

UNIVERSIDADE ESTADUAL PAULISTA

INSTITUTO DE BIOCÊNCIAS

BRUNO FRANCELINO DE MELO

**RELAÇÕES FILOGENÉTICAS NA SUPERFAMÍLIA
ANOSTOMOIDEA (OSTARIOPHYSI: CHARACIFORMES)**

DOUTORADO

BOTUCATU

2015

UNIVERSIDADE ESTADUAL PAULISTA

INSTITUTO DE BIOCIÊNCIAS

Bruno Francelino de Melo

**RELAÇÕES FILOGENÉTICAS NA SUPERFAMÍLIA
ANOSTOMOIDEA (OSTARIOPHYSI: CHARACIFORMES)**

Tese apresentada ao Instituto de Biociências de Botucatu, Universidade Estadual Paulista – UNESP, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas, Área de Concentração: Genética.

Orientador: Dr. Claudio de Oliveira

Co-orientador: Dr. Richard Peter Vari

BOTUCATU

2015

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Melo, Bruno Francelino de.

Relações filogenéticas na superfamília Anostomoidea
(Ostariophysi: Characiformes) / Bruno Francelino de Melo.
- Botucatu, 2015

Tese (doutorado) - Universidade Estadual Paulista
"Júlio de Mesquita Filho", Instituto de Biociências de
Botucatu

Orientador: Claudio de Oliveira
Coorientador: Richard Peter Vari
Capes: 20200005

1. Peixe de água doce. 2. Peixe - Classificação. 3.
Filogenia. 4. Characiformes.

Palavras-chave: Elementos ultraconservados; Filogenias;
Filogenômica; Peixes; Sistemática.

*“Aos meus pais, Osvaldo e Jaci,
exemplos de força, humildade e sabedoria...”*

AGRADECIMENTOS

Expresso aqui meus sinceros agradecimentos às instituições e pessoas que contribuíram para a realização deste trabalho.

Ao Prof. Claudio de Oliveira, pela orientação acadêmica em todas as fases do projeto, e às oportunidades oferecidas de se fazer pesquisa com qualidade, além da amizade, essenciais para minha formação.

Ao Dr. Richard P. Vari, pela co-orientação de suma importância pra este projeto, pela acolhida no Smithsonian Institution, pela valiosa e sólida colaboração científica que possuímos hoje, e pela rica e forte amizade.

Ao Dr. Brian L. Sidlauskas, por ter permitido realizar o doutorado-sanduíche na Oregon State University, por ser um dos responsáveis diretos pela minha inserção em projetos de alto nível científico e por ter aberto as portas para minha experiência internacional.

Ao Dr. Michael E. Alfaro, por me acolher na University of California, Los Angeles, por ter possibilitado a viabilização das modernas técnicas de sequenciamento de elementos ultraconservados, e por ter permitido que esta importante etapa do projeto tenha sido realizada com sucesso.

Aos colaboradores e amigos: Kendra Hoekzema, parceira de laboratório de biologia molecular; Ben Frable, parceiro de análises filogenéticas e discussões de resultados; Brant Faircloth, conhecedor dos elementos ultraconservados; Mike Burns, Casey Dillman, Mark Sabaj Perez, pelo conhecimento compartilhado referente a este projeto e pela receptividade nas instituições; José L. O. Birindelli, Raphael Covain e Ricardo M. C. Castro, pelo auxílio na identificação das espécies; ao Prof. Fausto Foresti pelos ensinamentos; ao Prof. Ricardo C. Benine, pelas grandiosas colaborações científicas de longa data.

A várias coleções científicas, seus curadores e pesquisadores que possibilitaram a ampliação de nossa amostragem: DZSJRP, ANSP, AUM, DePaul University, FMNH, INPA, LIRP, MCP, MHNG, MNHN, MZUSP, OSU, ROM, STRI, UNIR, USNM.

Aos amigos do Laboratório de Biologia e Genética de Peixes da UNESP e aos amigos de Botucatu pela contribuição e pelos inúmeros momentos de descontração. À Dra. Cristiane Shimabukuro-Dias e Amanda Alves pela preparação e envio de tecidos aos EUA.

A Universidade Estadual Paulista, Instituto de Biociências de Botucatu, Programa de Pós-Graduação em Ciências Biológicas (Genética) e Departamento de Morfologia, pela oportunidade e condições necessárias à realização deste estudo.

Agradecimento especial à Ana Paula Taioqui, minha linda namorada, que esteve ao meu lado nesta importante etapa da minha vida profissional. Obrigado pela companhia, paciência e amor. Te amo!

Agradecimento especial também aos meus pais Osvaldo e Maria Jaci, irmãos Ronaldo, Rosângela e Lucilene (e família: Ewerthon e meus lindos sobrinhos Bárbara e Bryan) e toda minha família Melo e Petrini, sempre oferecendo apoio incondicional.

Este projeto foi financiado pela agências de fomento: FAPESP (proc. 2011/08374-1 e BEPE proc. 2013/16436-2), CNPq (# 143365/2011-8 e # 140193/2015-4) e NSF (DEB-1257898).



AVISO

Esta tese é parte dos requerimentos necessários à obtenção do título de Doutor em Ciências Biológicas, Genética, e como tal, não deve ser vista como uma publicação no senso do Código Internacional de Nomenclatura Zoológica (apesar de disponível publicamente sem restrições). Desta forma, quaisquer informações inéditas, opiniões e hipóteses, bem como nomes novos, mudanças taxonômicas não estão disponíveis na literatura zoológica. Pessoas interessadas devem estar cientes de que referências públicas ao conteúdo deste estudo, na sua presente forma, somente devem ser feitas com a aprovação prévia do autor.

NOTICE

This thesis is a partial requirement for the PhD degree in Biological Sciences with emphasis in Genetics and, as such, should not be considered as a publication in the sense of the International Code of Zoological Nomenclature (although it is available without restrictions). Therefore, any new information, opinions, and hypotheses, as well as new names, taxonomic changes are not available in the zoological literature. Interested people are advised that any public reference to this study, in its current form, should only be done after previous acceptance of the author.

RESUMO

A superfamília Anostomoidea representa um grande grupo de peixes da ordem Characiformes, amplamente distribuído pelos ecossistemas de água-doce neotropicais e representa uma proporção significativa da biomassa em algumas regiões. Anostomoidea é reconhecidamente monofilética e formada por quatro famílias: Anostomidae, Chilodontidae, Curimatidae e Prochilodontidae. As relações filogenéticas neste grupo têm sido estudadas por diversos autores, mas as hipóteses apresentadas são ainda discordantes. Internamente, as hipóteses de relação próxima entre Anostomidae e Chilodontidae e entre Curimatidae e Prochilodontidae são suportadas por diversas sinapomorfias baseadas em caracteres morfológicos, porém não completamente corroboradas por estudos moleculares. A incongruência dos estudos prévios associada a uma pequena representatividade das espécies utilizadas nesses estudos evidencia a importância de um estudo filogenético aprofundado usando uma ampla amostragem de Anostomoidea. Neste contexto, o principal objetivo do presente projeto foi estudar as relações filogenéticas entre as famílias, os gêneros e as espécies de Anostomoidea com base em sequências de três loci nucleares e três mitocondriais. Nós também utilizamos, pela primeira vez em peixes neotropicais, novas metodologias filogenômicas usando elementos ultraconservados do genoma. Os resultados das análises de cerca de 1500 elementos ultraconservados em 69 táxons e das análises com seis loci usando 422 táxons (~70% das espécies) revelaram similarmente uma nova hipótese de relacionamento interfamiliar (Anostomidae (Prochilodontidae (Chilodontidae + Curimatidae))). Apresentamos também as filogenias multilocus para cada uma das quatro famílias discutindo as relações intergenéricas e interespecíficas e comparando-as com estudos morfológicos prévios. Muitas hipóteses de relação foram corroboradas e as discordâncias são extensivamente discutidas, incluindo novas propostas de classificação. Também discutimos os processos evolutivos que podem ter sido responsáveis pela diversificação, numa escala temporal determinada por árvores datadas com base no registro fóssil disponível. Nossos resultados deverão contribuir para o entendimento das relações evolutivas que ocorreram na radiação do grande número de espécies que compõe a superfamília Anostomoidea, assim como em outros grandes subgrupos de Characiformes.

Palavras-chave: Elementos ultraconservados, filogenias, filogenômica, peixes, sistemática.

ABSTRACT

The superfamily Anostomoidea represents one of the largest groups within the order Characiformes, widely distributed along Neotropical freshwater ecosystems, representing a significant biomass proportion in some regions. The Anostomoidea is recognizably monophyletic containing four families: Anostomidae, Chilodontidae, Curimatidae, and Prochilodontidae. Several authors have extensively studied the phylogenetic relationships within this group, albeit incongruences among the proposed hypotheses still exist. Several morphological synapomorphies support the close relationship between Anostomidae and Chilodontidae and this clade sister to Curimatidae and Prochilodontidae, whereas molecular phylogenies present distinct resolution. These incongruences associated to a low taxon sampling evidenced the necessity of an extensive phylogenetic study within the superfamily Anostomoidea using a broad taxon sampling. Herein, we aimed to suggest new hypotheses about the phylogenetic relationships among families, genera and species of Anostomoidea using sequences of three nuclear and three mitochondrial loci. We also used, for the first time in Neotropical fishes, modern phylogenomic methods that capture genomic-wide ultraconserved elements. Results from approximately 1500 ultraconserved elements spanning 69 taxa and from six genes with 422 taxa (~70% species) similarly agree with a novel interfamilial hypothesis (Anostomidae (Prochilodontidae (Chilodontidae + Curimatidae)). We also presented multilocus phylogenies for each of the four family and we discussed intergeneric and interspecific relationships comparing with previous morphological studies. Many hypotheses of relationships are corroborated and the discordances are extensively discussed, including new proposals of classification. We also discussed evolutionary processes that might explain the species diversification in a temporal scale determined by time-scaled trees using the available fossil record. Our results will contribute to understand the evolutionary relationships driving the radiation of the large number of species of the superfamily Anostomoidea as well as in other major subgroups of Characiformes.

Keywords: Phylogenies, phylogenomics, fishes, systematics, ultraconserved elements.

SUMÁRIO

PREFÁCIO	9
INTRODUÇÃO	12
Estudos filogenéticos em Characiformes	12
Superfamília Anostomoidea	16
Prochilodontidae + Curimatidae	19
Anostomidae + Chilodontidae	21
JUSTIFICATIVA	24
OBJETIVOS	25
MATERIAL E MÉTODOS, RESULTADOS E DISCUSSÃO	26
Phylogenomic analysis reveals multiple episodes of diversification and convergence within the radiation of South American anostomoid fishes (Ostariophysi: Characiformes)	28
Miocene radiations in the family Prochilodontidae (Ostariophysi: Characiformes) revealed by a time calibrated molecular phylogeny	52
The Neotropical fish family Curimatidae (Teleostei: Ostariophysi: Characiformes): classification and phylogenetic relationships based on molecular data	82
Molecular phylogenetics of the family Anostomidae (Teleostei: Characiformes)	116
LITERATURA CITADA	141
ANEXO 1: The first molecular phylogeny of Chilodontidae (Teleostei: Ostariophysi: Characiformes) reveals cryptic biodiversity and taxonomic uncertainty	157
ANEXO 2: New species of <i>Cyphocharax</i> (Characiformes: Curimatidae) from the upper rio Negro, Amazon basin	166
ANEXO 3: Produção Acadêmica e Científica no Doutorado: 2011–2015	173

PREFÁCIO

Estudos abordando a história evolutiva através de reconstrução filogenética das famílias de Characiformes foram, até o início do novo século, predominantemente baseados em caracteres morfológicos. Buscando contribuir para o conhecimento da evolução de grandes subgrupos de Characiformes, esta tese objetivou analisar as relações filogenéticas de quatro famílias (Anostomidae, Chilodontidae, Curimatidae e Prochilodontidae) que formam a superfamília Anostomoidea, com base em caracteres moleculares utilizando diferentes metodologias. Os resultados apresentados aqui são frutos de uma importante rede de colaboração científica que foi implementada para compreender os processos evolutivos responsáveis pela enorme radiação de espécies da superfamília Anostomoidea. Reitero que estes achados são cooperativamente divididos entre os membros deste grupo de pesquisa. Da mesma forma, esta tese é parte do trabalho intelectual de todos os membros desta equipe e de pesquisas constantes que, indubitavelmente, irão se manter ao longo dos próximos anos.

Inicialmente, apresento uma introdução geral com detalhes de todo o histórico de estudos realizados e cada hipótese levantada, utilizando diferentes metodologias, tais como uso de caracteres morfológicos e moleculares. Muitas destas hipóteses foram primariamente propostas em estudos de longa data por Richard Vari (USNM), que se dedicou imensamente em compreender as relações entre esses táxons. Apresento seguidamente a justificativa e os objetivos deste trabalho.

Em seguida, apresento quatro capítulos inéditos contendo suas metodologias específicas, os resultados encontrados com detalhadas discussões. No primeiro, apresento os resultados das primeiras análises utilizando filogenômica com elementos ultraconservados do genoma. Neste, que é o primeiro estudo filogenômico com peixes Neotropicais, utilizamos cerca de 1500 *loci* ortólogos, distribuídos ao longo do genoma de 69 táxons, e reconstruímos as relações entre as famílias e entre a maioria dos gêneros de Anostomoidea. A árvore final obtida foi datada, com base no registro fóssil, para identificar, temporalmente, os eventos que promoveram a diversificação na superfamília. Comparo também com a topologia final da filogenia multilocus com 422 táxons e 70% de cobertura das espécies. A filogenia de elementos ultraconservados é a principal hipótese molecular fortemente sustentada das relações interfamiliares que nosso grupo de pesquisa já obteve.

No segundo capítulo, apresento a filogenia molecular da família Prochilodontidae utilizando dados multilocus, com 90% de representatividade das espécies. Mostro também os resultados das análises de relógio molecular, datado com o registro fóssil, e os eventos que podem ter proporcionado a radiação das espécies da família.

No terceiro capítulo, apresento a primeira filogenia molecular dos peixes detritívoros da grande família Curimatidae, com 75% de cobertura das espécies. Neste capítulo, apresentamos novas hipóteses de relacionamento entre gêneros e espécies. Também sugerimos mudanças na classificação dos táxons, com propostas de sinonimização de um dos gêneros e de realocação de algumas espécies, com o objetivo de se reconhecer agrupamentos monofiléticos.

No quarto e último capítulo, apresentamos as relações entre representantes da família Anostomidae, a maior família entre as quatro de Anostomoidea, com um total de 62% de cobertura das espécies. Novos relacionamentos são propostos, assim como o reconhecimento de vários clados monofiléticos dentro da linhagem que contém o gênero *Leporinus*. Em síntese, apresentamos também as relações entre todos os táxons de Anostomoidea utilizando dados multilocus e discutimos brevemente as principais discrepâncias em relação aos resultados com elementos ultraconservados.

Ao final, incluí dois artigos já publicados. O Anexo 1 contém o trabalho das relações filogenéticas em Chilodontidae. Neste artigo, sustentamos vários agrupamentos previamente propostos mas descobrimos várias espécies crípticas dentro dos dois gêneros que compõem a família, através de análises multilocus. O Anexo 2 contém um artigo de descrição de uma nova espécie de *Cyphocharax* da bacia amazônica, fruto também das nossas pesquisas com taxonomia em Curimatidae. O Anexo 3 mostra a minha produção acadêmica e científica durante este doutorado. Assim, finalizamos essa contribuição para o entendimento das relações filogenéticas na superfamília Anostomoidea.

INTRODUÇÃO

Estudos filogenéticos em Characiformes

A fauna de peixes neotropicais de água-doce continentais é composta por mais de 7.000 espécies, sendo de longe a mais rica fauna de vertebrados continentais no planeta (Albert and Reis 2011a, 2011b, e referências citadas). A ictiofauna é dominada, tanto em termos de diversidade taxonômica quanto em biomassa, por peixes da superordem Ostariophysi, série Otophysi, que alcançam aproximadamente 73% das espécies descritas, divididas primariamente entre Siluriformes (~37% das espécies) e Characiformes (~33% das espécies) e, secundariamente, por Gymnotiformes (~3% das espécies); as espécies restantes dividem-se entre Cichlidae (~9%), Cyprinodontiformes (~12%), ambas da superordem Acanthopterygii, e ainda todo um conjunto (~7% do total de espécies) formado por vários grupos taxonômicos (Potamotrygonidae, Lepidosirenidae, Osteoglossidae, Engraulidae, Clupeidae, Synbranchidae, Sciaenidae, Gobiidae, Nandidae, Belonidae, Tetraodontidae, etc.).

