C, L, and P specify the kind of data available for each terminal: C = molecular, L = morphological from Lehmann 2006, and P = morphological from Pereira 2008.

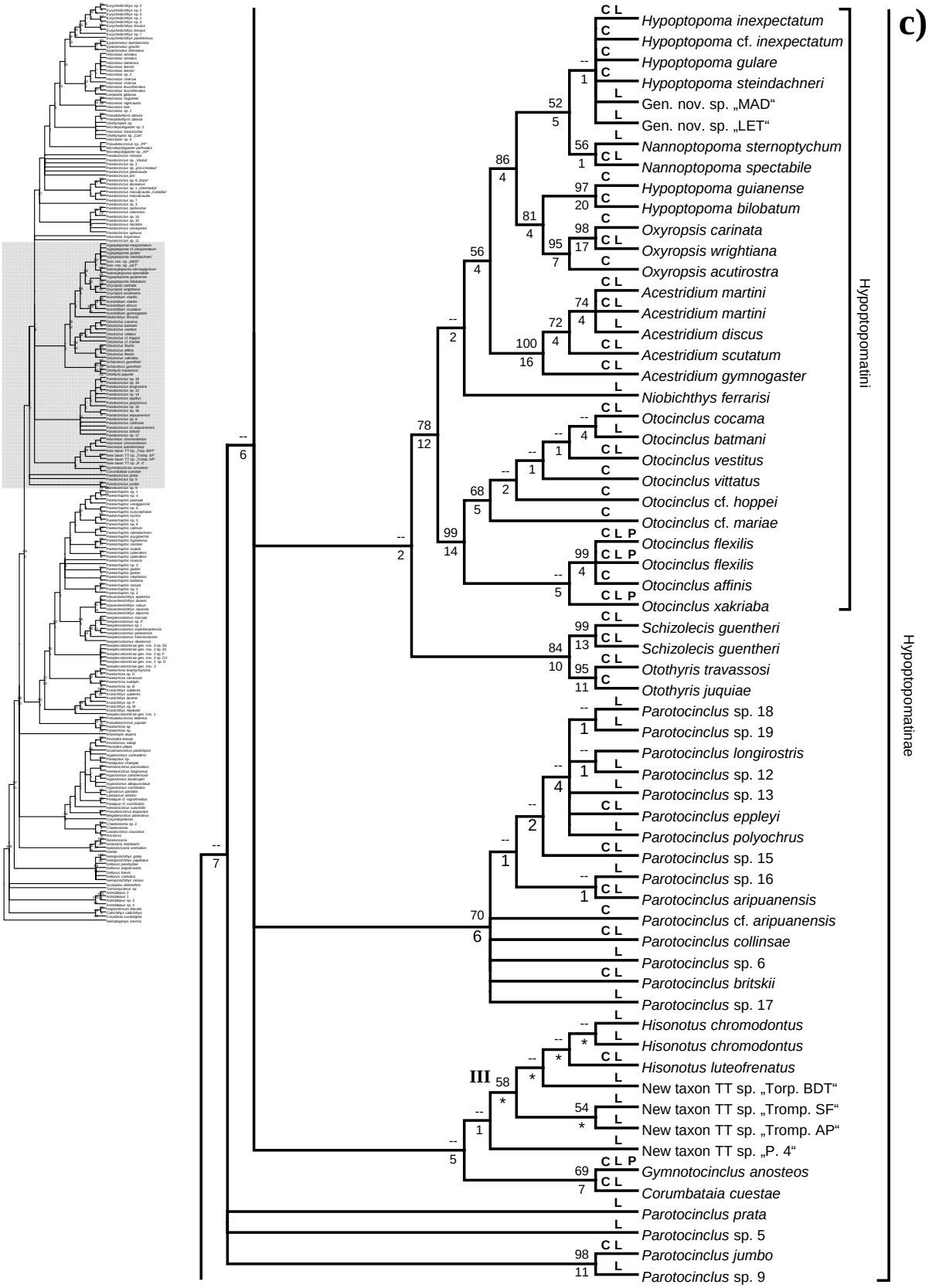

Fig. 10c Third part of the strict consensus of the 150 most parsimonious trees (11135 steps; CI: 0.28) with the jumping taxa (Table 3) excluded. Numbers above branches are values from 2000 bootstrap replicates, numbers below are Bremer support (clades marked with * were only resolved after the exclusion of the taxa from Table 3). Remainder of the tree in Fig. 10a, b, and d. Roman numbers name clades. C, L, and P specify the kind of data available for each terminal: C = molecular, L = morphological from Lehmann 2006, and P = morphological from Pereira 2008.

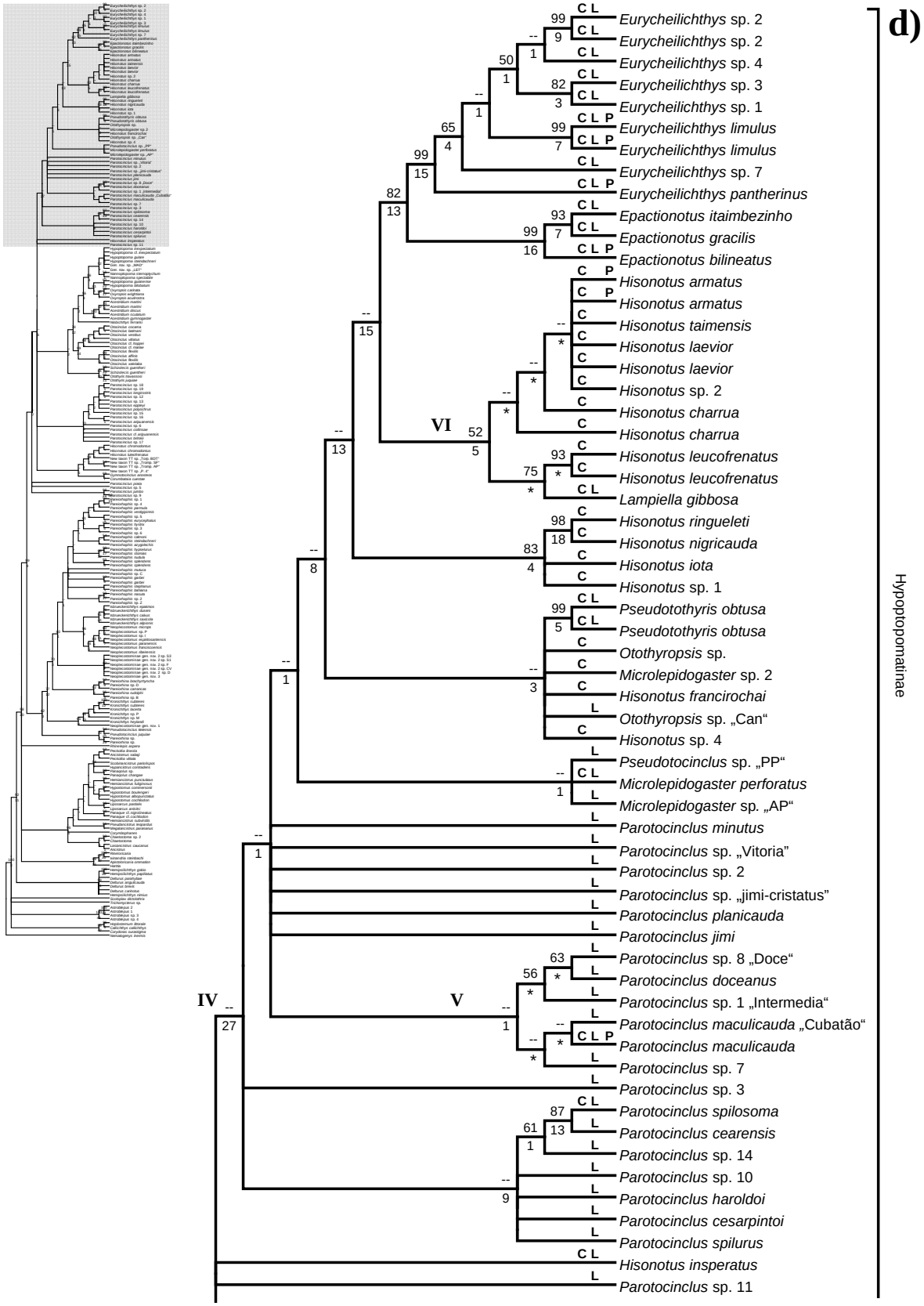

Fig. 10d Fourth part of the strict consensus of the 150 most parsimonious trees (11135 steps; CI: 0.28) with the jumping taxa (Table 3) excluded. Numbers above branches are values from 2000 bootstrap replicates, numbers below are Bremer support (clades marked with * were only resolved after the exclusion of the taxa from Table 3). Remainder of the tree in Fig. 10a-c. Roman numbers name clades. C, L, and P specify the kind of data available for each terminal: C = molecular, L = morphological from Lehmann 2006, and P = morphological from Pereira 2008.

Our initial strict consensus contained some large polytomies. We detected eight "jumping taxa" (see Table 3), species whose phylogenetic position were very unstable, causing large polytomies. After their exclusion from the consensus, most of these polytomies could be resolved. We suspect that most of the uncertain placements were due to lack of compatible data: Hypoptopomatinae sp. Surinam, *Microlepidogaster* sp. 1, and *Parotocinclus bidentatus* were only represented by molecular data, being placed in a larger group of taxa where only morphological data were available; the contrary is the case for *Hisonotus notatus* and New taxon 22 "Microhypostomus", which were only represented by morphological data among species with molecular data only. *Pareiorhaphis cerosus* is only known from two type specimens and *P. regani* by the holotype only. Because of these circumstances, only characters from external morphology could be included for these two species. Nevertheless, we see no risk of biased groupings caused by the presence of only one kind of data as most clades have been found in similar ways by previous studies.

Below we will discuss our results beginning on the bottom of the tree, climbing upwards.