A ordem Characiformes constitui um dos maiores e mais diversificados grupos de peixes de água-doce contendo atualmente cerca de 2.100 espécies válidas, alocadas em cerca de 270 gêneros e 23 famílias (Eschmeyer e Fong 2015). Possui distribuição ampla nas maiores drenagens da África subsaariana, América do Sul (exceto o sul da Argentina e Chile), América Central, México e o sul dos Estados Unidos. É representada por espécimes de diversos tamanhos, desde miniaturas (*sensu* Weitzman e Vari 1988) como algumas espécies de Crenuchidae, Cheirodontinae e Lebiasinidae até alguns de grande porte, atuando como importantes recursos para pesca (e.g. *Brycon*, *Hydrocinus*, *Leporinus*, *Piaractus*, *Prochilodus*, *Salminus*). A idade mínima das principais linhagens de peixes neotropicais (ordens Characiformes, Siluriformes e Gymnotiformes) data do período Cretáceo, entre 90 e 112 milhões de anos (Ma) (Lundberg *et al.* 1998). Estudos recentes, utilizando o registro fóssil com datação de relógios moleculares, corroboraram a origem de diversificação de Characiformes no Cretáceo há aproximadamente 108 Ma (Near *et al.* 2012; Arroyave *et al.* 2013).

Espécies pertencentes à ordem Characiformes formam um agrupamento natural, como proposto por Müller (1842) e reafirmado por Müller e Troschel (1845) e, um século depois, por Greenwood *et al.* (1966). Após o advento da metodologia cladística ou filogenética (Hennig

1950, 1966), a ordem Characiformes foi analisada por Fink e Fink (1981), que propuseram sua monofilia com base em sete sinapomorfias. No entanto, as relações entre ordens de Ostariophysi, assim como o estabelecimento da monofilia de cada uma delas, não estão completamente esclarecidas. Nakatani *et al.* (2011; Fig. 2) resumem as incongruências no relacionamento entre as ordens de Ostariophysi apresentadas por diferentes hipóteses (Fink e Fink 1981; Dimmick e Larson 1996; Ortí e Meyer 1996; Ortí e Meyer 1997; Saitoh *et al.* 2003; Peng *et al.* 2006; Lavoué *et al.* 2008; Li *et al.* 2008; Poulsen *et al.* 2009). Utilizando mitogenomas de mais de 100 espécimes de Otophysi, os autores também não encontraram suporte para a monofilia de Characiformes, sugerindo que a subordem Characoidei (Characiformes americanos) é mais relacionada à Siluriformes do que Citharinoidei (Characiformes africanos), tornando os Characiformes parafiléticos. Estudos futuros são necessários para testar a monofilia de Characiformes utilizando um maior número de caracteres em escala genômica.

Somente uma publicação até agora analisou as relações de alto nível entre os principais componentes de Characiformes com base em dados morfológicos (Buckup 1998). Este estudo analisou 80 caracteres de 27 grupos internos com representantes de todos os Characiformes conhecidos menos Cynodontidae, Gasteropelecidae e Serrasalminae (previamente reconhecidas como subfamílias de Characidae) e apenas sete gêneros de Characidae. Outros estudos morfológicos focaram em questões variando da relação entre algumas famílias a relações dentro de famílias ou entre seus componentes. Estes incluem os Alestidae (Zanata e Vari 2005), Anostomidae (Vari 1983; Sidlauskas e Vari 2008), Characidae (Reis 1989; Lucena e Menezes 1998; Malabarba 1998; Toledo-Piza 2000; Vari e Harold 2001; Malabarba e Weitzman 2003; Mirande 2009, 2010; Mattox e Toledo-Piza 2012), Chilodontidae (Vari 1983; Vari *et al.* 1995), Citharinidae (Vari 1979), Crenuchidae (Buckup 1998), Curimatidae (Vari 1983, 1989a), Distichodontidae (Vari 1979), Erythrinidae (Oyakawa e Mattox 2009; Mattox *et al.* 2014), Hemiodontidae (Langeani 1998), Prochilodontidae (Vari 1983; Castro e Vari 2004), dentre outros.

Métodos moleculares têm sido utilizados há vários anos para estudar as relações internas em Characiformes. Os primeiros estudos foram publicados por Ortí e Meyer (1997). Baseados em sequências parciais dos genes 12S e 16S rRNA (cerca de 880 pb), os autores procuraram investigar as relações dentro das ordens de Ostariophysi, em famílias em Characiformes e entre gêneros em Serrasalminae (=Serrasalminidae). Ainda que uma hipótese filogenética de

relacionamento entre os Characiformes seja apresentada, Ortí e Meyer (1997) notaram que as relações entre as famílias não puderam ser reconstruídas com segurança. Entre as hipóteses interessantes propostas nestes estudos estão a posição basal das famílias africanas Citharinidae e Distichodontidae, a hipótese de que a união de grupos africanos e neotropicais não constituem grupos naturais e a hipótese de que Serrasalminidae não é intimamente relacionada com Characidae.

Calcagnotto *et al.* (2005) apresentaram um estudo filogenético amplo com base na análise de dois genes mitocondriais e quatro genes nucleares em 124 táxons de Characiformes, incluindo 59 representantes de famílias africanas, embora não tenham incluído representantes das famílias Curimatidae e Gasteropelecidae. O grande número de táxons africanos analisados neste estudo permitiu corroborar a natureza monofilética das famílias Citharinidae, Distichodontidae, Alestidae e Hepsetidae (sendo a última a única monotípica) e novamente rejeitando o conceito de que grupos africanos e neotropicais formam unidades monofiléticas. Entretanto, o pequeno número de táxons neotropicais analisado, principalmente Characidae, não permitiu uma melhor resolução da hipótese apresentada.

Characidae, a maior família de peixes de água-doce Neotropical, ainda é a que apresenta os maiores problemas taxonômicos e sistemáticos. Utilizando dados morfológicos, Miranda (2009, 2010) suporta a monofilia da família com base em oito sinapomorfias, embora sete delas possuam homoplasias. Com dados moleculares, Javonillo *et al.* (2010) propuseram três unidades monofiléticas definidas então como clados A, B e C dentro de Characidae. Subsequentemente, Oliveira *et al.* (2011) apresentaram a primeira filogenia de Characiformes incluindo representantes de todas as famílias e uma grande amostragem de Characidae. Os autores utilizaram dois *loci* mitocondriais e três nucleares de 213 espécies representando todas as famílias então reconhecidas, e apresentaram uma nova hipótese de classificação dentro da ordem Characiformes (Fig. 1). Nesta hipótese, diversos grupos antes considerados como membros de Characidae são alocados em outros grupos monofiléticos e Characidae é redefinido como um grande grupo monofilético com quatro subclados monofiléticos.

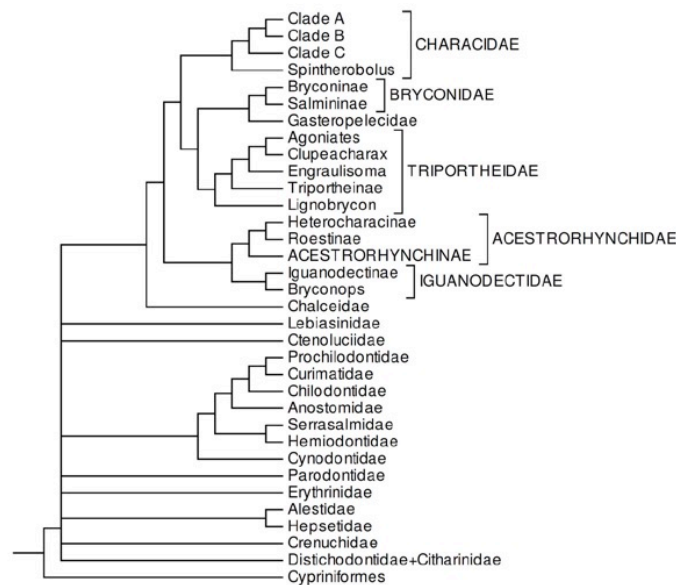


Figura 1. Relações filogenéticas em Characiformes com base em dados moleculares (Oliveira *et al.* 2011).

Filogenias moleculares recentes vêm sendo publicadas para diversos subgrupos de Characiformes. Dentro de Characidae, estudos com Aphyocharacinae (Tagliacollo *et al.* 2012) e Cheirodontinae (Mariguela *et al.* 2013b) permitiram uma melhor definição dessas subfamílias com propostas de organização de diversos gêneros, mas também apontaram para diversos possíveis problemas taxonômicos não suspeitados até o momento. A nível de gênero, Mariguela *et al.* (2013a) analisaram 29 espécies de *Moenkhausia* e identificaram cinco unidades monofiléticas que mostram o gênero como polifilético. Por outro lado, o emprego de dados moleculares tem-se mostrado muito útil, permitindo uma maior segurança na descrição de espécies (Benine *et al.* 2009; Melo *et al.* 2011; Silva *et al.* 2013) e até mesmo gêneros (Netto-Ferreira *et al.* 2013) em Characidae. Assim, estudos com maior número de gêneros e outros marcadores moleculares podem ajudar a elucidar as relações existentes dentro da ordem Characiformes e ainda permitir o reconhecimento de novos grupos taxonômicos.

Estudos extensivos em outras famílias de Characiformes também têm mostrado resultados relevantes. Arroyave e Stiassny (2011) estudaram a família africana Alestidae e não recuperaram sua monofilia pois o gênero *Chalceus* foi alocado dentro do grupo de Characiformes sul-americanos. Além disso, os autores não recuperaram a monofilia de vários dos seus gêneros. Subsequentemente, Arroyave *et al.* (2013) publicaram um trabalho contendo muitos representantes de Distichodontidae no qual corroboram a monofilia da família e de vários

gêneros, mas também suportam o parafiletismo de alguns gêneros como *Phago*, *Nannocharax*, e *Hemigrammocharax*, este recentemente sinonimizado em *Nannocharax* (Jerep e Vari 2014). A filogenia molecular de Gasteropelecidae (Abe *et al.* 2013) mostrou que vários grupos (gêneros e espécies) não são monofiléticos, havendo portanto a necessidade de uma revisão da família. Em Bryconidae (Abe *et al.* 2014), os resultados mostraram que o principal gênero, *Brycon*, não é monofilético, mas quatro grandes grupos foram identificados na família, sendo *Salminus* e *Brycon pesu* os únicos grupos monofiléticos. A filogenia molecular de Chilodontidae (Melo *et al.* 2014) corroborou a monofilia de cada um dos gêneros mas revelou também a presença de várias espécies crípticas e a necessidade de revisão taxonômica. Um estudo recentemente publicado em Serrasalminidae (Thompson *et al.* 2014) sugere que essa família tenha se originado no período Cretáceo, mas a diversificação das piranhas (o grupo mais numeroso) seria mais recente, tendo se originado durante o Eoceno.

Superfamília Anostomoidea

A relação próxima entre as famílias Anostomidae, Chilodontidae, Curimatidae e Prochilodontidae dentro da ordem Characiformes foi primeiramente estudada por autores pré-cladistas (Günther 1864; Boulenger 1904; Regan 1911; Eigenmann 1917; Gregory e Conrad 1938; Roberts 1973; Géry 1977) que, por muitas vezes incluíram outras famílias nas análises, como Parodontidae, Lebiasinidae e Hemiodontidae. Entretanto, foi num extenso estudo baseado principalmente em osteologia que Vari (1983) propôs o monofiletismo do grupo das quatro famílias, denominado superfamília Anostomoidea, sustentado por quatro sinapomorfias. O autor também propõe a relação ((Curimatidae + Prochilodontidae)(Anostomidae + Chilodontidae)) sustentadas por 11 e 15 sinapomorfias para o primeiro e o segundo clado, respectivamente (Fig. 2). Essas relações interfamiliares foram subsequentemente corroboradas por vários estudos morfológicos (Buckup 1998; Castro e Vari 2004; Moreira 2007; Sidlauskas 2008).

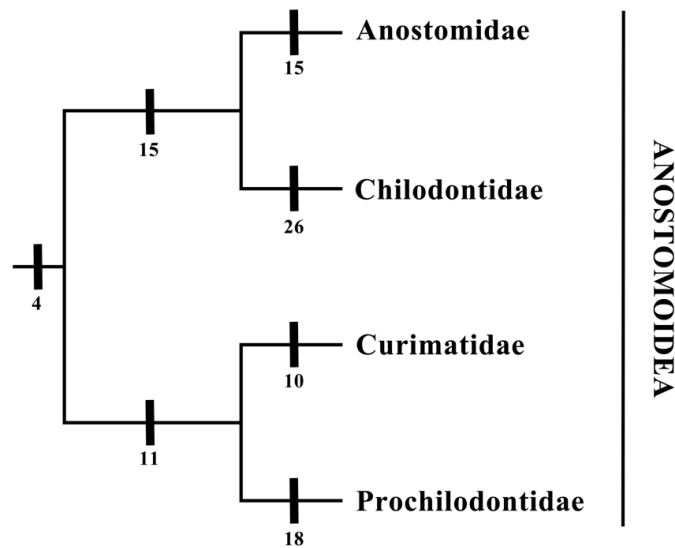


Figura 2. Relações filogenéticas entre as quatro famílias de Anostomoidea com dados morfológicos, modificada de Vari (1983). Números representam as respectivas sinapomorfias que sustentam cada clado.

A monofilia de Anostomoidea, proposta pelos trabalhos anteriores e sustentada por caracteres morfológicos foi, entretanto, rejeitada por Ortí e Meyer (1997) usando dados moleculares. O estudo corrobora, no entanto, o agrupamento formado por Prochilodontidae e Curimatidae proposto por Vari (1983), e este aparecendo como grupo-irmão de Hemiodontidae. A família Chilodontidae surpreendentemente agrupa-se com Crenuchidae, ambas relacionadas com Anostomidae. Assim, as relações em Anostomoidea aparecem de forma distinta das propostas anteriores. Embora os autores tenham incluído representantes das 18 famílias então reconhecidas de Characiformes (*sensu* Nelson 2006) e vários grupos de Characidae, os genes mitocondriais escolhidos (12S e 16S) não resolveram as relações devido, reconhecidamente, à saturação das mudanças nucleotídicas e/ou ao modo de diversificação evolutiva dos táxons, como discutido pelos próprios autores (Ortí e Meyer 1997: 91).

Diferentemente do estudo anterior, Calcagnotto *et al.* (2005) corroboraram Anostomoidea (*sensu* Vari 1983), porém os autores não usaram nenhum representante de Curimatidae, impossibilitando se testar a hipótese de relações próximas entre as quatro famílias. A topologia apontou discordância com os estudos anteriores, evidenciando Chilodontidae irmão de Prochilodontidae, e estes relacionados com Anostomidae. Outro resultado interessante refere-se ao grupo-irmão de Anostomoidea, composto por Parodontidae e Hemiodontidae, previamente

indicados como aparentados em estudos pré-cladistas (*e.g.* Boulenger 1904) e em estudos recentes (*e.g.* Moreira 2007).

Utilizando uma ampla amostragem dentro de Characiformes e Characidae, Oliveira *et al.* (2011) corroboram a monofilia de Anostomoidea (Fig. 1) com altos índices de suporte estatístico, sendo este agrupado num grande clado composto por Cynodontidae, Serrasalminae, Hemiodontidae e Parodontidae. O agrupamento formado por Prochilodontidae e Curimatidae dentro de Anostomoidea é corroborado. Entretanto, este trabalho não resolve completamente a posição de Chilodontidae, sendo esta alocada em diferentes clados nas diferentes análises (Bayesiana, máxima verossimilhança e máxima parcimônia). Os autores sugerem que a hipótese de relacionamento das famílias proposta por Vari (1983) seja testada com a inclusão de mais táxons.

Melo *et al.* (2014; Anexo 1) testaram as hipóteses de relacionamento na família Chilodontidae propostas por Vari *et al.* (1995) corroborando o monofiletismo da família e dos dois gêneros, mas também evidenciou a existência de diversas espécies crípticas além de uma necessidade de revisão taxonômica, principalmente em *Caenotropus labyrinthicus* e *Chilodus punctatus sensu lato*. Mesmo que os autores tenham utilizado um número relativamente baixo de representantes de outras famílias de Anostomoidea, as relações entre as quatro famílias novamente não foram reconstruídas como a proposta de Vari (1983). Pela primeira vez, a relação se apresentou como (Prochilodontidae (Chilodontidae + Curimatidae)) e Anostomidae como um clado não resolvido na base da filogenia. Os autores destacam, considerando esta hipótese molecular, duas hipóteses para explicar tal arranjo: todos os caracteres comuns a Anostomidae e Chilodontidae são convergentes, ou eles estiveram presentes no ancestral comum de Anostomoidea e foram subsequentemente perdidos durante a evolução de Curimatidae e Prochilodontidae.

Embora o trabalho de Ortí e Meyer (1997) não recupere a monofilia de Anostomoidea, todos os demais estudos moleculares corroboram este agrupamento. Assim, nos quatro trabalhos utilizando caracteres moleculares, as posições de cada uma das famílias de Anostomoidea ainda permanecem incertas e a hipótese de Vari (1983) ainda não foi satisfatoriamente refutada. Em suma, cinco diferentes padrões de relacionamento (Fig. 2 do Capítulo 1) entre as famílias de Anostomoidea foram propostas até o presente, através de estudos morfológicos realizados por Vari (1983) e corroborados por outros estudos morfológicos (*e.g.* Buckup 1998) e moleculares

realizados por Ortí e Meyer (1997), Calcagnotto *et al.* (2005), Oliveira *et al.* (2011) e Melo *et al.* (2014).

Prochilodontidae + Curimatidae

Estudos anteriores já haviam mostrado relação próxima entre essas duas famílias, mesmo no período pré-cladístico. Günther (1864) foi um dos primeiros a relacionar *Curimatus* (atuais Curimatidae) com *Prochilodus* num clado chamado “grupo Curimatina”, além de diversos outros rearranjos propostos por outros autores, envolvendo Parodontidae, Citharinidae e Hemiodontidae (ver revisão em Vari 1983). No entanto, análises baseadas em caracteres morfológicos realizadas por Vari (1983) apontaram Prochilodontidae como mais relacionada à Curimatidae sustentados por 11 sinapomorfias (Fig. 2). Este relacionamento é corroborado por Vari (1989a) que incluiu mais quatro novas sinapomorfias, e também por Ortí e Meyer (1997), Buckup (1998) e Oliveira *et al.* (2011). Alternativamente, Calcagnotto *et al.* (2005) diferem dos anteriores, indicando uma relação de Prochilodontidae com Chilodontidae por uma nítida razão: a ausência de Curimatidae. Melo *et al.* (2014, Anexo 1) encontraram uma nova reconstrução, com Curimatidae sendo irmão de Chilodontidae, e este clado irmão de Prochilodontidae com moderado suporte estatístico, embora os autores tenham apenas usado uma grande amostragem de Chilodontidae, foco principal do estudo.

Vari (1983) sustentou a monofilia de Prochilodontidae baseando-se em 18 sinapomorfias associadas principalmente aos arcos branquiais e hióide, suspensório e maxilas. Num extenso trabalho, Castro e Vari (2004) utilizaram 125 caracteres e corroboraram esta hipótese adicionando 40 outras sinapomorfias para a família. Estes autores delimitam a família em três gêneros monofiléticos com um total de 21 espécies, em que *Prochilodus* é considerado grupo-irmão do clado *Semaprochilodus* + *Ichthyoelephas* (Fig. 3). No entanto, as relações interespecíficas não foram completamente resolvidas em *Prochilodus* e *Semaprochilodus*.

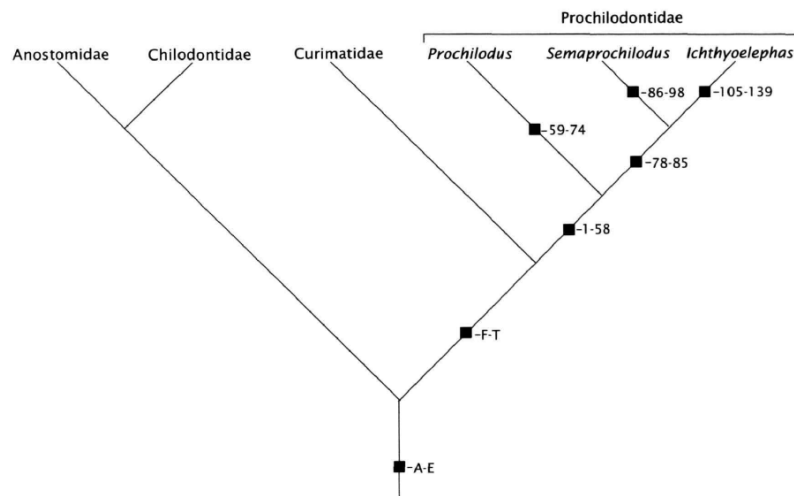


Figura 3. Relações intergenéricas em Prochilodontidae com dados morfológicos (Castro e Vari 2004).

Curimatidae é considerada monofilética e sustentada por dezenove sinapomorfias (Vari 1983, 1989a). As relações intra e intergenéricas são compreendidas devido a uma série de trabalhos realizados por Vari (1982a, 1983, 1984, 1989a, 1989b, 1989c, 1989d, 1991, 1992a, 1992b). Embora as relações mais basais tenham sido mais resolutive, os gêneros *Curimatella*, *Cyphocharax*, *Pseudocurimata* e *Steindachnerina* formam um clado não resolvido e sem monofiletismo estabelecido (Vari 1989a) (Fig. 4). A família possui, atualmente, 105 espécies válidas distribuídas em oito gêneros, e diversas espécies novas têm sido constantemente descritas (e.g. Vari *et al.* 2010, 2012; Netto-Ferreira e Vari 2011; Melo e Vari 2014, Anexo 2).

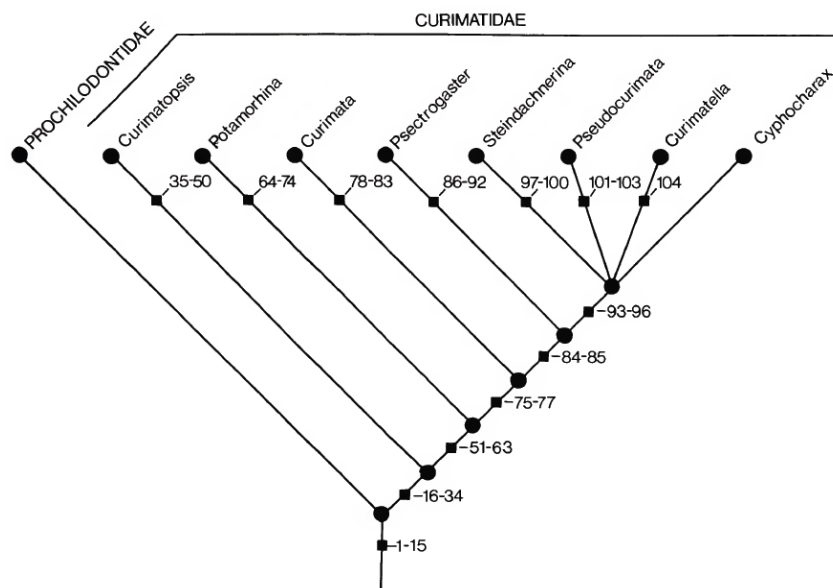


Fig. 4. Relações intergenéricas em Curimatidae com dados morfológicos (Vari 1989a).

Anostomidae + Chilodontidae

Vari (1983) sustentou a monofilia deste clado com 15 sinapomorfias associadas ao aparato branquial e aos sistemas internos do corpo (Fig. 2). Posteriormente, esta proposta foi corroborada por outros estudos morfológicos (Buckup 1998; Moreira 2007; Sidlauskas 2008), mas não por estudos moleculares, os quais indicam grupos diferentes como irmãos de Chilodontidae e de Anostomidae. Ortí e Meyer (1997), por exemplo, recuperaram a relação (Anostomidae (Chilodontidae + Crenuchidae)). Calcagnotto *et al.* (2005) encontraram a relação (Anostomidae (Chilodontidae + Prochilodontidae)) com baixo suporte estatístico. Oliveira *et al.* (2011) também não recuperaram a hipótese de Vari (1983) sendo que Anostomidae e Chilodontidae aparecem com posição indefinida na base do clado de Anostomoidea. Melo *et al.* (2014) novamente não recuperaram a relação de Anostomidae com Chilodontidae, sendo que esta última família é resolvida como irmã de Curimatidae. Embora esses estudos mostrem avanços no nosso conhecimento sistemático em Characiformes, muitos deles possuem similaridades nos caracteres moleculares, uma vez que pertencem aos mesmos *loci*, e também devido ao foco dos estudos não serem exatamente as famílias de Anostomoidea. Assim, a hipótese de Vari (1983) ainda não foi corroborada com caracteres moleculares.

Diversos estudos desde Günther (1864) com o ‘grupo Anostomina’ até Géry (1977) têm sido realizados com os representantes da atual família Anostomidae. No contexto cladista, Vari (1983) sustenta sua monofilia com base em 15 sinapomorfias, tendo Chilodontidae como grupo-irmão (Fig. 2). Internamente, Winterbottom (1980) estabelece a monofilia da subfamília ‘Anostominae’ que engloba *Anostomus*, *Gnathodolus*, *Pseudanos*, *Sartor* e *Synaptolaemus*, externamente distinta dos demais gêneros pela posição superior da boca, porém com poucas espécies da atual composição de toda a família. Revisões completas foram realizadas nos gêneros *Abramites* (Vari e Williams 1987) e *Laemolyta* (Mautari e Menezes 2006), enquanto que parciais vem sendo feitas em *Leporinus* (e.g. Garavello 1979; Britski *et al.* 2012; Birindelli e Britski 2013), *Pseudanos* (Sidlauskas e Santos 2010; Birindelli *et al.* 2012) e *Schizodon* (e.g. Bergmann 1988; Garavello e Britski 1990). Recentemente, Sidlauskas e Vari (2008) realizaram uma extensiva análise filogenética na família Anostomidae baseada em caracteres morfológicos, sustentando a monofilia de todos os gêneros da família, exceto *Leporinus* (Fig. 5). O estudo é bastante resolutivo nas relações entre os gêneros *Anostomoides*, *Rhytiodus*, *Schizodon*, *Laemolyta* e na posição de *Leporellus* como grupo-irmão de todos os demais membros da

família. Os autores descrevem *Petulanos* como um novo gênero alocado na subfamília Anostominae e ressuscitam o gênero *Hypomasticus*. No entanto, os resultados mostram *Abramites* e os demais gêneros (*Anostomoides*, *Laemolyta*, *Rhythiodus*, *Schizodon* e os membros de Anostominae) alocados dentro do clado contendo as espécies de *Leporinus*, evidenciando seu parafiletismo. Além disso, os autores amostraram poucas espécies de *Leporinus* (14 de ~90 válidas), evidenciando que mais estudos filogenéticos são necessários para entender as relações intergenéricas em Anostomidae. A família possui atualmente 14 gêneros (*sensu* Sidlauskas e Vari 2008) contendo cerca de 150 espécies válidas.

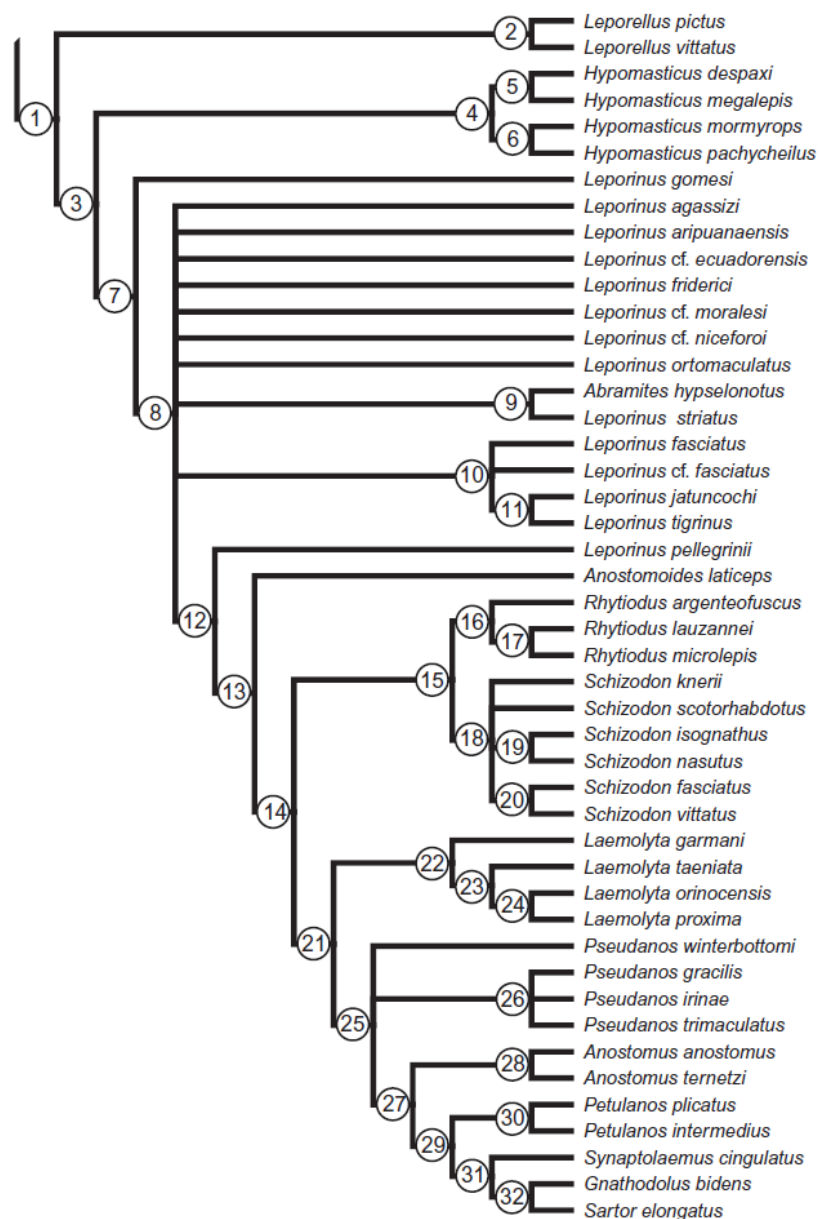


Fig. 5. Relações filogenéticas em Anostomidae baseada em caracteres morfológicos (Sidlauskas e Vari 2008).

Com dois gêneros (*Caenotropus* e *Chilodus*) e oito espécies, algumas de interesse para a aquariofilia, a família Chilodontidae é distribuída pelas bacias do Orinoco, Amazonas, rios independentes das Guianas e no rio Parnaíba. Com base em 26 sinapomorfias, Vari (1983) sustenta a monofilia de Chilodontidae (Fig. 2), sendo corroborada por Vari *et al.* (1995) que adicionam dez sinapomorfias para o grupo. Os autores ainda propõem *Chilodus* e *Caenotropus* como grupos monofiléticos sustentados por oito sinapomorfias cada (Fig. 6), esclarecem ainda as relações intragenéricas em *Caenotropus* e discutem a biogeografia de Chilodontidae com base nas suas relações filogenéticas. Isbrücker e Nijssen (1988) revisaram o gênero *Chilodus*, Vari e Ortega (1997) descreveram *Chilodus fritillus* e Scharcansky e Lucena (2007) descrevem *Caenotropus schizodon* e comentaram sobre as relações interespecíficas em *Caenotropus*. Melo *et al.* (2014, Anexo 1) apresentaram a primeira filogenia molecular de Chilodontidae e corroboraram a hipótese de monofiletismo de cada um dos gêneros (Vari *et al.* 1995), mas encontraram evidências que suportam a hipótese da presença de espécies crípticas dentro do clado *Chilodus punctatus sensu lato* e ainda a necessidade de revisões taxonômicas dentro da família.

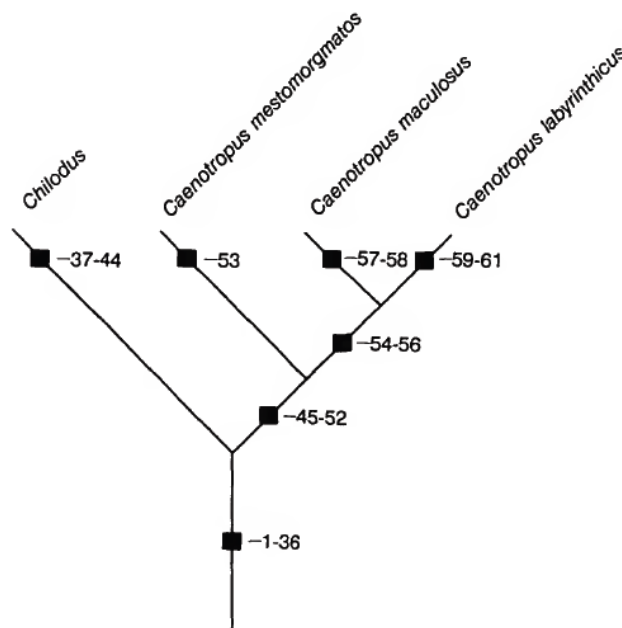


Figura 6. Relações filogenéticas em Chilodontidae com dados morfológicos (Vari *et al.* 1995).

JUSTIFICATIVA

A utilização de caracteres moleculares nas análises filogenéticas em peixes tem crescido consideravelmente nas últimas décadas, desde os primeiros trabalhos, como o de Bermingham e Avise (1986), até aqueles envolvendo peixes neotropicais (Chiachio *et al.* 2008; López-Fernández *et al.* 2010; Lundberg *et al.* 2011; Arce H *et al.* 2013; Roxo *et al.* 2014). Mais especificamente, diversos estudos vêm sendo publicados com diferentes famílias de Characiformes (Calcagnotto *et al.* 2005; Javonillo *et al.* 2010; Arroyave e Stiassny 2011; Oliveira *et al.* 2011; Tagliacollo *et al.* 2012; Abe *et al.* 2013, 2014; Arroyave *et al.* 2013; Mariguela *et al.* 2013a, 2013b; Thompson *et al.* 2014; Melo *et al.* 2014). Esses trabalhos têm contribuído significativamente para uma melhor compreensão das relações entre grupos complexos ainda não esclarecidos. Contudo, não existem estudos em sistemática molecular abordando as relações internas de nenhuma das famílias contidas em Anostomoidea, exceto o trabalho recentemente publicado da família Chilodontidae (Melo *et al.* 2014; Anexo 1).