Delturinae

The monophyletic Delturinae is represented by all described species and was resolved as the most basal loricariid subfamily, corroborating earlier publications (e.g. Lehmann 2006; Cramer et al. 2008; Pereira 2008; Cramer et al. this volume). This well supported group can easily be recognized by the combination of a high preadipose keel, formed by the azygous preadipose plates and almost symmetrically bifid jaw teeth (Reis et al. 2006). The lack of resolution within this clade might be due to an incomplete character sampling. *Delturus carinotus* is the only species where fresh tissue for molecular analyses was available, but at the same time, it is the only species where no specimens for morphological examinations

could be obtained. Morphologically, both genera can be easily distinguished: *Delturus* have a strong and massive body (vs. slender and elongate) and the dorsal-fin membrane is extended posteriorly, contacting the first preadipose plate (vs. not or slightly extended and never in contact with first preadipose plate) (Reis et al. 2006).

Loricariinae

Our results show the Loricariinae as a strongly supported monophyletic group. Since this subfamily was recovered as a natural group by all anterior studies (e.g. Schaefer 1987; Rapp Py-Daniel 1997; Fichberg 2008), we included only a few species as representatives. Members from this subfamily can be easily recognized by their long and flattened caudal peduncle and the absence of an adipose fin (Covain and Fisch-Muller 2007). Another particularity of this group is the diversity in lips structure, which can be strongly papillose, filamentous or smooth (Covain and Fisch-Muller 2007).

Hypostominae

Concordant with Cramer et al. (this volume), the Hypostominae is monophyletic, with the exception of *Rhinelepis aspera* being reallocated outside the subfamily, though no morphological data for *Rhinelepis* have been available. In spite of a high bootstrap value, the clade gets only low Bremer support. Hypostomines are typically bulkier than other loricariids and generally have thicker plates than neoplecostomines. They can be distinguished from other loricariids by the development of the spinelet that is large and V-shaped and clearly slides under the nuchal plate, whereas it is square or absent in most other loricariids and, when present, does not slide under the nuchal plate. The few hypoptopomatines that have a triangular spinelet can be distinguished from the Hypostominae by a completely or nearly completely exposed pectoral girdle (vs. at most some odontodes supported by the coracoid

strut) and an adductor fossa of the pectoral girdle covered by bone (vs. wholly exposed) (Armbruster 2004).

Like already stated elsewhere (Ferraris 2007; Cramer et al. this volume), there seems to be no closer connection between *Panaque* and the genera *Panaqolus* and *Scobinancistrus*, contradicting the hypothesis of these genera being synonyms or subgenera (Armbruster 2004). Several genera appeared as non-monophyletic, such as *Hemiancistrus*. But since the Hypostominae is not the main focus of the present study, only few taxa were included and further relationships inside this subfamily should be analyzed in a future study with a more complete taxon sampling.

Our strict consensus resulted in a polytomy for the Loricariinae, the Hypostominae, *Rhinelepis*, and the Neoplecostominae + Hypoptopomatinae. That way, we could not resolve the relationships between these groups.

Neoplecostominae

The Neoplecostominae and the Hypoptopomatinae were recovered as monophyletic sister groups. The Neoplecostominae, however, was found to be monophyletic only when including the hypoptopomatine genus *Pseudotocinclus* (Fig. 10b), getting weak support but corroborating the results of Cramer et al. (2008) and Chiachio et al. (2008). In the last years there has been some confusion about the definition and the composition of the Neoplecostominae. Based on morphological data, Armbruster (2004) recovered the Neoplecostominae as monophyletic enclosing the Hypoptopomatinae, Lehmann (2006) revealed this subfamily as paraphyletic, and Pereira (2008) showed it to be monophyletic, but excluding the genera *Kronichthys* and *Pareiorhina* that were recovered as one clade sister taxon to the Hypoptopomatinae; though the latter study did not include *Pseudotocinclus*. Based on DNA sequence data, Chiachio et al. (2008) and Cramer et al. (2008) found a monophyletic Neoplecostominae, including *Pseudotocinclus*, but these studies did not include

any representatives of or *Kronichthys* or *Isbrueckerichthys*. Cramer et al. (this volume), including all genera, came to a similar result, but unexpectedly excluding the species *Pareiorhaphis garbei* from the subfamily.

To justify the inclusion of the genus *Pseudotocinclus* in a monophyletic Neoplecostominae, Chiachio et al. (2008) found three non-exclusive morphological characters for this clade (dorsally positioned eyes, exposed preopercle, and incomplete fusion of the anterior abdominal dermal bony plates). Until a more complete morphological study (in preparation by EHLP) is available, we adopt these diagnostic characters here.

Inside the Neoplecostominae, as the most basal clade, we found *Pseudotocinclus* to be a sister taxon of part of *Pareiorhina*, though the latter was revealed as polyphyletic (see also Cramer et al. this volume). Such polyphyly might be reverted by the inclusion of morphologic data of *Pareiorhina* sp. (in preparation by EHLP), not available so far. An alternative topology with an enforced monophyly of *Pareiorhina* resulted in 49 additional steps and maintained *Pareiorhina* and *Pseudotocinclus* as sister taxa. This constrained topology was statistically refused by the KH test (p=0. 0012).

To discard the possibility of a misidentification, we examined the voucher and other available specimens of *Pareiorhina* sp. and found them to share the non-exclusive synapomorphies (Pereira 2008) of *Pareiorhina*. The remaining species of *Pareiorhina* were recovered as a strongly supported monophyletic group. Future analyses (in preparation by EHLP) will bring more light into this question.

The major clade of the Neoplecostominae, containing all taxa but *Pseudotocinclus* and *Pareiorhina* sp., got high Bremer support.

Kronichthys was recovered as monophyletic, being sister to the new genus 1. Lehmann (2006) already found them to be related in some proximity, but not as sister clades.

The new genera 2 and 3 form a monophyletic group consisting of one large polytomy. Probably, that was caused by the character sampling as molecular data were only available for one species of the new genus 2 and no morphological data were available for the new genus 3.

Concordant with Pereira (2008), the genera *Isbrueckerichthys* and *Neoplecostomus* are sister taxa. Both were recovered as well supported monophyletic groups, contrary to Cramer et al. (this volume), who found *Neoplecostomus ribeirensis* inside the genus *Isbrueckerichthys*, but without any morphological evidence for this finding.

Forming the most derived clade, the genus *Pareiorhaphis* was recovered as monophyletic. Cramer et al. (this volume) were the only ones who revealed it as polyphyletic, contrary to other studies that included more than only a few species (Cramer et al. 2008; Pereira 2008). As described above, only few characters could be included for the two species *Pareiorhaphis cerosus* and *P. regani.* For this reason, their placement inside the genus could not be resolved in the complete analyses, causing large polytomies. Therefore we decided to exclude these two species from the strict consensus in order to gain a better resolution (Fig. 10b) and to discuss the results of the separate analyses of the genus including all species (see Fig. 11).

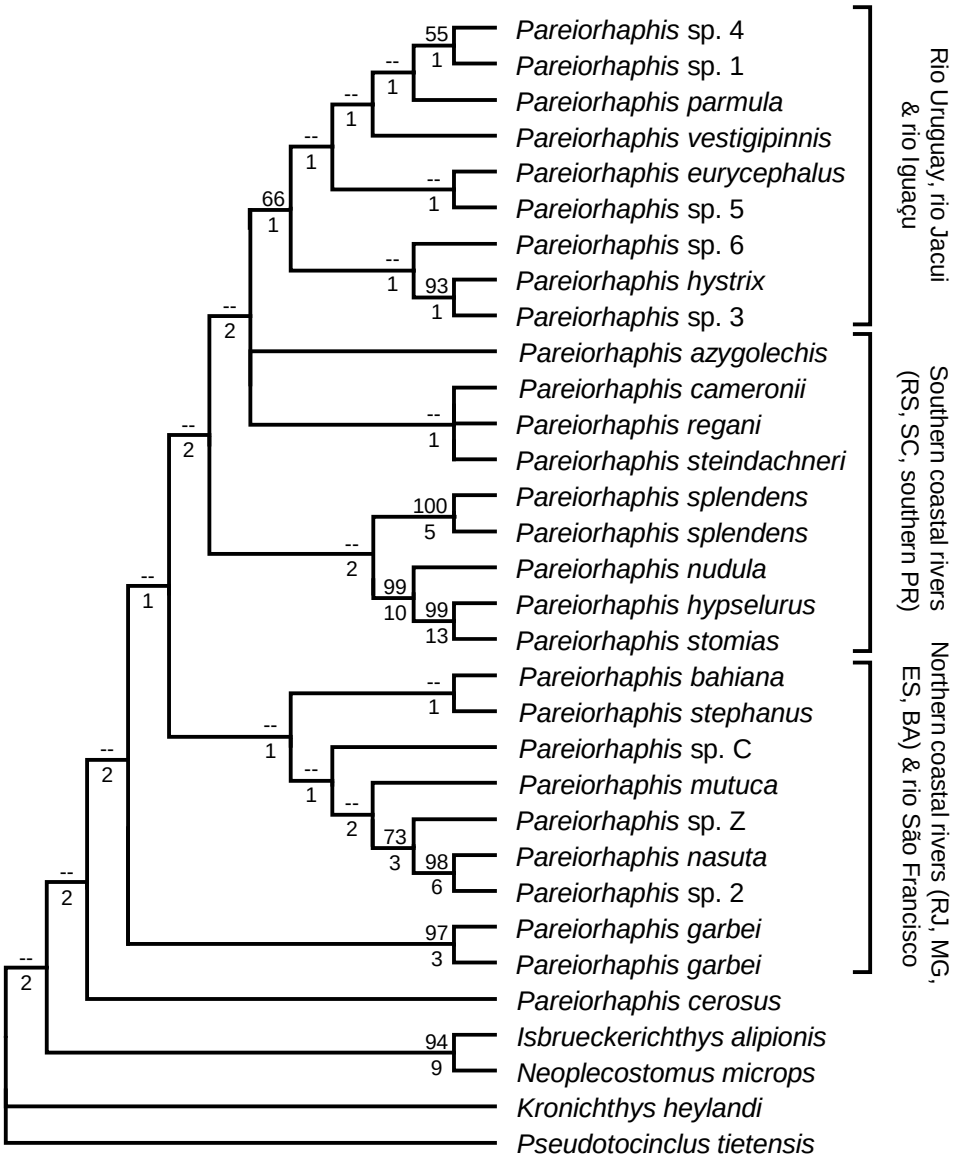

Fig. 11 Strict consensus of the three best trees (1628 steps; CI: 0.50) from the separate analysis of the genus *Pareiorhaphis*, including *P. cerosus* and *P. regani*. Numbers above branches are values from 1000 bootstrap replicates, numbers below are Bremer support.

Pareiorhaphis cerosus came out as the most basal taxon. However its status is highly uncertain as this species is only known from the two type specimens without a type locality and only few external morphological characters could be obtained.

An interesting finding is that the remaining species of *Pareiorhaphis* clustered into about three geographically delimited clades (Fig. 11 and Fig. 12). The first group is from the northern coastal rivers (states Rio de Janeiro, Espirito Santo, and Bahia) and the headwaters of the rio São Francisco (Minas Gerais). *Pareiorhaphis garbei* is the most basal species with

known origin. It occurs in the rio Macaé and rio Macacu (Rio de Janeiro) and thus is also geographically separated from its congeners, even being more closely related to the monophyletic northern clade.

The second non-monophyletic group occurs in the southern coastal rivers (states Rio Grande do Sul, Santa Catarina, and Paraná). Inside this group, the three species from the rivers Maquiné, Três Forquilhas, Mampituba, and Araranguá (*Pareiorhaphis hypselurus*, *P. nudula*, and *P. stomias*) form a clade. These rivers are considered as a region with high degree of endemism (Malabarba and Isaia 1992; Reis and Schaefer 1998), and recently were recognized as a distinct freshwater ecoregion in the WWF/TNC mapping project (Abell et al. 2008). *Pareiorhaphis regani* was described from the rio Curicuriari (rio Negro basin), but as the description is based on one single specimen and there are no other Neoplecostominae known from the whole Amazon region, this locality is highly doubtful. In spite of having longer odontodes than known from *P. steindachneri*, these two species might be synonyms. But only the examination of the holotype may resolve this question.

The third geographical clade is formed by the species distributed in the basins that drain the southern Brazilian highlands westwards. *Pareiorhaphis parmula* occurs in the upper rio Iguaçu, *P.* sp. 4 in the upper rio Jacui and *P. hystrix* is known from the upper reaches of both the rio Jacui and rio Uruguay. The remaining species are distributed in the upper rio Uruguay basin.

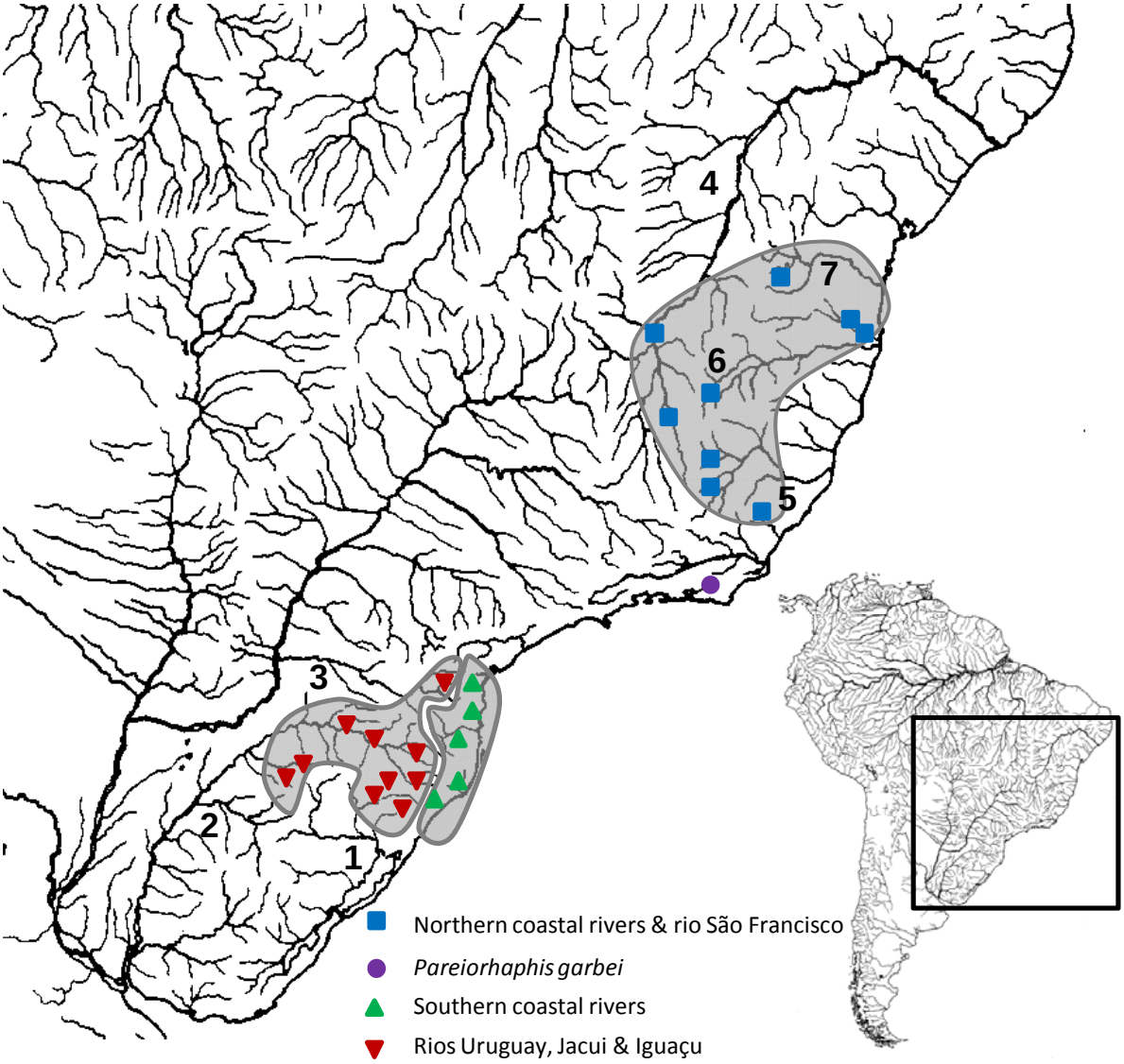

Fig. 12 Map showing the distribution of the species from the genus *Pareiorhaphis* (without the species *P. cerosus* and *P. regani* which do not have confirmed localities). 1 = Rio Jacui, 2 = rio Uruguai, 3 = rio Iguaçu, 4 = rio São Francisco, 5 = rio Doce, 5 = rio Jequitinhonha, 6 = rio Contas.

Hypoptopomatinae

All morphological phylogenies of members of the Hypoptopomatinae (Schaefer 1991; Schaefer 1997; Schaefer 1998; Armbruster 2004; Lehmann 2006; Pereira 2008) have recovered the subfamily as monophyletic. Contrastingly, none of the solely molecular analyses so far conducted (Montoya-Burgos et al. 1998; Chiachio et al. 2008; Cramer et al. 2008, this volume) was able to uncover a monophyletic Hypoptopomatinae. The molecular

analyses consistently find the Neoplecostominae amidst the Hypoptopomatinae (Figs. 6, 7, and 9). Our total evidence analysis, however, recovered the Hypoptopomatinae as a monophyletic group with moderately high Bremer support. A characteristic of this subfamily is the ventral surface of the pectoral fin skeleton covered by thin skin and usually bearing numerous odontodes, such that the bone appears to be exposed on the ventral surface (Schaefer 2003).

Schaefer (1991) divided the subfamily into two purportedly monophyletic clades, the tribes Hypoptopomatini and Otothyrini. Our results confirm his findings for the former, concordant with some previous studies (based on morphology: Schaefer 1997, 1998; Armbruster 2004; Lehmann 2006; based on molecular data: Chiachio et al. 2008). But we could not corroborate the monophyly of the Otothyrini, agreeing with Gauger and Buckup (2005), Cramer et al. (2008), Lehmann (2006), Pereira (2008), and Cramer et al. (this volume) but contrary to Chiachio et al. (2008) and Schaefer (1997, 1998). Even with the Otothyrini being polyphyletic, here we continue using the denomination Hypoptopomatini to refer to this monophyletic clade.

Like already demonstrated by other studies (Gauger and Buckup 2005; Lehmann 2006; Cramer et al. 2008; Cramer et al. this volume), we found *Parotocinclus* to be a highly paraphyletic group, albeit some monophyletic clades were recovered. For the strongly supported *Parotocinclus jumbo* and *P.* sp. 9 clade, a new genus is being described (PL and RER, in preparation). The species from northeastern Brazil, with the exclusion of *Parotocinclus jumbo* that is clearly different from this group, compose a well supported clade (*P. cearensis*, *P. cesarpintoi*, *P. haroldoi*, *P. spilosoma*, *P. spilurus*, *P.* sp. 10, and *P.* sp. 14), likely representing a second new genus. Lehmann (2006) already suspected such a relationship but could not resolve them as monophyletic. Another geographical group is formed by the species from the Guyana Shield (*Parotocinclus aripuanensis*, *P. britskii*, *P. collinsae*, *P. eppleyi*, *P. longirostris*, *P. polyochrus*, and the undescribed species 6, 12, 13, and

15 – 19), as also found by Lehmann (2006) and Cramer et al. (this volume). These species differ clearly from its congeners in their smaller size, body shape, and coloration and likely comprise a third new genus.