Adicionalmente, as relações filogenéticas entre as famílias de Anostomoidea propostas por Vari (1983) ainda não foram testadas utilizando uma ampla amostragem, como já abordado nesta introdução. Os únicos estudos que estudaram as relações nos Anostomoidea foram aqueles realizados com grupos abrangentes, suprafamiliares (Ortí e Meyer 1997; Buckup 1998; Calcagnotto *et al.* 2005; Moreira 2007; Oliveira *et al.* 2011) ou intrafamiliares (Vari 1989a; Vari *et al.* 1995; Castro e Vari 2004; Sidlauskas e Vari 2008; Melo *et al.* 2014). Após o estudo de Vari (1983), nenhum outro objetivou testar as relações filogenéticas entre as famílias de Anostomoidea e de seus grupos relacionados. A reconstrução filogenética é fundamental para o entendimento das relações evolutivas entre as famílias que compõe Anostomoidea, e consequentemente entre os gêneros de cada uma delas. A incongruência entre os resultados propostos, associada a uma pequena representatividade da diversidade de espécies da superfamília nestes estudos, evidencia a importância de um estudo aprofundado utilizando uma grande amostragem. Nesse ponto, análises filogenéticas utilizando dados moleculares se tornam muito importantes para um melhor entendimento da diversidade e relações na superfamília Anostomoidea.

OBJETIVOS

Baseando-se nas hipóteses prévias de relacionamento entre membros da superfamília Anostomoidea, e desta com outras famílias, o presente estudo teve como objetivos:

- 1) Testar as hipóteses de relações filogenéticas entre as famílias de Anostomoidea com base em caracteres moleculares;
- 2) Testar as hipóteses de relações filogenéticas entre os gêneros e espécies de cada uma das famílias de Anostomoidea com base em caracteres moleculares;
- 3) Testar novas metodologias filogenômicas e novos métodos de análise filogenética e de calibração de relógio molecular na superfamília Anostomoidea;
- 4) Desenvolver novas filogenias intrafamiliares mais bem resolvidas para servir de base para futuros estudos taxonômicos e identificar clados problemáticos que requerem análises mais aprofundadas;
- 5) Gerar filogenias consistentes que poderão ser usadas em futuros estudos evolutivos.

MATERIAL E MÉTODOS, RESULTADOS E DISCUSSÃO

As sessões de Material e Métodos, Resultados e Discussão da presente tese estão contidos em quatro manuscritos, que seguem os padrões atuais de publicação científica. Os manuscritos estão organizados da seguinte forma:

- 1) Phylogenomic analysis reveals multiple episodes of diversification and convergence within the radiation of South American anostomoid fishes (Ostariophysi: Characiformes)
- 2) Miocene radiations in the family Prochilodontidae (Ostariophysi: Characiformes) revealed by a time-calibrated molecular phylogeny
- 3) The Neotropical fish family Curimatidae (Teleostei: Ostariophysi: Characiformes): classification and phylogenetic relationships based on molecular data
- 4) Molecular phylogenetics of the family Anostomidae (Teleostei: Characiformes)

Além deles, apresentamos em dois Anexos, o artigo publicado sobre a filogenia molecular de Chilodontidae, e o artigo de descrição de uma nova espécie de *Cyphocharax*, sendo parte dos resultados desta tese.

Capítulo 1

Phylogenomic analysis reveals multiple episodes of diversification and convergence within the radiation of South American anostomoid fishes (Ostariophysi: Characiformes)

Kendra Hoekzema¹, Bruno F. Melo², Michael E. Alfaro³, Michael D. Burns¹, Brant C. Faircloth^{3,4}, Benjamin W. Frable¹, Claudio Oliveira², Richard P. Vari⁵, and Brian L. Sidlauskas^{1,5*}

¹Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR, USA.

²Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, SP, Brazil.

³Department of Ecology and Evolutionary Biology, University of California Los Angeles, Los Angeles, CA, USA.

⁴Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA.

⁵Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington DC, USA.

*Corresponding author: B. L. Sidlauskas (brian.sidlauskas@oregonstate.edu)

Target journal: *Current Biology*

Running title: Phylogenomics of Anostomoid Fishes

Highlights:

- First phylogenomic study for Anostomoidea, a major clade of Neotropical fishes
- Novel ostariophysan probe set capable of capturing 1500 ultraconserved *loci*
- Remarkable morphological convergences in gill arches and oral jaws
- The ecomorphologically diverse Anostomidae may represent a rare riverine adaptive radiation.

Keywords: Headstanders, Anostomoidea, Neotropics, Convergent Evolution, Next-generation Sequencing, UCEs, Osteology, Tempo and Mode, Adaptive Radiation

Abstract

Background. Using a phylogenomic approach spanning approximately 1000 ultraconserved elements in a new ostariophysan-specific array, we present the first genus-level molecular phylogeny for the Neotropical fishes in superfamily Anostomoidea. This clade of approximately 300 species includes three primarily or exclusively detritivorous families, plus Anostomidae, an ecomorphologically diverse and species-rich family of omnivores, insectivores and herbivores. Together these four families are among the most important components of South American fish diversity.

Results. Phylogenomics confirm the monophyly of all four anostomoid families, and return a topology that is largely congruent with an earlier osteological dataset spanning nearly 500 characters. However, novel placements of family Chilodontidae and subfamily Anostominae imply two instances of remarkable morphological convergence, one related to gill arch morphology and the other involving repeated evolution of upturned oral jaws. Anostomidae also has a much younger crown group than does the detritivorous clade, implying unexpectedly swift evolution of species and morphologies within the family of greatest ecomorphological diversity.

Conclusions. Phylogenomics reveal several surprising convergences in this diverse clade of fishes, and contradict previous macroevolutionary hypotheses that assumed roughly equal ages of the two main subclades. Given its young age and quick genesis of morphological and species diversity, family Anostomidae may represent a rare example of a riverine adaptive radiation. Fully testing this hypothesis will require linking morphological and ecological data to a species-level phylogeny. Given the high support for relationships at deep and shallow nodes obtained in this study, UCE-based phylogenomics will provide an excellent method for generating such a densely-sampled phylogeny in the future.

Introduction

Systematic biology aims to reconstruct the evolutionary relationships among organisms in order to understand the genesis of biodiversity. Recent technological improvements have catalyzed remarkable progress in this field by permitting researchers to sequence genome-level data, harness increasingly powerful computers, and communicate effortlessly across the globe (Chen and Mayden 2010; Faircloth *et al.* 2012; Lemmon *et al.* 2012). With these new phylogenomic tools, systematics have forged new collaborations between biologists and informaticians to assemble unprecedentedly large and well-supported phylogenies for groups of organisms ranging from plants (Timme *et al.* 2012) to mammals (Meredith *et al.* 2011; McCormack *et al.* 2012). However, phylogenomic methods have not yet been applied to many clades, including some of the most prevalent evolutionary radiations in the hyperdiverse tropics. By targeting such understudied areas and organisms, we move towards a more complete understanding of the distribution of diversity across the global tree-of-life.

The approximately 300 fish species of superfamily Anostomoidea represent one of the most diverse, ubiquitous and important evolutionary radiations within the Neotropical ichthyofauna, which is the richest fish fauna in the world (Albert and Reis 2011). Their ranks include large migratory prochilodontid species that support commercial fisheries, the popular headstanders of the aquarium trade, the brightly colored and ecomorphologically diverse members of family Anostomidae, and numerous cryptic species of detritivorous curimatids (Fig. 1). Perhaps because of their exceptional ecological and morphological diversity, anostomoids have been frequently treated in systematic (Winterbottom 1980; Vari 1983, 1989a; Vari *et al.* 1995; Castro and Vari 2004; Sidlauskas and Vari 2008; Melo *et al.* 2014), taxonomic (Britski and Garavello 1993; Garavello 2000; Mautari and Menezes 2006; Birindelli *et al.* 2013), evolutionary (Sidlauskas 2007, 2008) and biogeographic (Vari 1988; Sidlauskas and Vari 2012) studies, and they rank among the most intensively-studied South American fishes.

Each of the four anostomoid families has a highly distinct head anatomy (Vari 1983), and the approximately 150 known species of Anostomidae have diversified into a wide range of mouth positions (Winterbottom 1980; Sidlauskas 2007), tooth configurations (Géry 1961, 1977; Sidlauskas and Vari 2008) and diets (Santos and Rosa 1988; Balassa *et al.* 2004), while species within the other three families vary much less in head shape and trophic position (Sidlauskas 2007, 2008).

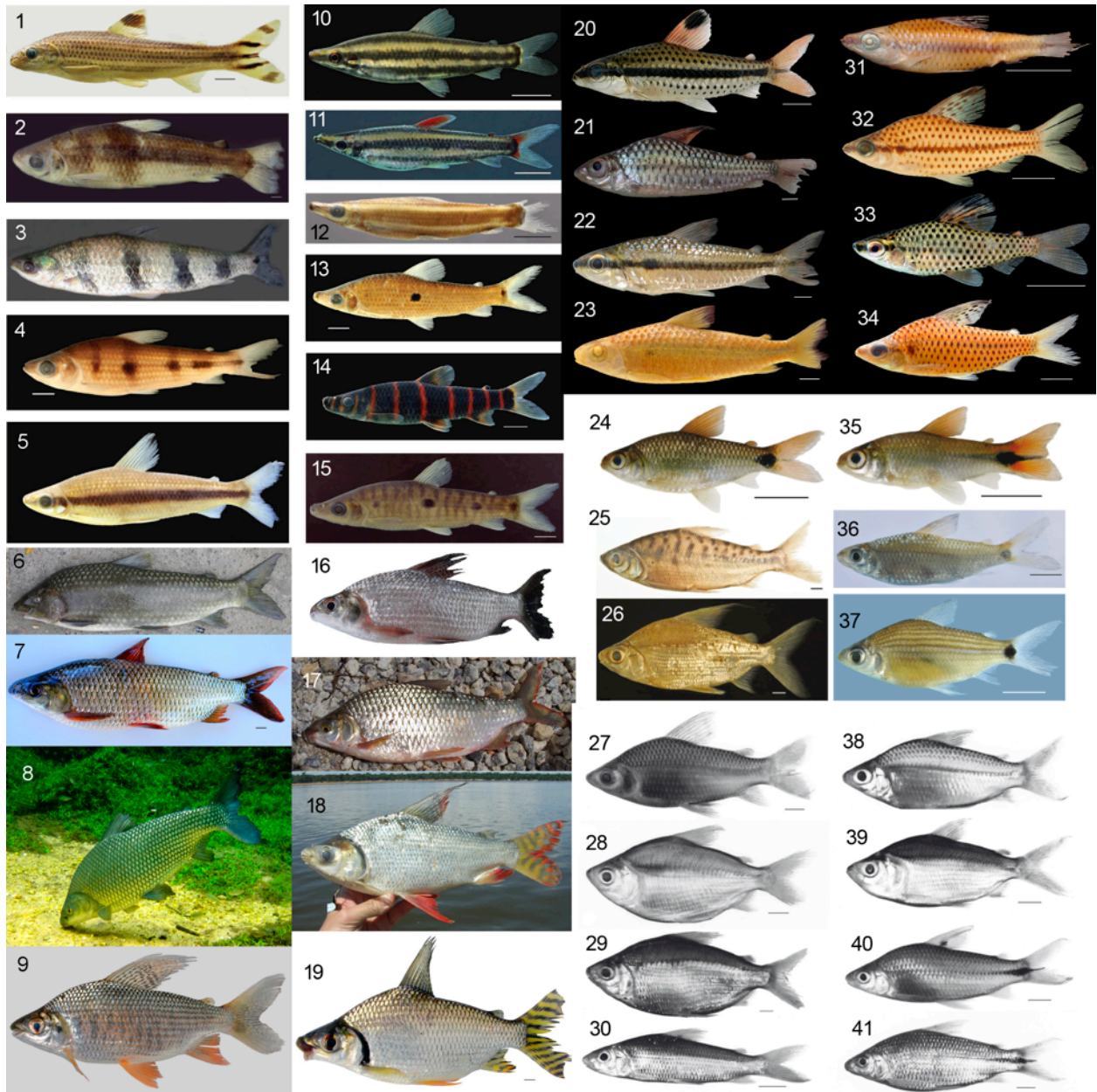


Figure 1. Diversity of anostomoid fishes. 1. *Leporellus vittatus*; 2. *Anostomoides laticeps*; 3. *Schizodon fasciatus*; 4. *Schizodon vittatus*; 5. *Laemolyta proxima*; 6. *Ichthyoelephas longirostris*; 7. *Prochilodus hartii*; 8. *Prochilodus lineatus*; 9. *Prochilodus nigricans*; 10. *Anostomus brevior*; 11. *Anostomus anostomus*; 12. *Anostomus ternetzi*; 13. *Pseudanos irinae*; 14. *Synaptolaemus latofasciatus*; 15. *Petulanos plicatus*; 16. *Prochilodus rubrotaeniatus*; 17. *Prochilodus vimboides*; 18. *Semaprochilodus laticeps*; 19. *Semaprochilodus varii*; 20. *Caenotropus maculosus*; 21. *Caenotropus mestomorgmatos*; 22. *Caenotropus labyrinthicus*; 23. *Caenotropus schizodon*; 24. *Curimatopsis crypticus*; 25. *Curimata vittata*; 26. *Curimata cyprinoides*; 27. *Curimatella alburna*; 28. *Psectrogaster ciliata*; 29. *Curimata roseni*; 30. *Steindachnerina planiventris*; 31. *Chilodus gracilis*; 32. *Chilodus punctatus*; 33. *Chilodus fritillus*; 34. *Chilodus zunevei*; 35. *Curimatopsis crypticus*; 36. *Cyphocharax spilurus*; 37. *Cyphocharax helleri*; 38. *Curimatella immaculata*; 39. *Psectrogaster essequibensis*; 40. *Steindachnerina guentheri*; 41. *Steindachnerina varii*. Scale bars indicate one centimeter. Photographs of Anostomidae (1-5; 10-15) and Curimatidae (24-30; 35-41) are from Sidlauskas and Vari (2012), and photographs of Chilodontidae (20-23; 31-34) are from Melo *et al.* (2014); photographs of Prochilodontidae (6-9; 16-19) will be published by Melo *et al.* (in prep., Cap. 2).

The skeletal and muscular diversity that characterize all four families has been described and analyzed in exceptional detail, and a family-level phylogeny originally inferred from 104 morphological characters (Vari 1983) and expanded to nearly 500 characters (Vari 1982, 1984, 1989a, 1989b, 1989c, 1989d, 1991; Vari *et al.* 1995; Castro and Vari 2004; Sidlauskas and Vari 2008) has stood as canon for nearly forty years. In turn, that phylogeny provided the conceptual framework for studies suggesting that the remarkable morphological diversity in a clade containing Anostomidae and Chilodontidae resulted from a lack of constraint and a greater tendency towards innovation, rather than a faster rate of change than in the two exclusively detritivorous families, Curimatidae and Prochilodontidae (Sidlauskas 2007, 2008).

Given the taxonomic richness, ecological diversity and evolutionary interest of these fishes, it is remarkable that no one has ever proposed a comprehensive molecular phylogeny for them. A few multilocus molecular studies at higher (Calcagnotto *et al.* 2005; Oliveira *et al.* 2011) or lower (Melo *et al.* 2014) taxonomic levels have included a handful of anostomoid taxa, and several of these have obtained novel relationships among the four anostomoid families by placing Chilodontidae, the smallest of the four, as sister to Prochilodontidae (Calcagnotto *et al.* 2005) or Curimatidae (Melo *et al.* 2014) or in a polytomy with those families (Oliveira *et al.* 2011), rather than as sister to Anostomidae, which is the preferred placement based on morphological (Roberts 1969; Vari 1983; Buckup 1998; Sidlauskas 2008; Sidlauskas and Vari 2008) and mitochondrial ribosomal data (Ortí and Meyer 1997) (Fig. 2). Such a novel placement would challenge our understanding of morphological evolution by implying numerous convergences or reversals in body systems ranging from the bones of the upper gill arches and the pharyngeal dentition to the intercostal ligaments connecting the ribs (Vari *et al.* 1995). However, because previous molecular studies (Ortí and Meyer 1997; Calcagnotto *et al.* 2005; Oliveira *et al.* 2011; Melo *et al.* 2014) have sampled extremely few taxa and employed no more than six *loci*, they cannot support a definitive revision of relationships. Formal tests of convergence and congruence between molecules and morphology demand the same rigor in genetic analysis that has already been achieved for morphology.