The type species, *Parotocinclus maculicauda*, clustered with *P. bidentatus*, *P. doceanus*, the new species 1, 7, and 8, and *P. maculicauda* "Cubatão" (clade V, Fig. 10d), getting only low support. The latter probably is identical with the type species and was only separated because of some minor morphological differences. Unfortunately, we could not resolve which other species make part of this *Parotocinclus sensu strictu*, as there was a large polytomy on the base of this clade, containing *P. jimi*, *P. minutus*, *P. planicauda*, and some additional undescribed species. The inclusion of DNA sequence data for more species might resolve this problem.

Most unexpectedly, the "jumping" taxon *Microlepidogaster* sp. 1 was also recovered inside the clade V. This species was preliminary identified as a *Microlepidogaster*, but it is presently being described as a new genus (in preparation by Martins and Langeani, personal communication). The position of *Parotocinclus cristatus* (clade IV, Fig. 10d) could not be resolved as it is one of the jumping species that have been excluded from the consensus.

Another well supported clade is formed by *Corumbataia cuestae* and *Gymnotocinclus*, which were recovered as sister to a new genus, here entitled New taxon TT. This genus (under description by Buckup, Britto, and Reis) currently comprises six species. Four of them are new and two have recently been described as *Hisonotus*, albeit the authors already stated that there are uncertainties about this placement and that the genus *Hisonotus* is in need of a taxonomic revision (Britski and Garavello 2007). A seventh species, Hypoptopomatinae sp. Surinam, probably the same species as shown in Le Bail et al. (2000: p. 262) and Schaefer (1998: taxon 3), was found to belong to this group, but was excluded from the strict consensus. A lack of compatible data frustrated a precise grouping.

The next clade is formed by *Otothyris* and *Schizolecis*, getting high Bremer support, and being placed as sister to the Hypoptopomatini. This weakly supported position differs from all previous studies, although Lehmann (2006) came to a similar result, but including also the genera *Corumbataia* and *Pseudotothyris*.

Hypoptopomatini

Schaefer (1991) found this clade as monophyletic and described it as a new tribe, a result further corroborated by the present and most previous studies. The few studies that found a non-monophyletic Hypoptopomatini (Cramer et al. 2008; Cramer et al. this volume), obtained weak support for alternative resolutions. Schaefer (1991) gives the following characteristics for this tribe: Derived absence of a *levator arcus palatini* crest on the hyomandibula, reduced levator muscle, and presence of a few, relatively large plates at the anterior snout margin.

Our topology is very similar to Schaefer's (1991), recovering *Otocinclus* and *Acestridium* as monophyletic genera and revealing *Niobichthys* as sister to all hypoptopomatin genera but *Otocinclus*. On the other hand, we recovered the genus *Hypoptopoma* as polyphyletic, concordant with Chiachio et al. (2008) and Cramer et al. (this volume). Since the results of Chiachio et al. (2008) placed *Oxyropsis* as a separate clade, those authors tried to resolve the paraphyly of *Hypoptopoma* treating *Nannoptopoma* as a synonym of *Hypoptopoma*, suggesting that the former is a neotenic form of the latter. However, as they included only few species of both genera in their analysis, it is no surprise that our inclusion of more taxa resulted in different topologies, an effect already described by Schaefer (1998), coincidently using hypoptopomatine taxa as examples. In their revision of *Oxyropsis*, Aquino and Schaefer (2002) considered the genus as valid and well defined. As we could not include the type species *Hypoptopoma thoracatum*, we prefer to maintain *Nannoptopoma* and *Oxyropsis* as distinct monophyletic genera and to postpone the decision whether to split

Hypoptopoma or to synonymize the other two genera till a more complete dataset is available. The taxonomic status of the possible new genus also could not be clarified as its two species were placed into a polytomy with part of the species of *Hypoptopoma*.

Isbrücker and Seidel (in Isbrücker et al. 2001) described the genus *Macrotocinclus* for the species *Otocinclus affinis* and *O. flexilis*, repeating basically Schaefer's definition of the *Otocinclus affinis* complex (Schaefer 1997). Based on our results (Lehmann 2006; Lehmann et al. submitted; Cramer et al. this volume; this study), *Otocinclus* is a strongly supported clade and there is no need for a split in two genera. Moreover, the recognition of *Macrotocinclus* as defined by its authors would turn *Otocinclus* paraphyletic, as *O. xakriaba*, *O. mimulus*, and *O. arnoldi* are more closely related to *O. affinis* and *O. flexilis* than to the remaining species of *Otocinclus* (Lehmann et al in press). Therefore, we consider *Macrotocinclus* as a synonym of *Otocinclus*.

Going on with the remaining taxa of the Otothyrini (sensu Schaefer 1991), *Hisonotus insperatus* is placed in a larger polytomy, far from other congeners. This is not unexpected, because, even considering the necessity of a taxonomic revision of *Hisonotus* (Britski and Garavello 2007), this species can be clearly distinguished from the remaining species (e.g. having one internasal plate [vs. three] that does not contact the rostral plate [vs. in contact]; predorsal plates fused and unique [vs. not fused and paired] [Lehmann 2006]) and is more akin to *Parotocinclus*.

Our clade IV received very high Bremer support and might serve as a base for a future description of a new subfamily. Inside clade IV, besides the bulk of species of *Parotocinclus*, treated above, the next monophyletic group comprises *Microlepidogaster perforatus*, *M.* sp. "AP", and *Pseudotocinclus* sp. "PP". The latter was preliminary assigned to the genus *Pseudotocinclus* by Lehmann (2006), because his results showed it as sister to

Pseudotocinclus tietensis. At present, the genus *Microlepidogaster* is being revised (in preparation by B. Calegari, personal communication) and the species "PP" is likely to make part of it (Calegari, personal communication). Respecting the ongoing revision and the low support of the clade, we do not make changes in this species generic assignment.

A heterogeneously composed clade is formed by *Pseudotothyris obtusa*, two undescribed species of *Otothyropsis*, *Microlepidogaster* sp. 2, *Hisonotus francirochai*, and *Hisonotus* sp. 4. As already postulated by its authors (Ribeiro et al. 2005), *Otothyropsis*, which is currently being revised (in preparation by B. Calegari, personal communication), and *Pseudotothyris* were found as closely related. Based on this grouping, we reexamined the voucher specimens of *Microlepidogaster* sp. 2 and *Hisonotus francirochai*. Both species appear to be more closely related to *Otothyropsis* than to the assigned genera (Calegari, personal communication). For example they share a crest with odontodes on the tip of the supraoccipital plate. Unfortunately, the voucher of *Hisonotus* sp. 4 was not available, but from its collecting locality in the rio Alambari in the rio Tietê basin, it might be a *Otothyropsis*, as the type species was described from this basin.

Concordant with earlier studies (Britski and Garavello 2007; Cramer et al. 2008; Cramer et al. this volume), the genus *Hisonotus* was revealed as polyphyletic, comprising at least two clades, each with moderate support. However, we could not find any characters to distinguish the monophyletic groups. Even the species from the rio Uruguai basin (Carvalho and Reis 2009) could not be joined in one group (*Hisonotus* sp. 1 occurs in the rio Jacui basin; *Hisonotus charrua* is distributed in the rio Uruguai basin, but was placed in the other clade, separated with high decay index). Additionally, *Lampiella gibbosa* was found as sister to *Hisonotus leucofrenatus*. Currently, the status of *Lampiella* is uncertain. The species was first described as *Otocinclus*, and Chiachio et al. (2008) found it to be sister to that genus. Lehmann (2006) showed it as more proximally related to *Hisonotus* than to *Otocinclus*, and our earlier molecular results resolved it as sister to either *Otocinclus* or *Hisonotus*

leucofrenatus (Cramer et al. this volume). Based on the lack of strong evidence for a taxonomic change, we maintain its generic status as separated from *Otocinclus* and *Hisonotus*.

The last clade of our consensus tree is composed by *Epactionotus* and *Eurycheilichthys* as sister taxa in a highly supported group. Both genera are clearly monophyletic, concordant with Lehmann (2006) and Cramer et al. (2008, this volume). Remarkably, based purely on morphological evidence, Lehmann (2006) recovered *Eurycheilichthys* as the most basal taxon in the Hypoptopomatinae, and not closely related to *Epactionotus*, contrary to the results of Chiachio et al. (2008), Cramer et al. (2008; this volume) and the ones presented here. These two genera occur in small rivers draining the southern portion of the Brazilian Shield. A common pattern for the distribution of fishes is that basal taxa are distributed on the highlands, such as the Brazilian and the Guyana shields, and the more derived taxa are spread in the lowlands, such as the Amazon and the Orinoco basins. This pattern is caused by the older age of the shields and repeated incursions of marine water in the lower areas till the late Miocene (Lundberg et al. 1998; Hulka et al. 2006), inhibiting the life of strict freshwater inhabitants. That way, diversification began on the shields and only later, the lowlands were invaded. Among others, Ribeiro et al. (2005) showed this for the genera *Creagrutus* and *Piabina*, and Menezes et al. (2008) for the Glandulocaudinae, but there are several examples for loricariids, as well. The two most basal subfamilies, Lithogeninae and Delturinae, are solely distributed on the Guyana and the Brazilian shield, respectively (Reis et al. 2006; Schaefer and Provenzano 2008). Inside the Loricariinae, *Harttia* is one of the most basal taxa (Rapp Py-Daniel 1997), with its species occurring mostly on the Guyana and the Brazilian shields (Covain et al. 2006), whereas *Pseudohemiodon* as one of the most derived loricariine taxa is mainly distributed in the Amazon lowlands. The same is true for the Hypostominae, with the members from the most basal tribe Corymbophanini (Armbruster 2004) exclusively dispersed on the Guyana shield and the most derived tribe Ancistrini having its highest diversity in the Amazon region.

Further on this biogeographic evidence, morphology within the Hypoptopomatinae is highly variable and is also in disagreement with the phylogenetic findings. For instance, *Eurycheilichthys* and *Epactionotus* are very generalized in their morphology, being hardly distinguishable from neoplecostomines or basal hypostomines. On the other hand, genera like *Hypoptopoma*, *Nannoptopoma*, *Oxyropsis*, and especially *Acestridium* are highly modified morphologically from the general pattern of basal loricariids with some bizarre body shapes and other specializations.

The entire Neoplecostominae is restricted to the Brazilian shield. Therefore it is a somewhat unexpected finding that the Hypoptopomatini with species exclusively from the lowlands was recovered as one of the most basal clades inside the Hypoptopomatinae, and *Epactionotus* + *Eurycheilichthys*, both from the highlands, were recovered as the most derived taxa.

Comparing the results from solely molecular or morphologic analyses with the ones obtained from the total evidence approach, several advantages of the latter were found. Other studies on the phylogeny of fishes already have come to the same conclusion, but only used few species (e.g. Betancur-R et al 2007; Pretti et al. 2009). With the present study probably being the largest total evidence analysis conducted for fishes so far, we would like to highlight the importance and the benefits of this method.

Results from solely molecular or morphological data have not been able to fully resolve the loricariid phylogeny, and different studies have come to conflicting results, even using the same type of data, whereas our total evidence phylogeny seems to be the best approach. Our results combine resolutions from previous analyses, resolving most suprageneric groups as monophyletic. The Neoplecostominae and the Hypoptopomatinae are groups where molecular and morphological studies came to highly controversial results. Our total evidence solution shows both as separate monophyletic clades, including *Pareiorhina* and *Kronichthys* in the

Neoplecostominae, as already proposed by Armbruster (2004), but with the addition of the genus *Pseudotocinclus*.

Doubts about the influence of the different quantities of molecular and morphological characters apparently are without fundament, as our matrix comprised above two times more parsimony informative molecular characters than morphologic ones (1089 vs. 472). Nevertheless, the comparison of the different results shows that morphological characters have a strong influence on the result of the total evidence analysis. Solely molecular data did not recover the monophyly of the Hypoptopomatinae or the genera *Neoplecostomus*, *Isbrueckerichthys*, and *Pareiorhaphis*, whereas they become monophyletic using the combined data set. Another finding that only the jointed data revealed, is the biogeographic pattern found for the genus *Pareiorhaphis*. Even groups with relatively scarce molecular data, such as the *Parotocinclus* from northeastern Brazil, benefited from the total evidence approach as morphology alone did not recover its monophyly.

Summarizing, we show that a total evidence approach is the most adequate method to explore the loricariid phylogeny since our combined dataset was able to resolve several of the known problems for the subfamilies Hypoptopomatinae and Neoplecostominae. The Neoplecostominae was resolved as monophyletic, including *Pseudotocinclus*, as well as *Kronichthys* and *Pareiorhina* that had been considered as hypoptopomatines by Pereira (2008). Most of its genera were found to be monophyletic, except for *Pareiorhina* that requires additional morphological data. Both, the Hypoptopomatinae and the Hypoptopomatini were also recovered as monophyletic. Conversely, the Otothyrini was found to be a polyphyletic taxon, corroborating other recent studies (Armbruster 2004; Lehmann 2006; Cramer et al. 2008; Pereira 2008; Cramer et al. this volume). Because of a larger polytomy in our consensus tree, we still cannot offer an approach for a split into well defined

monophyletic groups, though our clade IV is a strong candidate for a future suprageneric taxon.

The polyphyly of *Parotocinclus* could be partly resolved as we found three monophyletic groups that represent undescribed genera. Nevertheless, the species around *P. maculicauda* (the *Parotocinclus sensu strictu*) remain unresolved.

The status of the "Otothyrini" and the genera *Hisonotus*, *Hypoptopoma*, and *Parotocinclus* should be resolved by a further improvement of the character as well as the taxon sampling. Our results suggest that filling the gaps in our matrix (especially for the jumping taxa from Table 3), as well as the inclusion of additional (e.g. currently still only half of the species of *Hisonotus* are represented) and especially some critical taxa (e.g. *Hypoptopoma thoracatum*) will amend our understanding of the phylogeny of the remaining polyphyletic taxa.

To test the influence of the characters that were used by Lehmann (2006) and Pereira (2008) and that way were included twice in our matrix, two additional analyses were run, using only one of the morphological data sets (Fig. 13 and 14, Appendix 2 and 3).

The first one, with the data from Lehmann (2006), resulted in a strict consensus tree containing a large polytomy inside a clade containing the Hypoptopomatinae + Neoplecostominae, probably caused by a lack of sufficient morphological data for the Neoplecostominae. The Hypoptopomatini (sensu Schaefer 1991) was recovered as monophyletic group.

The second one, with data from Pereira (2008), recovered the Neoplecostominae as monophyletic, but split the Hypoptopomatinae in three clades. The genus *Otocinclus* was separated from the Hypoptopomatini (sensu Schaefer 1991).

As expected, most clades that were resolved in all three analyses and got high Bremer support using all data, got lower support in the additional analyses (e.g. Eurycheilichthys + Epactionotus: 13 vs. 8 and 4; Isbrueckerichthys: 24 vs. 4 and 1), caused by the removal of

duplicated characters that supported these groups. But there are other cases where clades got identical support (e.g. Hypoptopomatinae + Neoplecostominae: 7 vs. 9 and 7) or even higher support in the additional analyses (e.g. *Otocinclus*: 5 vs. 13 and 25). In the former case, the removed characters were partly conflicting and their removal did not alter the result, in the latter case, they were in conflict with the resolution and their exclusion improved the results by getting higher support.

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Appendix 1 Species, depository information and GenBank accession numbers for the additional COI sequences from Cramer et al. (2008) used in this study.

Eurycheilichthys sp. 2 MCP 22374 EU370992. *Eurycheilichthys* sp. 2 MCP 22800 EU370994. *Eurycheilichthys* sp. 3 MCP 35049 EU370999. *Eurycheilichthys* sp. 4 MCP 22199 EU370991. *Eurycheilichthys* sp. 7 MCP 35071 EU370997.

Appendix 2 Results of the additional analysis including only sequence data and morphological data from Lehmann (2006)

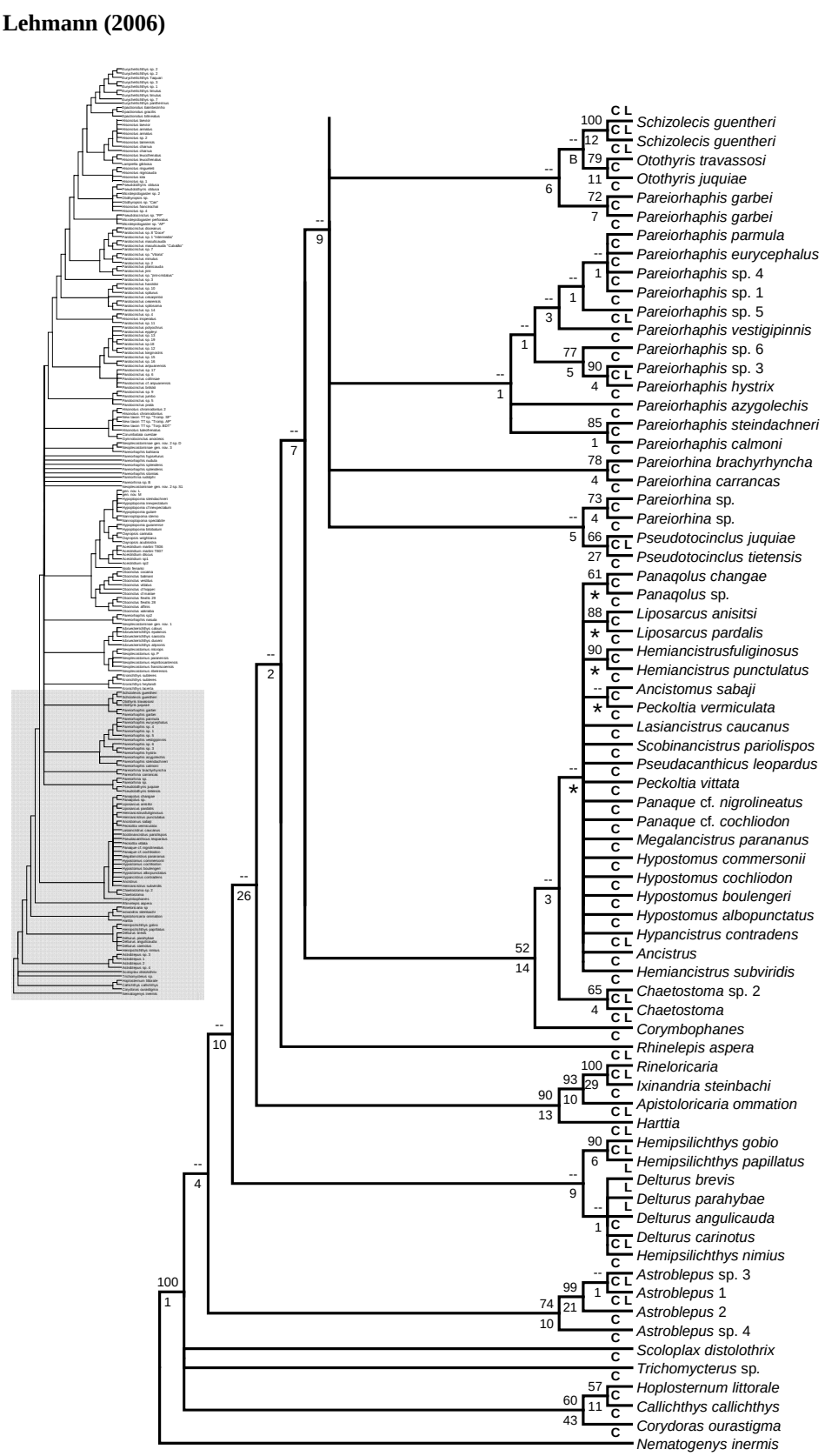

Fig. 13a First part of the strict consensus of the 216 most parsimonious trees (8810 steps) with the jumping taxa (Table 3) excluded. Numbers above branches are values from 2000 bootstrap replicates, numbers below are Bremer support (clades marked with * were only resolved after the exclusion of the taxa from Table 3). Remainder of the tree in Fig. 13b and c. C and L specify the kind of data available for each terminal: C = molecular, L = morphological from Lehmann 2006.