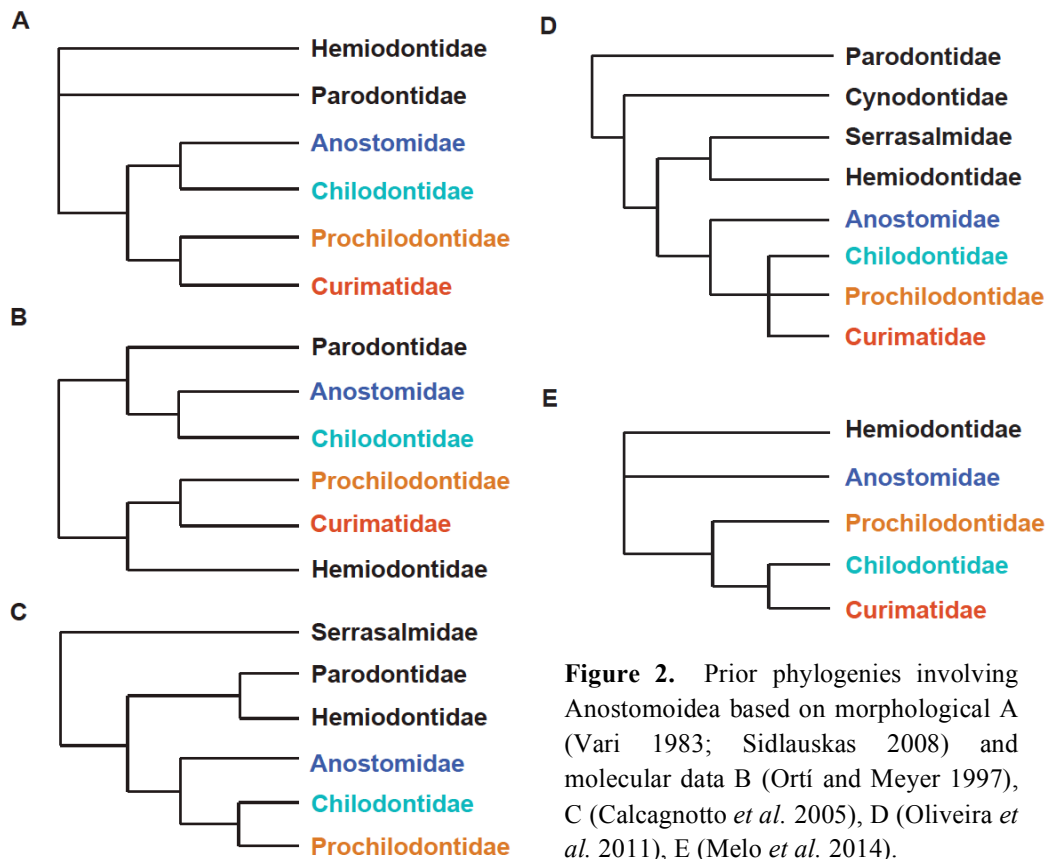


Figure 2. Prior phylogenies involving Anostomoidea based on morphological A (Vari 1983; Sidlauskas 2008) and molecular data B (Ortí and Meyer 1997), C (Calcagnotto *et al.* 2005), D (Oliveira *et al.* 2011), E (Melo *et al.* 2014).

Next-generation sequencing and phylogenomics allow for unprecedented resolution of relationships within many groups across the Tree of Life (Faircloth *et al.* 2012; Lemmon *et al.* 2012; Lemmon and Lemmon 2013). One such method uses ultraconserved elements (UCEs) (Bejerano *et al.* 2004; Stephen *et al.* 2008; Faircloth *et al.* 2012), which can be found across the genome and are, as the name implies, highly conserved across a broad taxonomic range (Stephen *et al.* 2008; Faircloth *et al.* 2012). These elements are flanked by variable nuclear regions, which can be enriched, sequenced, and analyzed using phylogenetic methods (Faircloth *et al.* 2012). Ultraconserved elements datasets dwarf traditional Sanger matrices, and have strongly supported phylogenetic reconstructions for insects (Faircloth *et al.* 2015), fishes (Faircloth *et al.* 2013), reptiles/birds (Crawford *et al.* 2012; McCormack *et al.* 2013; Smith *et al.* 2014), and mammals (McCormack *et al.* 2012), even for frequently debated or difficult questions such as the phylogenetic placement of turtles (Crawford *et al.* 2012), the relationship between parrots and passerines (McCormack *et al.* 2013) or the monophyly of holostean fishes (Faircloth *et al.* 2013).

Here, we apply the UCE approach to the superfamily Anostomoidea with a novel ostariophysan-specific set of sequence capture probes targeting more than 1000 UCE regions.

The phylogenomic perspective provided by these data permits the first rigorous molecular test of the morphological hypothesis of relationships of these fishes, and reveals the temporal context and progression of this fascinating evolutionary radiation. While confirming substantial congruence between the two datasets, the UCE data also provide strong support for several novel groupings of taxa, illustrate at least two remarkable instances of convergence, reveal an unexpectedly young crown radiation of the ecomorphologically diverse family Anostomidae, and confirm the power of this new approach to phylogenetics.

Methods

Taxon sampling

We sampled 69 ingroup species (43 specimens from Anostomidae, five from Chilodontidae, 27 from Curimatidae, and six from Prochilodontidae) and six representants of five characiform families (see Table S1 for museum catalog numbers). Taxa were chosen to achieve the widest possible coverage at the level of genera given the available tissues. Taxonomic identifications are based on physical or photographic examination of voucher specimens (Fig. S2).

Target enrichment and assembly

We extracted DNA using a DNeasy Tissue kit (Qiagen Inc.) following the instructions of the manufacturers or a modified NaCl extraction protocol (Lopera-Barrero *et al.* 2008). We prepared sequencing libraries from purified and sheared DNA using Illumina library preparation kits (Kapa Biosystems), following Faircloth *et al.* (2013). Libraries were PCR amplified (14 cycles) and brought to 147 ng/μL using a Speed-Vac before target enrichment. We pooled libraries to a total of 500ng of DNA per group and enriched the pooled libraries using a probe set designed for ostariophysian fishes ('MYBaits'; MYcroarray, Inc.). Identification of UCEs and design of the silico probes is detailed in Faircloth and Alfaro (in prep.). We quantified enriched, indexed libraries using qPCR (Kapa Biosystems), and we sequenced equimolar pools of enriched, indexed libraries using one lane of paired-end, 100 bp sequencing on an Illumina HiSeq 2000 in the Center for Genome Research and Biocomputing at Oregon State University.

After sequencing, we trimmed adapter contamination, low quality bases, and sequences containing ambiguous base calls using the Illumiprocessor workflow (available at

<https://github.com/faircloth-lab/illumiprocessor>), and assembled reads into contigs using Trinity (https://github.com/faircloth-lab/phylyuce/blob/master/bin/assembly/assembly_trinity.py).

Following assembly, we used PHYLUCE (<https://github.com/faircloth-lab/phylyuce>) and a custom Python program (`match_contigs_to_probes.py`) integrating LASTZ (Harris 2007) to align contigs to the set of probes/UCEs we used for enrichment while removing reciprocal and non-reciprocal duplicate hits from the data set. We used additional components of PHYLUCE to query the database, generate fasta files for the UCE *loci*, align contigs with MAFFT (Katoh *et al.* 2005) and trim contigs representing UCEs, in parallel, across the selected taxa prior to phylogenetic analysis following the procedure described in Faircloth *et al.* (2012).

Phylogenetic analysis

For Bayesian analysis, we used a custom script (`run_mraic.py`) wrapping a modified MrAIC 1.4.4 (Nylander 2004) to find the best-fitting, finite-sites substitution model for each UCE locus. We grouped *loci* having similar substitution models (selected by AICc) into the same partition, and we assigned the partition specific substitution model to all *loci* concatenated within each partition. For maximum likelihood analyses, we maintained the partitions identified in the Bayesian analysis and we modeled each partition using GTR+GAMMA. We performed Bayesian analysis of the concatenated data set using MrBayes 3.1 (Ronquist and Huelsenbeck 2003) and two independent runs (4 chains each) of 5,000,000 iterations each, sampling trees every 500 iterations, to yield a total of 10,000 trees. We sampled the last 5,000 trees after checking results for convergence by visualizing the log of posterior probability within and between the independent runs for each analysis, ensuring the average standard deviation of split frequencies was ≤ 0.001 , and ensuring the potential scale reduction factor for estimated parameters was approximately 1.0. We performed maximum likelihood analysis of the concatenated data in RAxML (Stamatakis 2006) using the standard bootstrapping algorithm and 500 bootstrap replicates. We time-calibrated the maximum likelihood phylogeny using a penalized likelihood approach (Sanderson 2002; Paradis 2013) as implemented in Chronos (Paradis 2013) in the R (R Development Core Team 2013) package ‘ape’ (Paradis *et al.* 2004). Four fossils provided calibrations (Supplementary material 3) and we chose a moderate value of lambda (R Development Core Team 2013).

Results

Following enrichment and sequencing, we obtained an average of 5,347,980 reads per species, which we assembled into an average of contigs having an average length of 622,376 bp (Table 1). After removing contigs that matched no UCEs and UCE *loci* that matched multiple contigs, we enriched unique contigs matching UCE *loci* from each species. This dataset was filtered to 75% completeness, which resulted in 1,077 UCE *loci*, totaling 469,960 bp with an average of 436 bp per contig; the complete dataset had only 68 UCE *loci* and 40,730 bp.

Table 1. Summary values describing the analysis of the UCE data.

	Incomplete dataset	75% complete dataset
Alignment summary		
[Alignment] number of <i>loci</i>	2302 <i>loci</i>	1077 <i>loci</i>
[Alignment] total length	774,792 bp	469,960 bp
[Alignment] mean bp per locus	336.57 bp	436.36 bp
[Alignment] min bp per locus	109 bp	147 bp
[Alignment] max bp per locus	1996 bp	1996 bp
Taxon summary		
[Taxa] mean	53.23 taxa	80.17 taxa
[Taxa] min	3 taxa	65 taxa
[Taxa] max	87 taxa	87 taxa
Missing data from trim summary		
[Missing data] mean	6.19%	5.31%
[Missing data] min	0%	0.09%
[Missing data] max	26.57%	15.38%
Character count summary		
[Characters] total number	47,467,378 bp	38,241,583 bp
[Nucleotides] total number	41,006,322 bp	32,739,348 bp
Data matrix completeness summary		
Matrix 50%	1437 alignments	1077 alignments
Matrix 55%	1370 alignments	1077 alignments
Matrix 60%	1298 alignments	1077 alignments
Matrix 65%	1235 alignments	1077 alignments
Matrix 70%	1164 alignments	1077 alignments
Matrix 75%	1077 alignments	1077 alignments
Matrix 80%	1013 alignments	1013 alignments
Matrix 85%	930 alignments	930 alignments
Matrix 90%	789 alignments	789 alignments
Matrix 95%	591 alignments	591 alignments

Maximum likelihood analysis produced a single, completely resolved topology (Fig. 3) with slight support differences for Bayesian analysis (Fig. S4). 75% complete trees are shown as Figs. S4–S5. Before focusing on the novel arrangements implied by the phylogenomic results, it is important to acknowledge that much of this molecular topology agrees with earlier hypotheses

based upon morphology (Fig. 4). These results confirm the monophyly of all four families and of all genera not previously suspected to be paraphyletic on the basis of morphological analysis (e.g. *Leporinus* and *Cyphocharax*), and provide unprecedented resolution within the latter two genera, both of which presented insoluble challenges in earlier morphological studies. Affinities of many genera are exactly as expected, including the close relationships between *Anostomoides*, *Laemolyta*, *Rhytidodus* and *Schizodon*, the presence of a clade containing *Curimatella*, *Cyphocharax* and *Steindachnerina*, the recovery of the subfamily Anostominae *sensu* Winterbottom (1980) (*Anostomus*, *Pseudanos* and *Synaptolaemus*), and the close affinity of *Leporinus striatus* and *Abramites hypselonotus*, which in turn allows a very precise time calibration using the Miocene fossil †*Leporinus scalabrinii*, which shares their morphological synapomorphies. Within the two largest families, the position of *Leporellus* and *Curimatopsis* as sister to the majority of other genera is upheld. Overall, far more congruence than discord exists between the molecular and morphological datasets (Fig. 4). Of the several disagreements, the most remarkable is the novel placement of the small family Chilodontidae within a clade also containing Curimatidae and Prochilodontidae. It is most likely sister to Curimatidae, but that grouping is subtended by an exceptionally small internode, and these three families essentially form a polytomy. Nevertheless, the eight chilodontid species have long been considered as sister to Anostomidae, and their placement herein implies convergence in either the substantial set of characters that have traditionally united those two families, or those that have united Curimatidae and Prochilodontidae.

The grouping of the anostomine genera (*Anostomus*, *Pseudanos* and *Synaptolaemus*) with *Leporellus*, and not *Laemolyta*, is similarly unexpected. With the exception of *Leporellus*, all of these genera possess strongly upturned jaws and highly multicuspidate premaxillary teeth, and were reconstructed in a single clade in prior morphological analysis (Sidlauskas and Vari 2008). Their wide separation herein implies that anostomids have evolved apparently equivalent morphologies at least twice, and that morphological divergence within the clade containing *Leporellus* is particularly noteworthy, given that *Leporellus* possesses a downturned mouth entirely unlikely that of the upturned anostomines. Other conflicting relationships include the placement of the trans-Andean clade *Pseudocurimata* with *Psectrogaster* instead of as sister to the clade containing *Cyphocharax*, *Curimatella* and *Steindachnerina*, and the placement of

Ichthyoelephas, a genus with immensely hypertrophied lips, as sister to all other members of Prochilodontidae, rather than as sister to *Semaprochilodus* alone.

Time calibration revealed unexpectedly major differences in the age of the crown clades of the two halves of the anostomoid radiation (Fig. 5). The three detritivorous families split from each other around 90 million years ago, while diversification within the living Anostomidae dates back only half that long. Given the roughly equal species richnesses of the two major subclades, Anostomidae must have experienced a much faster net rate of cladogenesis. The extremely short internodes reconstructed within the paraphyletic genus *Leporinus* further support the inference of accelerated speciation within that portion of the tree.

Discussion

The novel scenarios proposed by the molecular results involve an exceptional amount of osteological convergence and call earlier macroevolutionary conclusions into question. Yet, they appear with high statistical support for the 1,077 UCE *loci* analysis, and must be taken seriously. While provocative, these data are based upon only 23% species diversity within Anostomoidea. We propose to evaluate those hypotheses using much greater taxon sampling reaching 80% taxon coverage.

Is Anostomidae an Adaptive Radiation? Possibly. The extremely young age and apparently rapid speciation within the most ecomorphologically diverse clade suggests that evolutionary dynamics in this portion of the tree differ remarkably from those in the detritivorous families. A match between rate of speciation and ecomorphological diversification is one of several predictions of adaptive radiation. Examples of adaptive radiation are well known for cichlid fishes in the African Great Lakes (Kocher 2004; Seehausen 2006; Turner 2007; Wagner *et al.* 2012) and were predicted by both ecological opportunity and sexual selection (Wagner *et al.* 2012). But, it is not clear whether the long apparent delay before the onset of speciation in Anostomidae is real, or reflects the extinction of early branching species. Rates of morphological and ecological change have not yet been formally measured, and functional implications of the morphological shifts have not been quantified.

Do UCEs provide an effective phylogenomic approach? Yes. The phylogenetic analyses showed extremely high support for almost every node in the phylogeny at both deep and shallow levels. Ultraconserved *loci* have clear ability to reconstruct a tree that makes good biological

sense and largely agrees with a wealth of prior knowledge about morphology. As its prevalence and application grows, phylogenomics promises to systematists a new age of biodiversity discovery (Pennisi 2013).

Comparisons between UCE and multilocus topologies. We incorporated preliminary results of the multilocus analysis and present for the first time the multilocus phylogeny of 422 with 13 outgroup taxa of the superfamily Anostomoidea using six *loci* (Fig. 6). As noted, the resolution among families is exactly the same than the UCE topology (Figs. 3–5) with (Anostomidae (Prochilodontidae (Chilodontidae + Curimatidae). Major discrepancies are the support values in which the UCE methodology shows very well supported clades in all instances of the tree (basal and derived clades) whereas multilocus analyses present some poorly-supported clades mainly at the familial resolution (weak clades of Fig. 6). Overall, the UCE topology obviously reconstructs the most likely molecular phylogeny by having the most robust molecular dataset.

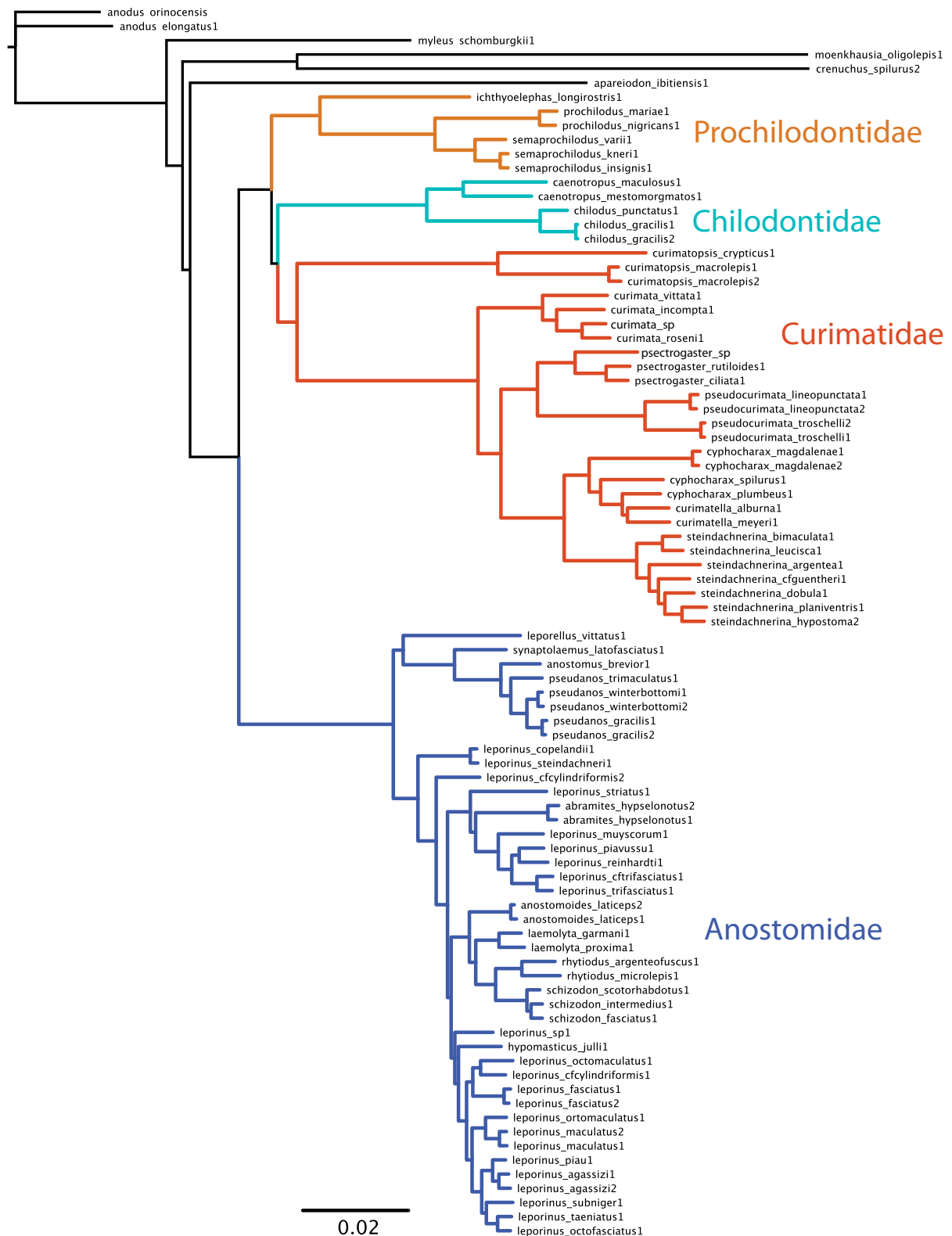


Figure 3. Phylogenetic relationships of Anostomoidea and outgroups based on a maximum likelihood analysis of 1,077 UCE loci.

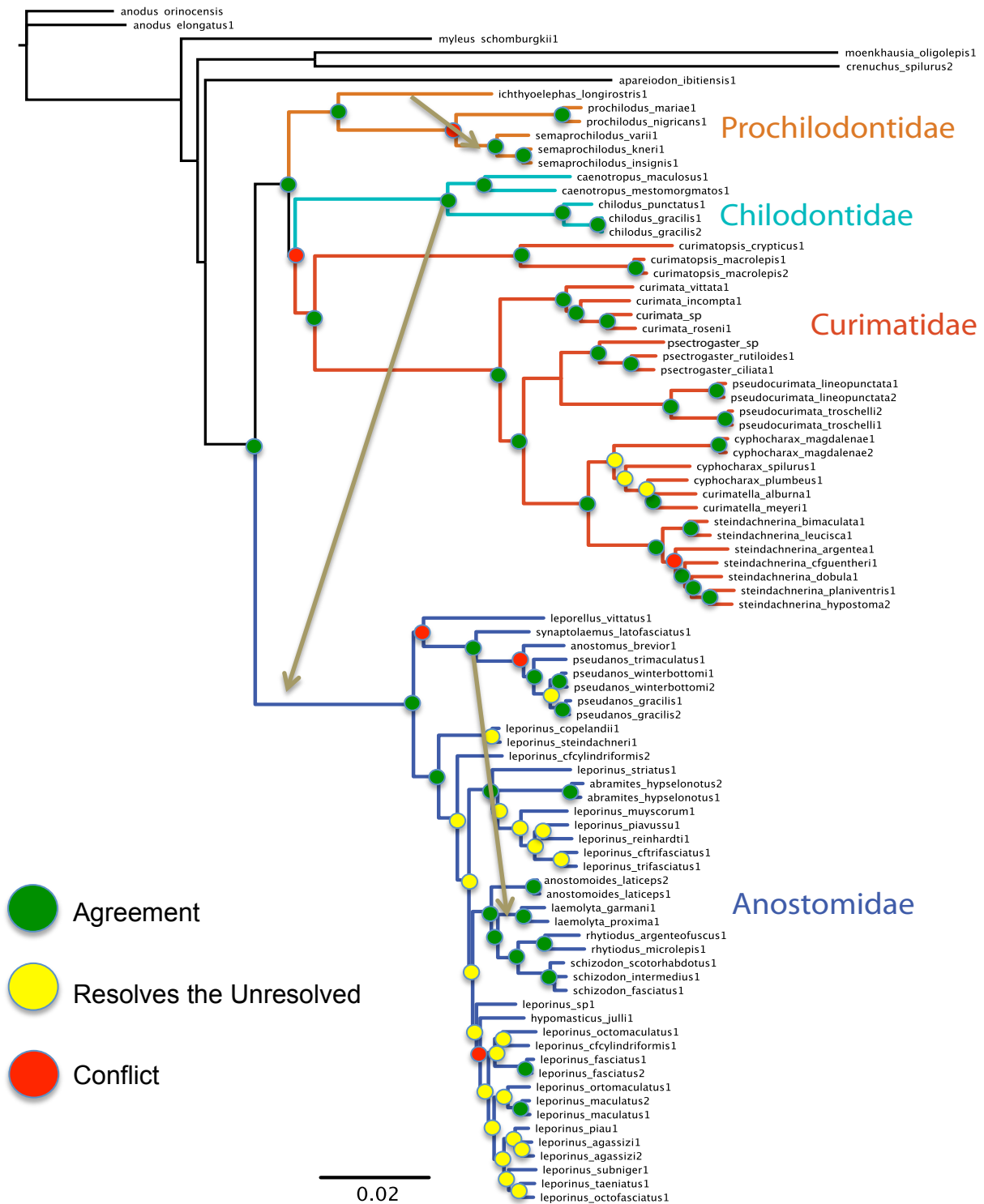


Figure 4. Bayesian phylogeny generated through ExaBayes evidencing clades in agreement or in conflict with prior morphological hypotheses (Vari 1983; Castro and Vari 2004; Sidlauskas and Vari 2008) and clades that first resolved the unresolved relationships.

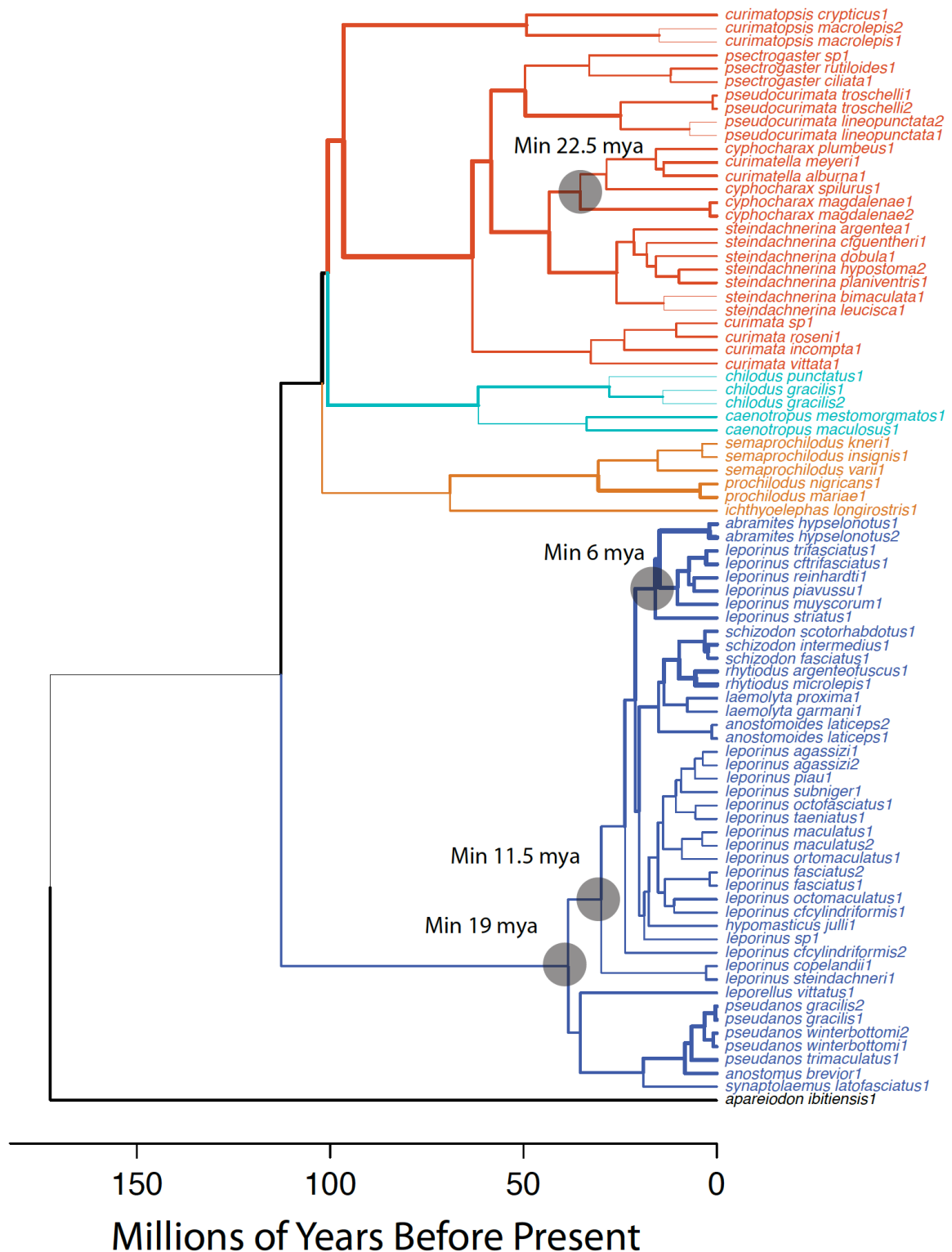


Figure 5. Time-calibrated phylogeny using four fossil calibrations (grey circles). Branch widths set proportional to inferred rate of molecular evolution. Branches of the four anostomoid families are colored as in previous figures. Numbers after names designate duplicated specimens.

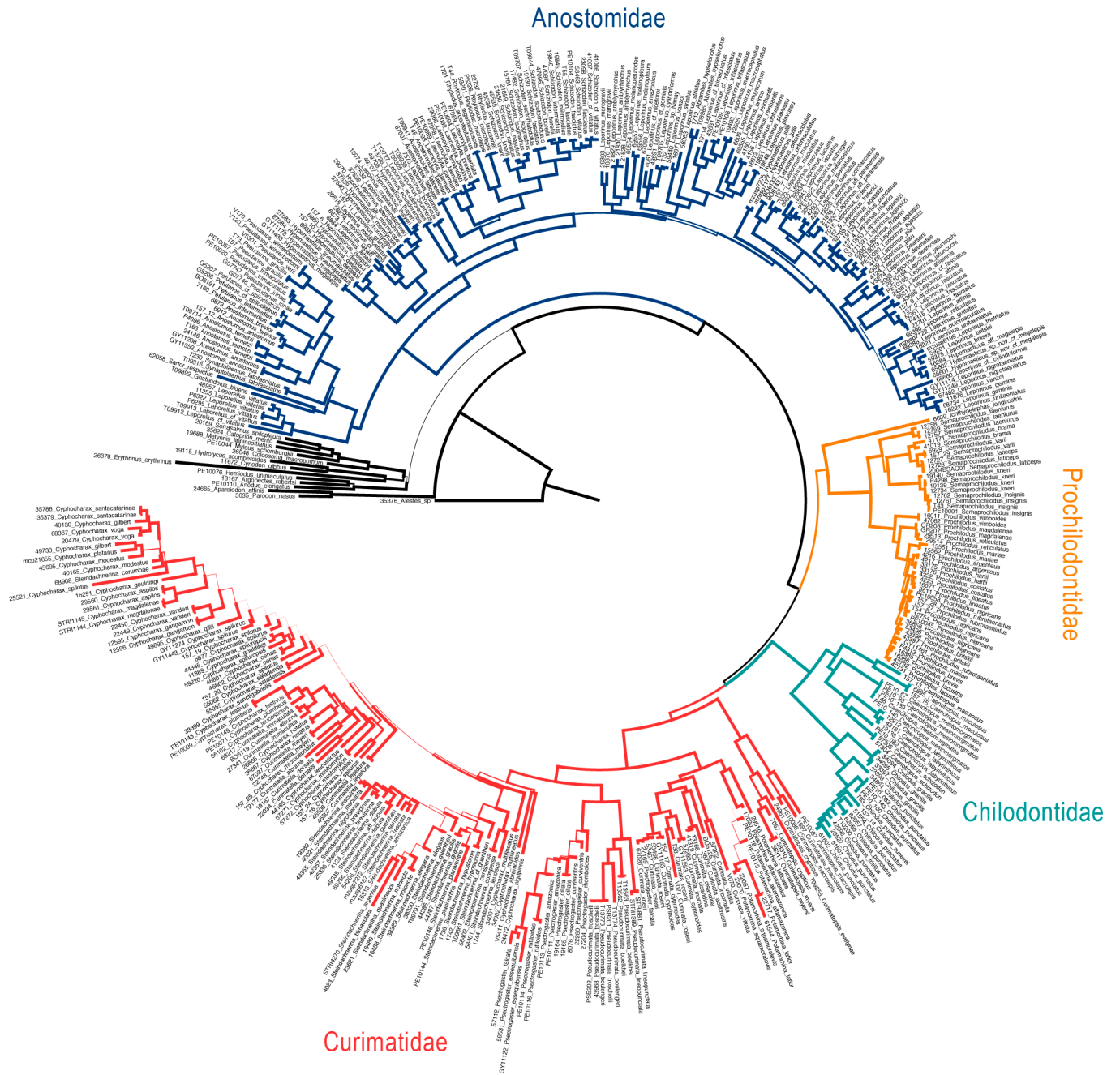


Figure 6. Best maximum likelihood tree including all analyzed taxa of Anostomoidea using a multilocus dataset showing the resolution (Anostomidae (Prochilodontidae (Chilodontidae + Curimatidae)). Branches of the four anostomoid families are colored as in previous figures. Branch weight is determined by bootstrap support.

Author Contributions

BLS, RPV, CO and MEA obtained funding and designed the project. BLS, RPV and CO facilitated the international exchange of samples. KH, BFM and MWB collected data. BCF and MEA wrote custom scripts and advised the phylogenomic analysis. KH, BFM, MEA, BCF, MWB and BLS performed analyses. BLS and RPV interpreted the results in the context of morphology. BLS and KH wrote most of the manuscript. BFM and RPV contributed sections of writing. BCF, BLS, BFM and KH prepared the figures. All authors edited and revised the manuscript.

Acknowledgements

We thank M. Sabaj-Perez (ANSP), N. Lujan and J. Armbruster (AUM), W. Aguirre (DePaul University), S. Mochel and K. Swagel (FMNH), M. de Pinna, O. Oyakawa, J. Birindelli and M. Gianeti (MZUSP) and R. Reina (STRI) for curatorial assistance and/or loan of tissues and vouchers. We especially thank C. Shimabukuro-Dias and A. Alves for helping with the preparation and shipment of tissues at LBP. Mark Dasenko and the CGRB CoreLabs at Oregon State provided assistance with the Illumina sequencing platform. NSF (DEB-1257898) and FAPESP (proc. 2011/08374-1 and proc. BEPE 2013/16436-2) funded this project.

Table S1. Voucher information for fish species used in this study. Collection acronyms follow Sabaj Pérez (2013).