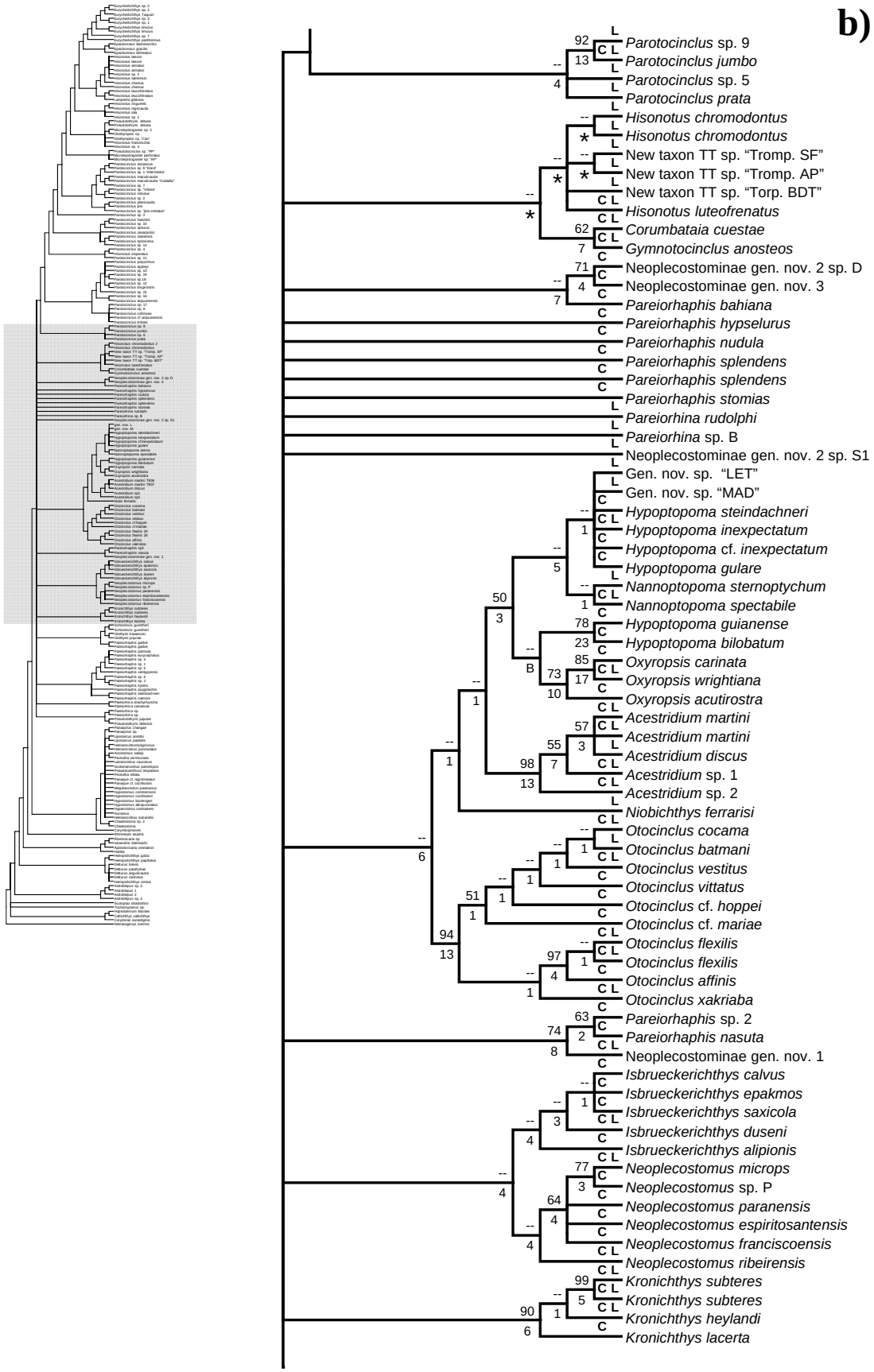

Fig. 13b Second part of the strict consensus of the 216 most parsimonious trees (8810 steps) with the jumping taxa (Table 3) excluded. Numbers above branches are values from 2000 bootstrap replicates, numbers below are Bremer support (clades marked with * were only resolved after the exclusion of the taxa from Table 3). Remainder of the tree in Fig. 13a and c. C and L specify the kind of data available for each terminal: C = molecular, L = morphological from Lehmann 2006.

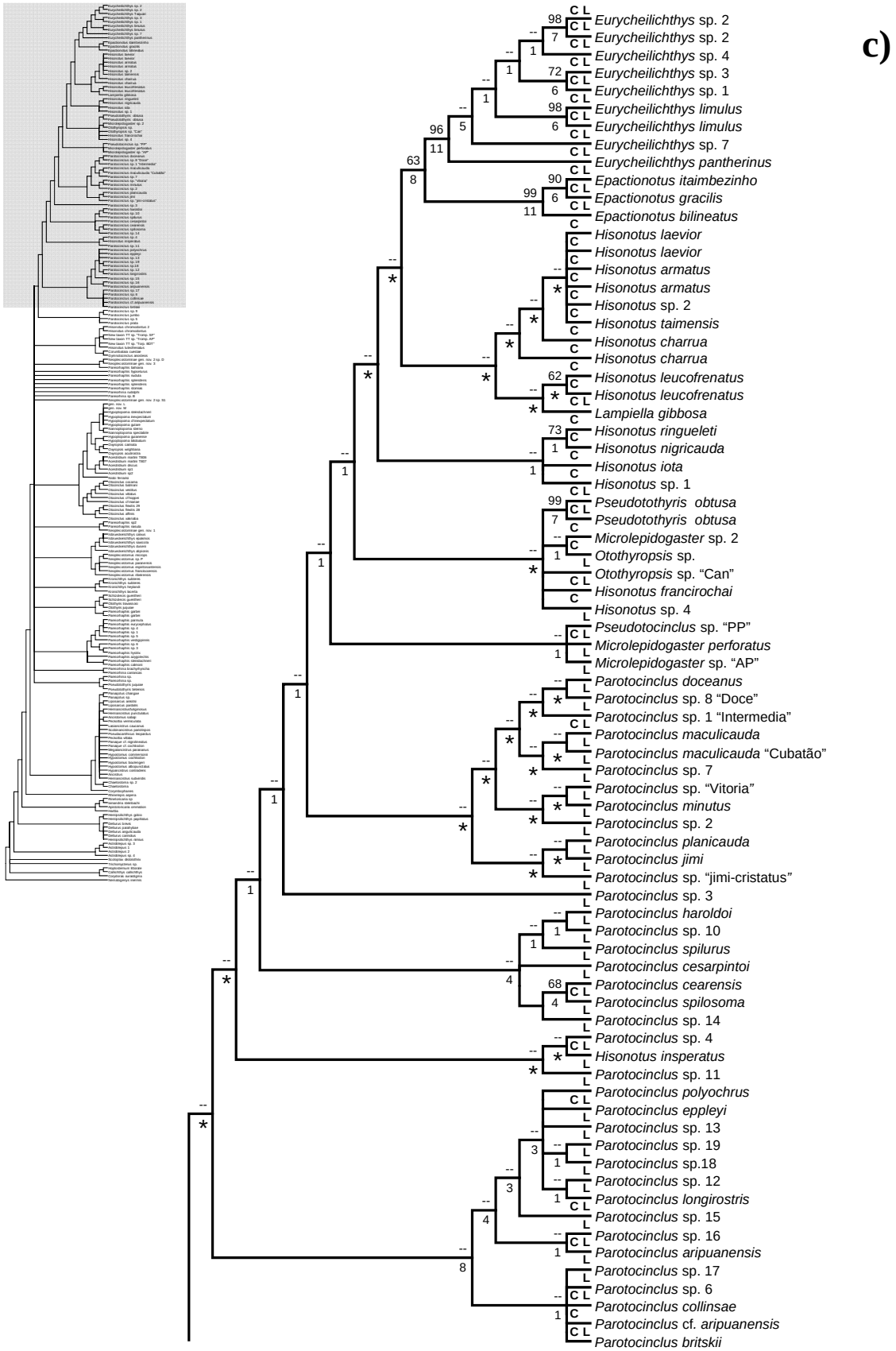

Fig. 13c Third part of the strict consensus of the 216 most parsimonious trees (8810 steps) with the jumping taxa (Table 3) excluded. Numbers above branches are values from 2000 bootstrap replicates, numbers below are Bremer support (clades marked with * were only resolved after the exclusion of the taxa from Table 3). Remainder of the tree in Fig. 13a and b. C and L specify the kind of data available for each terminal: C = molecular, L = morphological from Lehmann 2006.