Family	Species	Voucher	Tissue number
Anostomidae	<i>Abramites hypselonotus</i>	ANSP 178126	1712
	<i>Abramites hypselonotus</i>	AUM 53775	T08985
	<i>Anostomoides laticeps</i>	AUM 53813	T09941
	<i>Anostomoides laticeps</i>	FMNH 123875	T45
	<i>Anostomus brevior</i>	FMNH ex ANSP 189141	6879
	<i>Hypomasticus julii</i>	MZUSP 97206	7275
	<i>Laemolyta garmani</i>	OS 18777	PE10-089
	<i>Laemolyta proxima</i>	OS 18778	PE10-094
	<i>Leporellus vittatus</i>	ANSP 182609	P6322
	<i>Leporinus agassizi</i>	OS 18369	PE10-073
	<i>Leporinus agassizi</i>	OS 18775	PE10-090
	<i>Leporinus</i> cf. <i>arimaspi</i>	ANSP 191183	BO6133
	<i>Leporinus brunneus</i>	AUM 54394	T09305
	<i>Leporinus</i> cf. <i>trifasciatus</i>	LBP 12722	43631
	<i>Leporinus copelandii</i>	LBP 6449	29075
	<i>Leporinus fasciatus</i>	ANSP 180321	NS61
	<i>Leporinus fasciatus</i>	AUM 43300	P4315
	<i>Leporinus maculatus</i>	FMNH Ex ANSP 189041	7035
	<i>Leporinus maculatus</i>	ANSP 189041	6982
	<i>Leporinus muyscorum</i>	ANSP 192864	6606
	<i>Leporinus octofasciatus</i>	LBP 3939	19827
	<i>Leporinus octomaculatus</i>	LBP 13249	69389
	<i>Leporinus ortomaculatus</i>	ANSP 191182	BO6132
	<i>Leporinus piau</i>	LBP 340	4309
	<i>Leporinus piavussu</i>	LBP 3304	19848
	<i>Leporinus reinhardti</i>	LBP 259	4156
	<i>Leporinus</i> sp. “Nanay”	ANSP 180317	T32
	<i>Leporinus steindachneri</i>	LBP 8098	37533
	<i>Leporinus striatus</i>	LBP 3180	16871
	<i>Leporinus subniger</i>	OS 18726	PE10-022
	<i>Leporinus taeniatus</i>	LBP 329	4267
	<i>Leporinus trifasciatus</i>	OS 18311	PE10-108
	<i>Pseudanos gracilis</i>	FMNH113530	T57
	<i>Pseudanos gracilis</i>	ANSP 180320	T73
	<i>Pseudanos trimaculatus</i>	OS 18779	PE10-021
	<i>Pseudanos winterbottomi</i>	AUM 39855	V120
	<i>Pseudanos winterbottomi</i>	AUM 39292	V170
	<i>Rhytidodus argenteofuscus</i>	ANSP 180242	T44
	<i>Rhytidodus microlepis</i>	ANSP 182603	P6326
	<i>Schizodon fasciatus</i>	OS 18310	PE10-104
	<i>Schizodon intermedius</i>	LBP 3305	19845
	<i>Schizodon scotorhabdotus</i>	AUM 53654	T09707
	<i>Synaptolaemus latofasciatus</i>	MZUSP 97460	mz7230
Chilodontidae	<i>Caenotropus maculosus</i>	ANSP 189159	6999
	<i>Caenotropus mestomorgmatos</i>	OS 18772	PE10-093
	<i>Chilodus gracilis</i>	LBP 6962	33397
	<i>Chilodus gracilis</i>	LBP 6962	33398
Curimatidae	<i>Chilodus punctatus</i>	OS 18781	PE10-100
	<i>Curimata acutirostris</i>	LBP 5761	28139
	<i>Curimata incompta</i>	ANSP 191354	BO6125
	<i>Curimata roseni</i>	ANSP 180389	T38
	<i>Curimata roseni</i>	LBP 12864	53467

	<i>Curimata vittata</i>	LBP 13846	57302
	<i>Curimatella alburna</i>	LBP 5431	57171
	<i>Curimatella meyeri</i>	LBP 4253	22748
	<i>Curimatopsis crypticus</i>	ANSP 189091	7057
	<i>Curimatopsis macrolepis</i>	OS 18337	PE10-086
	<i>Curimatopsis macrolepis</i>	OS 18337	PE10-088
	<i>Cyphocharax magdalenae</i>		STRI-1001
	<i>Cyphocharax magdalenae</i>	BFD01631	STRI-1145
	<i>Cyphocharax plumbeus</i>	OS 18373	PE10-071
	<i>Cyphocharax spilurus</i>	ANSP 189157	6877
	<i>Psectrogaster amazonica</i>	OS 18313	PE10-111
	<i>Psectrogaster ciliata</i>	ANSP 191145	BO6120
	<i>Psectrogaster rutiloides</i>	ANSP178284	1576
	<i>Pseudocurimata lineopunctata</i>	BFD01628	STRI-1389
	<i>Pseudocurimata lineopunctata</i>	BFD01630	STRI-881
	<i>Pseudocurimata troschelli</i>	Univ Guayaquil 1542	PSB-201
	<i>Pseudocurimata troschelli</i>	Univ Guayaquil 1542	PSB-202
	<i>Steindachnerina argentea</i>	BFD01625	STRI-4270
	<i>Steindachnerina bimaculata</i>	LBP 173	4023
	<i>Steindachnerina guentheri</i>	AUM 54441	T09661
	<i>Steindachnerina dobula</i>	ANSP 180798	4123
	<i>Steindachnerina hypostoma</i>	ANSP 178124	1742
	<i>Steindachnerina hypostoma</i>	ANSP 178124	1736
	<i>Steindachnerina leucisca</i>	ANSP178125	1744
	<i>Steindachnerina planiventris</i>	OS 18324	PE10-150
Prochilodontidae	<i>Ichthyoelephas longirostris</i>	ANSP 192865	6609
	<i>Prochilodus mariae</i>	ANSP 40692	P4313
	<i>Prochilodus nigricans</i>	OS 18792	PE10-045
	<i>Semaprochilodus insignis</i>	ANSP 180205	T43
	<i>Semaprochilodus kneri</i>	ANSP 187277	P4298
	<i>Semaprochilodus varii</i>	ANSP 187435	6929
Serrasalminidae	<i>Myleus schomburgkii</i>	OS 18990	PE10-044
Characidae	<i>Moenkhausia oligolepis</i>	OS 18747	PE10-162
Hemiodontidae	<i>Anodus elongatus</i>	LBP 4244	22720
	<i>Anodus orinocensis</i>	LBP 2210	15614
Parodontidae	<i>Apareiodon ibitiensis</i>	LBP 2890	18635
Crenuchidae	<i>Crenuchus spilurus</i>	LBP 10622	24012



Supp. material 2. Representatives of the voucher specimens analyzed in this study: 1. *Moenkhausia oligolepis*, OS 18747, Peru, Rio Nanay, Amazon basin; 2. *Myiurus schomburgkii*, OS uncatalogued, Peru, Rio Nanay, Amazon basin; 3. *Anodus elongatus*, LBP 4244, Brazil, Rio Jurua, Amazon basin; 4. *Apareiodon ibitiensis*, LBP 2890, Brazil, Rio Grande, upper Parana basin; 5. *Laemolyta garmani*, OS 18777, Peru, Rio Nanay, Amazon basin; 6. *Laemolyta proxima*, OS 18778, Peru, Rio Nanay, Amazon basin; 7. *Leporinus agassizi*, OS 18369, Peru, Rio Nanay, Amazon basin; 8. *Leporinus agassizi*, OS 18775, Peru, Rio Nanay, Amazon basin; 9. *Leporinus copelandii*, LBP 6449, Brazil, Rio Paraiiba do Sul; 10. *Leporinus octomaculatus*, LBP 13249, Brazil, Rio Tapajós, Amazon basin; 11. *Leporinus pianu*, LBP 340, Brazil, Rio São Francisco; 12. *Leporinus reinhardti*, LBP 259, Brazil, Rio São Francisco; 13. *Leporinus subniger*, OS 18726, Peru, Rio Nanay, Amazon basin; 14. *Leporinus steindachneri*, LBP 8098, Brazil, Rio Mucuri; 15. *Leporinus striatus*, LBP 3180, Brazil, Rio Parapanema, upper Parana basin; 16. *Pseudanos trimaculatus*, OS 18779, Peru, Rio Nanay, Amazon basin; 17. *Leporinus octofasciatus*, LBP 3939, Brazil, Rio Parapanema, upper Parana basin; 18. *Leporinus cf. trifasciatus*, LBP 12722, Brazil, Rio Araguaia, Amazon basin; 19. *Leporinus trifasciatus*, OS 18311, Peru, Iquitos market, Amazon basin; 20. *Schizodon intermedius*, LBP 3305, Brazil, Rio Tietê, upper Parana basin; 21. *Schizodon fasciatus*, OS 18310, Peru, Iquitos market, Amazon basin; 22. *Caenotropus mesomorphnatus*, OS 18772, Peru, Rio Nanay, Amazon basin; 23. *Chilodus gracilis*, LBP 6962, Brazil, Rio Negro, Amazon basin; 24. *Chilodus gracilis*, LBP 6962, Brazil, Rio Negro, Amazon basin; 25. *Chilodus punctatus*, OS 18781, Peru, Rio Nanay, Amazon basin; 26. *Curimata acutirostris*, LBP 5761, Brazil, Rio Araguaia, Amazon basin; 27. *Curimata kneri*, LBP 12864, Brazil, Rio Tapajós, Amazon basin; 28. *Curimata vittata*, LBP 13846, Brazil, Rio Tapajós, Amazon basin; 29. *Curimatopsis macrolepis*, OS 18337, Peru, Rio Nanay, Amazon basin; 30. *Curimatopsis macrolepis*, OS 18767, Peru, Rio Nanay, Amazon basin; 31. *Curimatella meyeri*, LBP 4253, Brazil, Rio Jurua, Amazon basin; 32. *Steindachnerina bimaculata*, LBP 173, Brazil, Rio Purus, Amazon basin; 33. *Cyphocharax plumbeus*, OS 18373, Peru, Rio Nanay, Amazon basin; 34. *Psectrogaster amazonica*, OS 18313, Peru, Iquitos market, Amazon basin; 35. *Steindachnerina planiventris*, OS 18324, Peru, Iquitos market, Amazon basin; 36. *Prochilodus nigricans*, OS 18792, Peru, Rio Ilaya, Amazon basin.

Supplementary material 3

Fossil calibrations used in chronogram construction

†*Cyphocharax mosesi*, UNG-TA 854. Articulated complete skeleton. Late Oligocene or very early Miocene of the Taubaté basin, Tremembé formation, São Paulo, Brazil. Minimally 22.5 mya (Travassos and Santos 1955; Malabarba 1996; Reis 1998) used to calibrate minimum age of node containing all living *Cyphocharax* species. †*Plesiocurimata alvarengai* (Pz.DBAV.UERJ.405-A) from the same formation (Figueiredo and Costa-Carvalho 1999) is a member of Curimatidae, but its possession of teeth at adult stages of ontogeny (which no living member of the family does) suggests that it falls outside of the crown group. Thus, †*Plesiocurimata alvarengai* provides no additional information for the purposes of time-calibration.

Leporinus sp. Jaw teeth and pharyngeal teeth (Roberts 1975) remains from Cuenca basin of Ecuador at 19 million years ago. Most likely a *Leporinus* but possibly representative of *Leporellus* (Roberts 1975).

Leporinus sp. IGM 253295. Symphyseal premaxillary tooth from La Venta formation of Colombia (Lundberg 1997), between 13.5 and 11.5 mya (Guerrero 1997). Used to set the minimum age of the clade containing all living species of *Leporinus*.

†*Leporinus scalabrinii*. MACN A-9880. Incomplete articulated skull. Late Miocene deposits of Entre Ríos, Argentina between 6 and 9 mya (Bogan *et al.* 2012). Several osteological synapomorphies support the placement of this fossil within a clade containing *Leporinus striatus* and *Abramites hypselonotus* (Bogan *et al.* 2012). These two species appear in close proximity in the UCE phylogeny, and the fossil was used to set a minimum age for the node representing their common ancestor.

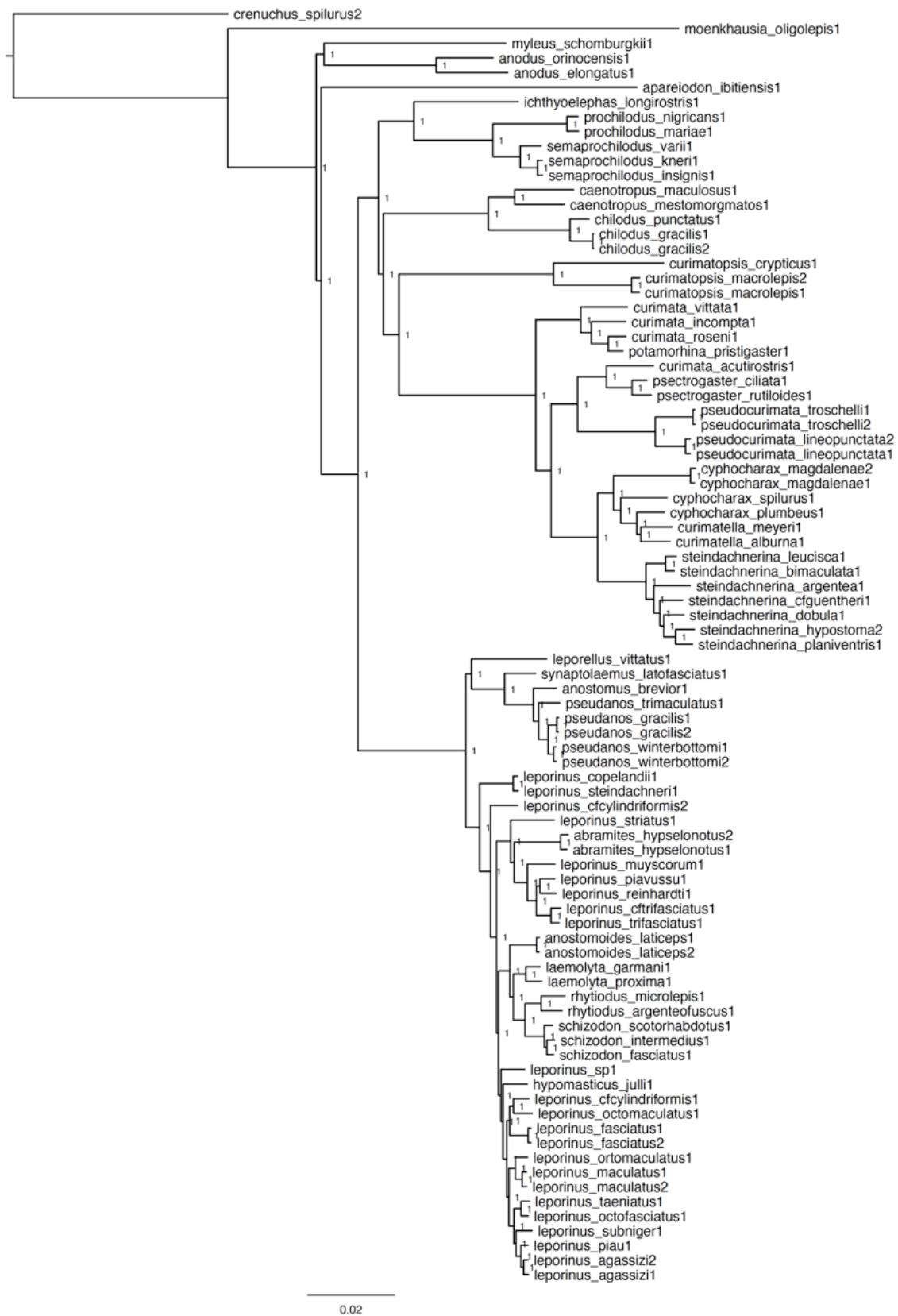


Fig. S4. Phylogenetic relationships of Anostomoidea and outgroups based on a Bayesian analysis of the 75% complete matrix. Numbers on branches represent posterior probabilities.

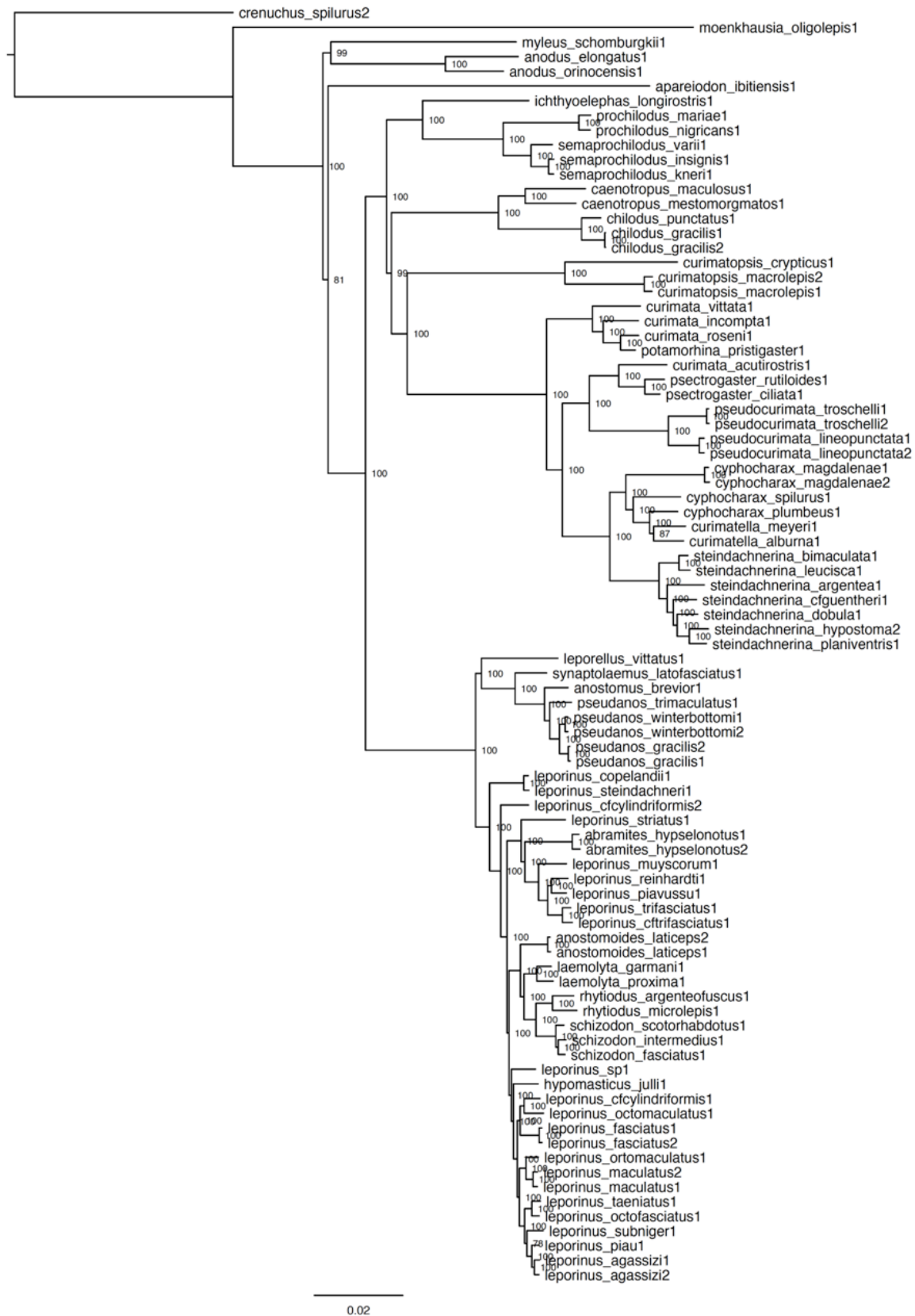


Fig. S5. Phylogenetic relationships of Anostomoidea and outgroups based on a maximum likelihood analysis of the 75% complete matrix. Numbers on branches represent percentages of bootstrap support.