Appendix 3 Results of the additional analysis including only sequence data and morphological data from Pereia (2008)

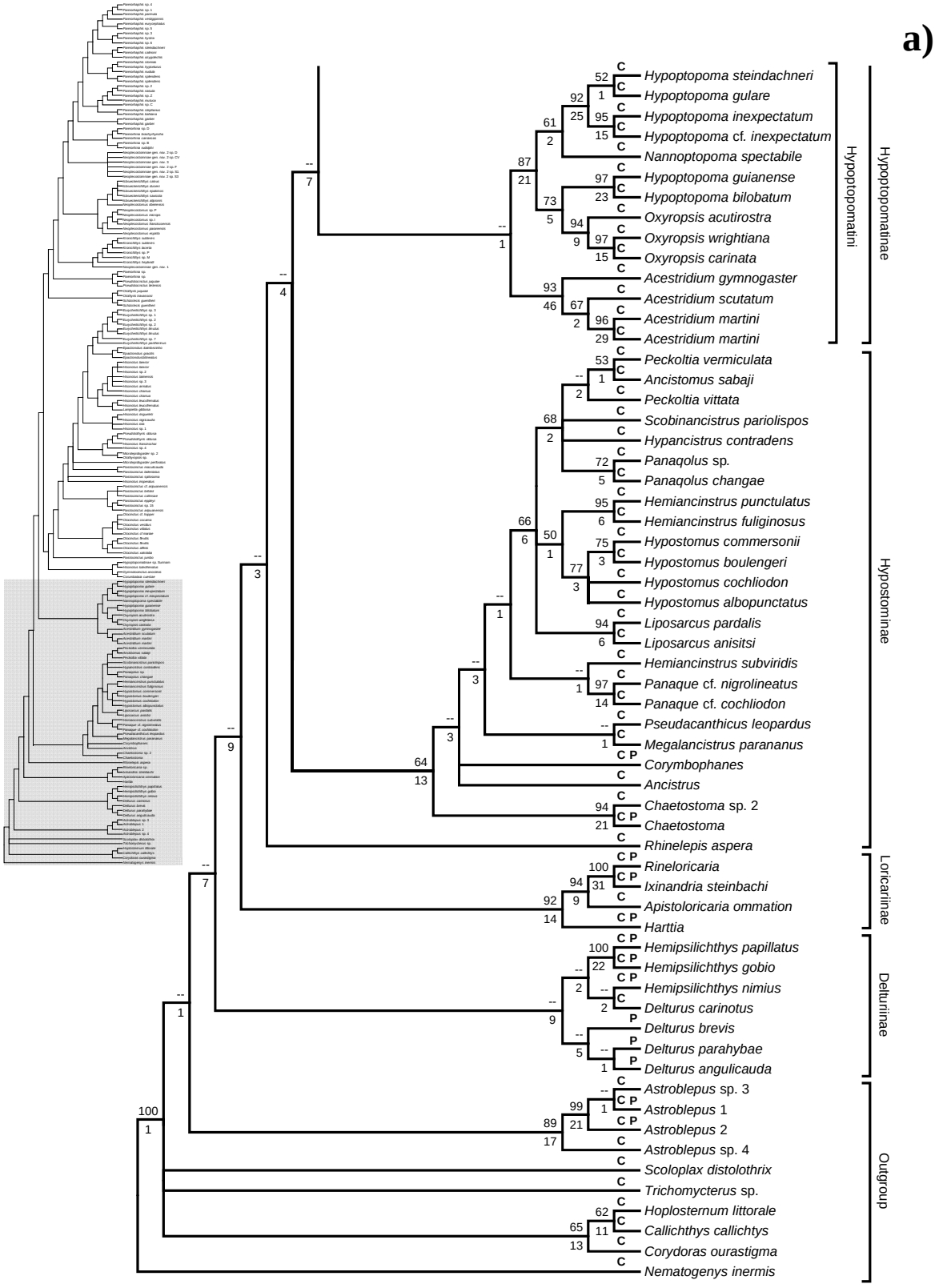

Fig. 14a First part of the strict consensus of the 95 most parsimonious trees (8826 steps) with the jumping taxa (Table 3) excluded. Numbers above branches are values from 2000 bootstrap replicates, numbers below are Bremer support (clades marked with * were only resolved after the exclusion of the taxa from Table 3). Remainder of the tree in Fig. 14b and c. C and L specify the kind of data available for each terminal: C = molecular, L = morphological from Lehmann 2006.

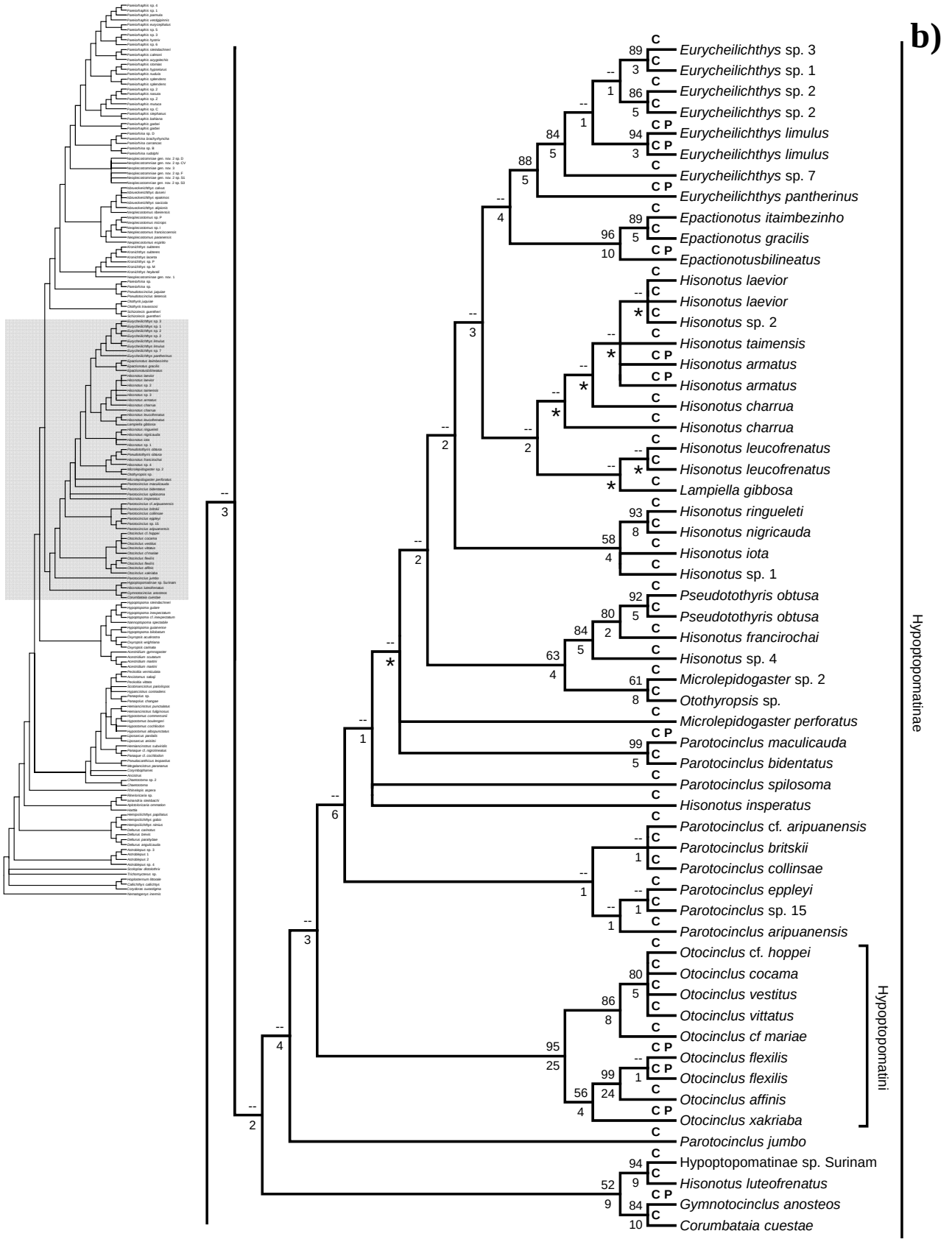

Fig. 14b Second part of the strict consensus of the 95 most parsimonious trees (8826 steps) with the jumping taxa (Table 3) excluded. Numbers above branches are values from 2000 bootstrap replicates, numbers below are Bremer support (clades marked with * were only resolved after the exclusion of the taxa from Table 3). Remainder of the tree in Fig. 14a and c. C and L specify the kind of data available for each terminal: C = molecular, L = morphological from Lehmann 2006.

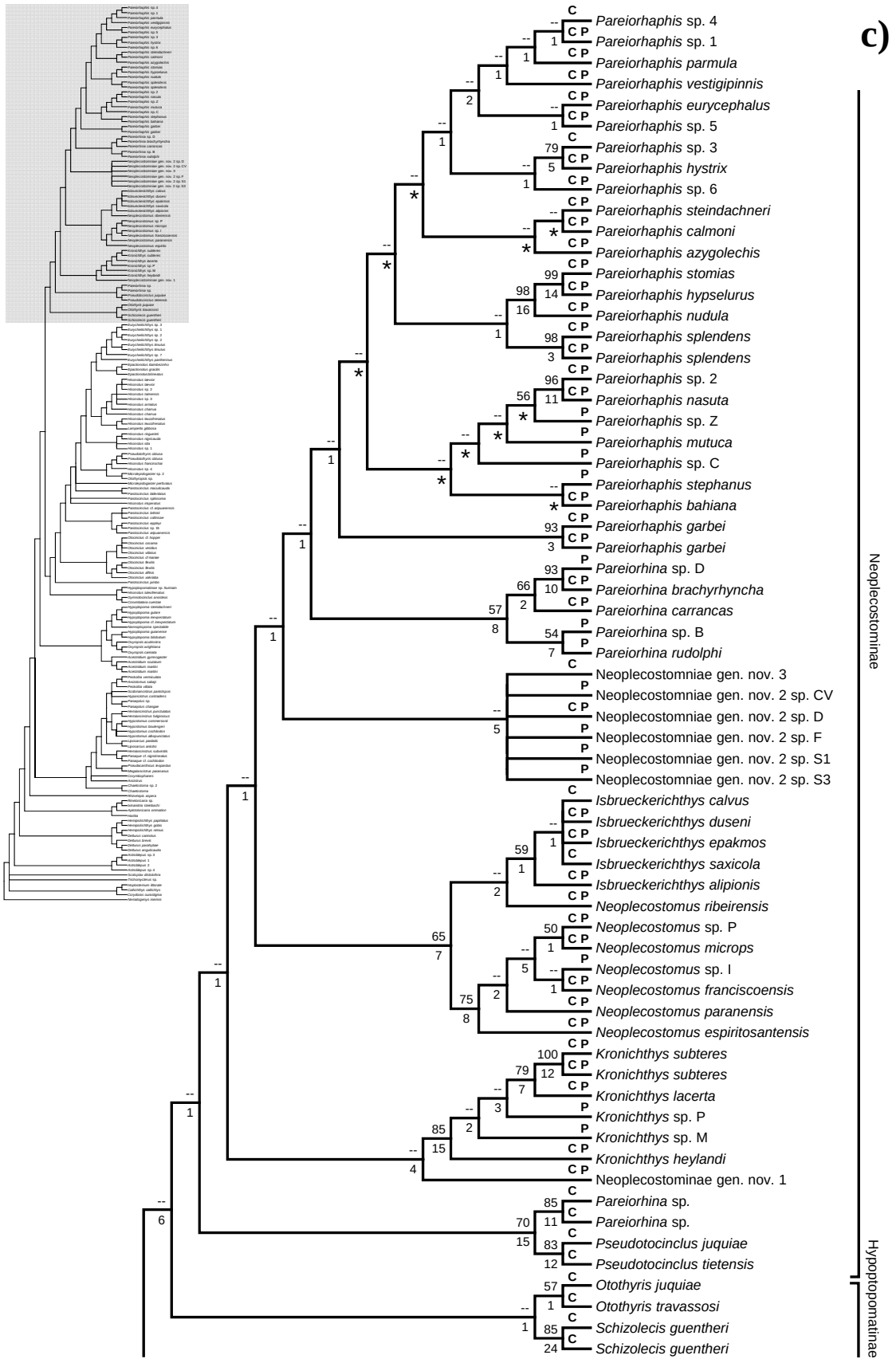

Fig. 14c Third part of the strict consensus of the 95 most parsimonious trees (8826 steps) with the jumping taxa (Table 3) excluded. Numbers above branches are values from 2000 bootstrap replicates, numbers below are Bremer support (clades marked with * were only resolved after the exclusion of the taxa from Table 3). Remainder of the tree in Fig. 14a and b. C and L specify the kind of data available for each terminal: C = molecular, L = morphological from Lehmann 2006.

Conclusões Gerais

No presente estudo foram elaboradas filogenias baseadas em sequências de DNA e de evidência total, incluindo dados morfológicos de estudos prévios. A análise de evidência total resultou na maior filogenia de bagres feita até o momento. Estes resultados foram comparados com as filogenias baseadas exclusivamente em dados morfológicos.

Usando somente dados moleculares, de um ou de múltiplos genes, vários grupos foram encontrados como não-monofiléticos, tais como a subfamília Hypoptopomatinae e os gêneros *Pareiorhina*, *Pareiorhaphis*, *Neoplecostomus*, *Hisonotus* e *Parotocinclus*. Ao contrário dos estudos morfológicos, o gênero *Pseudotocinclus* sempre foi revelado como membro da subfamília Neoplecostominae, e não de Hypoptopomatinae, corroborando publicações anteriores. Além disto, os gêneros *Kronichthys* e *Pareiorhina* são encontrados dentro de Neoplecostominae, confirmando a classificação de Armbruster (2004), mas contrariando Lehmann (2006) e Pereira (2008).

Com o objetivo de resolver estes conflitos entre os resultados de análises usando somente dados moleculares ou morfológicos, optou-se por fazer uma análise de evidência total. Os resultados deste conjunto de dados, incluindo 472 caracteres morfológicos e 2634 pares de bases de três genes para 207 espécies, conseguiram resolver vários dos conflitos conhecidos. As subfamílias Hypoptopomatinae e Neoplecostominae se apresentaram como monofiléticas, assim como a tribo Hypoptopomatini e todos os gêneros de Neoplecostominae, exceto *Pareiorhina*.

Oito espécies foram excluídas do consenso estrito, porque elas agruparam com várias espécies diferentes em árvores diferentes, resultando em grandes politomias. A escassez de dados disponíveis para estes táxons provavelmente foi responsável por isto. Depois de terem sido retirados do consenso, a maioria destas politomias foi resolvida.

Sem a possibilidade de incluir membros de Lithogeninae, a subfamília Delturinae foi encontrada como o clado mais basal da Loricariidae, corroborando Armbruster (2004) e Reis et al. (2006). Loricariinae foi encontrada monofilética, ficando em uma politomia junto com Hypostominae e Neoplecostominae + Hypoptopomatinae. Nas análises moleculares do capítulo II, *Rhinelepis aspera* foi encontrada fora de Hypostominae, contrariando Armbruster (2004).

Neoplecostominae e Hypoptopomatinae foram recuperadas como grupos-irmãos, a primeira incluindo o gênero *Pseudotocinclus*. Dentro de Neoplecostominae, todos os gêneros exceto *Pareiorhina* formam grupos naturais. Provavelmente, a polifilia de

Pareiorhina é causada por falta de dados morfológicos para uma espécie. O resultado de uma análise forçando *Pareiorhina* como monofilético foi refutado como sendo estatisticamente pior. Concordando com todas as análises moleculares, *Pseudotocinclus* é mais proximamente relacionado à *Pareiorhina*.

A análise de evidência total encontrou uma filogenia com um padrão biogeográfico antes desconhecido para o gênero *Pareiorhaphis*. Foram formados três grupos geográficos: dos rios costeiros do sul (RS, SC e PR), das cabeceiras dos rios drenando para o oeste (rios Uruguai, Jacui e Iguaçu) e dos rios costeiros no norte (RJ, ES, BA) + rio São Francisco (MG).

Contrariando estudos exclusivamente moleculares, mas confirmando resultados morfológicos, a subfamília Hypoptopomatinae foi recuperada como monofilética. Entretanto, das duas tribos descritas por Schaefer (1991), somente Hypoptopomatini forma um grupo natural, deixando Otothyrini polifilética. Os gêneros *Nannoptopoma* e *Oxyropsis* dividem as espécies do gênero *Hypoptopoma* em dois grupos. Chiachio et al. (2008) encontraram uma situação parecida e sinonimizaram *Nannoptopoma* com *Hypoptopoma*, alegando neotenia. À base dos presentes resultados, seria necessária a sinonimização adicional de *Oxyropsis* para tornar *Hypoptopoma* monofilético. Como não foi possível incluir a espécie tipo, *Hypoptopoma thoracatum*, optou-se pela manutenção de *Nannoptopoma* até que uma análise mais completa possa oferecer uma solução mais bem suportada.

Confirmando outros estudos (Gauger e Buckup, 2005; Lehmann, 2006; Britski e Garavello, 2007), os gêneros *Hisonotus* e *Parotocinclus* foram encontrados como polifiléticos. Porém, três grupos monofiléticos puderam ser encontrados no último: um clado com duas espécies do sul e do nordeste do Brasil, um clado da Amazônia e um clado do nordeste brasileiro. Mas, mesmo assim, as espécies em volta da espécie tipo permaneceram em uma politomia, provavelmente causada por falta de dados moleculares. Além das espécies que claramente não pertencem ao gênero *Hisonotus*, por exemplo, *H. francirochai* e *H. insperatus*, as espécies formam dois grupos, portanto, poucos dados morfológicos foram disponíveis para estas espécies.

Epactionotus e *Eurycheilichthys* formam os táxons mais derivados de Hypoptopomatinae, confirmando outros estudos exclusivamente moleculares, mas contrariando os resultados de Lehmann (2006). Isto é surpreendente porque contradiz um padrão geral na distribuição de peixes. Exemplos para vários grupos mostram que, geralmente, os táxons mais basais têm a sua distribuição nos escudos e os grupos mais

derivados são encontrados nas áreas baixas do continente, como a bacia do Amazonas ou do Orinoco. Mesmo dentro da Loricariidae este padrão é comum: as duas subfamílias mais basais, Lithogeninae e Delturinae, bem como os táxons mais basais de Loricariinae e de Hypostominae, são dos escudos; portanto, os táxons mais derivados das duas últimas têm sua maior diversidade nas áreas baixas. Desta forma, com Hypoptopomatini distribuída principalmente na Amazônia, mas sendo um táxon basal dentro da subfamília, e *Epactionotus* + *Eurycheilichthys* sendo recuperados como mais derivados, mas ocorrendo no escudo brasileiro, Hypoptopomatinae não se enquadra neste padrão mais geral.

O uso da evidência total resolveu vários conflitos das filogenias prévias e parece ser a melhor estratégia para acessar as relações dentro de Loricariidae, mesmo não tendo todos os tipos de dados para todos os táxons. Mesmo usando quase quatro vezes mais dados moleculares, a morfologia tem forte influência na topologia. Os dois tipos de dados contribuíram nas resoluções encontradas e, juntos, revelaram grupos antes não resolvidos (por exemplo, os *Parotocinclus* do nordeste) e o padrão biogeográfico do gênero *Pareiorhaphis*. Um futuro estudo deveria usar este método, incluindo as informações ausentes para os grupos que ainda não foram bem resolvidos, especialmente para estes onde principalmente apenas um tipo de dados está disponível (por exemplo, Hypostominae, *Hisonotus* e *Parotocinclus*). Além disto, algumas espécies importantes deverão ser acrescentadas. Por enquanto, por exemplo, não foi possível incluir a espécie-tipo de *Hypoptopoma*, o que impediu uma decisão sobre o futuro deste gênero, e somente metade das espécies de *Hisonotus* estavam disponíveis, impossibilitando uma conclusão final sobre este grupo parafilético.

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