Capítulo 2

Miocene radiations in the family Prochilodontidae (Ostariophysi: Characiformes) revealed by a time calibrated molecular phylogeny

Bruno F. Melo^{1*}, Kendra Hoekzema², Benjamin W. Frable², Richard P. Vari³, Brian L. Sidlauskas^{2,3}, Claudio Oliveira¹

¹Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu SP, Brazil

²Department of Fisheries and Wildlife, Oregon State University, Corvallis OR, USA.

³Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington DC, USA.

* Corresponding author: B. F. Melo (melo@ibb.unesp.br).

Target journal: *BMC Evolutionary Biology*

Running title: Molecular phylogeny of the characiform family Prochilodontidae

Highlights:

- First molecular phylogeny for Prochilodontidae.
- *Ichthyoelephas* sister to *Prochilodus* and *Semaprochilodus*.
- Most relationships corroborate previous morphological hypothesis and incongruences are discussed.
- Divergence between *Ichthyoelephas* and other genera in the Late Cretaceous and internal radiations during the Miocene.

Keywords: Diversification, evolution, multilocus, phylogeny, Neotropics.

Abstract

The large migratory detritivores in the characiform family Prochilodontidae represent one of the most commercially important fish groups in South America, but have never been the subject of a comprehensive molecular phylogenetic analysis. With three mitochondrial and three nuclear *loci* from 90% of the recognized prochilodontid taxa, we generated a new phylogenetic hypothesis, time-calibrated it using three fossils, and reconstructed the evolutionary history of the family. Our molecular phylogeny corroborated most previous morphological hypotheses of relationships and resolved some longstanding polytomies. Several morphologically similar species are embedded within the current concept of *Prochilodus nigricans*, and this group clearly merits taxonomic revision. The timetree shows that the split of the trans-Andean genus *Ichthyoelephas* from other prochilodontids occurring in the Late Cretaceous at approximately 80 million years ago (mya). Most internal radiations was recovered to be occurred during the Miocene since 30 mya and that the diversification within *Prochilodus* is much more recent than those within *Semaprochilodus*.

Introduction

Over 2,000 species from Africa, Central America and South America, including piranhas, tetras, and the impressive tigerfish, form the megadiverse ostariophysian order Characiformes. In the last decade, several molecular phylogenetic analyses (Ortí et al. 2008; Arroyave and Stiassny 2011; Oliveira et al. 2011; Tagliacollo et al. 2012; Abe et al. 2013, 2014; Arroyave et al. 2013; Mariguela et al. 2013; Melo et al. 2014; Thompson et al. 2014) have revisited inter- and intrafamilial relationships within this order that had previously received only morphological attention. Molecular and morphological datasets have agreed on numerous phylogenetic questions in Characiformes, for instance the monophyly of superfamily Anostomoidea, which includes the families Anostomidae, Chilodontidae, Curimatidae, and Prochilodontidae (Vari 1983; Sidlauskas 2008; Oliveira *et al.* 2011). In other instances, molecular and morphological hypotheses have diverged, as in the case of the African family Alestidae. Morphological studies (Zanata and Vari 2005; Mirande 2009, 2010) placed Alestidae sister to the South American genus *Chalceus* whereas molecular studies (Calcagnotto *et al.* 2005; Arroyave and Stiassny 2011; Oliveira *et al.* 2011) recovered Alestidae sister to the African Hepsetidae with *Chalceus* phylogenetically distant, as a newly proposed family Chalceidae (Oliveira *et al.* 2011). These later authors (Oliveira *et al.* 2011) also rejected the monophyly of Cynodontidae as proposed by Lucena and Menezes (1998), but corroborated Cynodontidae as proposed by Toledo-Piza (2000). Notwithstanding these advances, the position of many characiform families (*e.g.* Crenuchidae, Ctenoluciidae, Lebiasinidae, Serrasalminidae) remains uncertain. These discordances and uncertainties demonstrate the need to reexamine established characiform families via robust molecular datasets in order to understand the evolution of this major teleost lineage.

The characiform superfamily Anostomoidea, containing four trophically diverse families and about 300 species, has received extensive systematic and revisionary treatment (Vari 1983; Sidlauskas 2008) and its monophyly seems certain. However, its internal familial relationships have been explored in detail only with morphology (Vari 1992b, 1982, 1984, 1989a, 1989b, 1989c, 1989d, 1991, 1992a; Vari et al. 1995; Castro and Vari 2004; Sidlauskas and Vari 2008), and merit reexamination with molecular data. A recent study of the eight described species in Chilodontidae (Melo *et al.* 2014), the smallest of the four anostomoid families, reinforced the monophyly of the two genera but also revealed several cryptic species, suggested potential necessary synonymies, and differed from the established morphological hypothesis in the

placement of one species (Vari *et al.* 1995). That analysis further demonstrates the utility of molecular data in taxonomic and evolutionary studies within characiforms, and suggests that comparable studies of the three more species-rich families in the Anostomoidea (Anostomidae, Curimatidae and Prochilodontidae) will yield similar insights and surprises.

Herein, we generate the first comprehensive molecular phylogeny of the detritivorous family Prochilodontidae, commonly known as *curimbatás* and *jaraquis* in Portuguese, *bocachicos* in Spanish, and flannel-mouth characiforms in English (Castro and Vari 2003). The family includes three genera: the remarkable, trans-Andean *Ichthyoelephas* with a unique, highly developed upper lip (2 species, Fig. 1a), and two abundant, migratory, and commercially important genera, *Prochilodus* (13 species, Fig. 1b–f) and *Semaprochilodus* (6 species, 1g–h). *Prochilodus* is present in all major river systems of South America on both sides of the Andes while *Semaprochilodus* is broadly distributed east of the Andes, throughout the Amazon and Orinoco basins and some coastal rivers of the Guiana Shield.

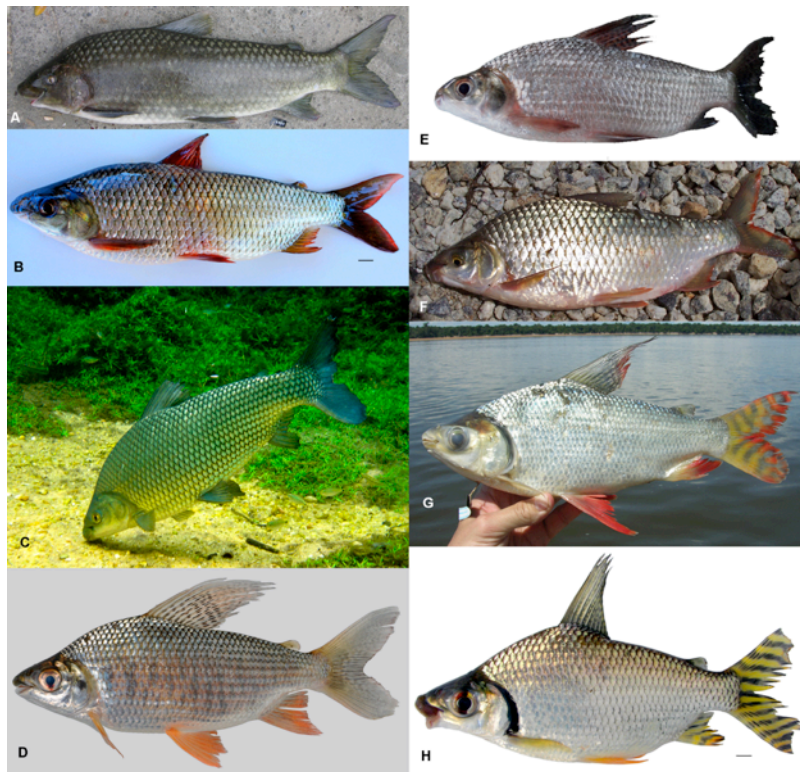


Figure 1. Live representative specimens of Prochilodontidae. (A) *Ichthyoelephas longirostris*, ANSP 192865, Colombia, Río Magdalena basin; (B) *Prochilodus hartii*, Brazil, Rio Jequitinhonha; (C) *P. lineatus*, Brazil, Rio Paraguai basin; (D) *P. nigricans*, Brazil, Rio Xingu, Amazon basin; (E) *P. rubrotaeniatus*, Brazil, Rio Branco, Amazon basin (F) *P. vimboides*, Brazil, Rio Mucuri; (G) *Semaprochilodus laticeps* (right after dead), MCNG 51963, Venezuela, Río Orinoco basin; (H) *S. varii*, ANSP 187435, Suriname, Marowijne river basin. Scale bars represent one centimeter. Photos by M. Sabaj Perez (A, D, G and H), T. C. Pessali (B), J. Sabino (C), B. F. Melo (E) and L. M. Sarmento-Soares (F).

Prochilodontidae is readily distinguished externally from all other characiforms by having numerous rows of relatively small teeth attached to the fleshy lips and structurally unique upper and lower jaws that support the expanded lips. These lips form an evertable (extendable outwards) oral disk (Castro and Vari 2004) which, in combination with the multiple tooth rows, serves to scrape periphyton and detritus off submerged surfaces and to suck detritus off the bottom of water bodies (Fig. 1c) (Bowen 1983; Bowen *et al.* 1984). Prochilodontids feed towards the base of the food chain and form a major link in the carbon flow within aquatic systems in the Neotropics (Taylor *et al.* 2006). Their specialization on a hyperabundant energy resource may account for the large bodies and enormous population sizes (Carolsfeld *et al.* 2003) of many species, which in turn allows these species to sustain the most important commercial and subsistence fisheries in many regions of South America (Bayley and Petrere Jr. 1989; Ribeiro and Petrere Jr. 1990; Camargo and Petrere Jr. 2001; Garcia *et al.* 2009). In many settings, prochilodontids additionally act as ecosystem engineers (Flecker 1996), and their removal can significantly impact broader riverine aquatic faunas (Ribeiro and Petrere Jr. 1990; Taylor *et al.* 2006). They are among the most important food resources for people across South America, albeit with anthropogenically induced declines in some fisheries (*e.g.* *Prochilodus lineatus* in the upper Río Paraguai (Mateus *et al.* 2004)).

Aside from their unusual feeding apparatus and incredible biomass, prochilodontids are famous for their large seasonal migrations which can span many hundreds of river kilometers (Paiva and Bastos 1981; Godinho and Kynard 2006). Because of those long migrations, natural populations of some prochilodontid species span enormous geographic ranges. For example, a study of population structure in *Prochilodus* (Sivasundar *et al.* 2001) suggested a remarkable intraspecific genetic homogeneity throughout each of the Magdalena, Orinoco, and Amazon basins, with major watershed boundaries structuring their diversity. Recent proposals to dam major portions of the Amazon and its tributaries (*e.g.* those under construction in the Madeira, Tapajós, and Xingu river systems) lead to serious concerns that these barriers will likely dramatically diminish, if not eliminate, flooded downstream areas and their marginal lagoons considered crucial for genetic diversity in *Prochilodus* (Melo *et al.* 2013). These major impoundments will also impede the long distance migrations prevalent in these ecologically important fishes with consequent fragmentation of populations. Such fragmentation may hold

unknown consequences for the reproduction and life history of one of the most important components of the artisanal and commercial fisheries in South America.

Despite the economic importance of Prochilodontidae, the systematics of this group remains incompletely resolved. Vari (1983) proposed monophyly of Prochilodontidae based on 18 synapomorphies related to dentition and cranial osteology, but examined only six species of the family. Later, in an extensive morphological phylogeny and taxonomic revision, Castro and Vari (2004) proposed 40 additional synapomorphies for the family, identified dozens of characters separating the three recognized genera, generated both generic and interspecific phylogenies, and hypothesized *Prochilodus* as sister to a clade containing *Ichthyoelephas* and *Semaprochilodus*. Despite their broad osteological sampling, Castro and Vari (2004) were able to identify only a few informative characters as to the relationships within *Prochilodus* and *Semaprochilodus* and only partially resolved the species-level phylogenies within those genera. This situation derives from the remarkable morphological similarity of species within each of these genera, with species differing primarily in scale counts, body morphometrics, nuanced aspects of coloration, and most significantly the watersheds to which they are endemic. Morphological stasis among species in the Prochilodontidae might be expected given their drainage-wide ranges and their low levels of sympatry, which would imply a primarily allopatric speciation mechanism without requisite major morphological evolution during speciation. Although biologically predictable, the lack of informative morphological characters at the species-level presents a significant obstacle in reconstructing evolutionary history based on such data sources. Molecular approaches, thereby, offer the surest path forward.

In this study, we combine mitochondrial and nuclear *loci* to revisit the phylogenetics of Prochilodontidae and advance our understanding of their evolution. We develop a time-scaled phylogeny for the family and outgroups using molecular-clock Bayesian estimation methods. With nearly complete taxon sampling, we test Vari's (1983) hypothesis of the monophyly of the family, Castro and Vari's (2004) hypotheses of relationships among genera and species, and present the first fully resolved species-level phylogeny for the family.

Material and Methods

Taxon sampling

We sampled 51 terminal taxa covering 19 of 21 known species (90%; only *Prochilodus britskii* and *Ichthyoelephas humeralis* missing) and all three genera. The outgroup taxa included species from each of the other three anostomoid families (Anostomidae, Chilodontidae, Curimatidae), representatives of three families (Hemiodontidae, Parodontidae and Serrasalminidae) previously hypothesized to be closely related to Anostomoidea, and one specimen of *Brycon pesu*, which was used to root the trees. We used tissues preserved in 95% ethanol or a saturated DMSO/NaCl solution, primarily from specimens deposited in museum and university collections (Table 1; abbreviations follow Sabaj Pérez (2013)). The tissue sample of *Ichthyoelephas longirostris* (ANSP 192865) has only a photographic voucher (Fig. 1a), but the highly distinctive morphology of this species leaves no doubt as to its proper identification. The Fig. 2 represents sampling localities for each analyzed prochilodontid species and was produced with QuantumGIS 10.07.28.

Multilocus sequencing

We extracted DNA from tissue muscles or fins with a DNeasy Tissue kit (Qiagen Inc.) following manufacturer's instructions or a modified NaCl extraction protocol adapted from Lopera-Barrero *et al.* (2008). Following recent phylogenetic studies with characiforms (Oliveira *et al.* 2011; Abe *et al.* 2013; Melo *et al.* 2014), we amplified partial sequences of the mitochondrial genes *16S rRNA* (16S, 510 bp), *cytochrome oxidase C subunit 1* (COI, 656 bp) and *cytochrome B* (Cytb, 990 bp) using one round of polymerase chain reaction (PCR). Additionally, we obtained sequences of the nuclear *myosin heavy chain 6 gene* (Myh6, 710 bp), *recombination activating gene 1* (Rag1, 1378 bp), and *recombination activating gene 2* (Rag2, 1029 bp) through nested-PCR following Oliveira *et al.* (2011). Primers used in this study are listed on Table 2. We did 12.5 µl reactions containing 9.075 µl of double-distilled water, 1.25 µl 5x reaction buffer, 0.375 MgCl₂, 0.25 µl dNTP mix at 8 mM, 0.25 µl of each primer at 10 µM (list of primers in Table 2), 0.05 µl Platinum Taq DNA polymerase enzyme (Invitrogen; www.invitrogen.com) and 1.0 µl genomic DNA (10–50 ng). The PCR consisted of an initial denaturation (4 min at 95°C) followed by 28–30 cycles of chain denaturation (30 s at 95°C), primer hybridization (30–60 s at 52–54°C), and nucleotide extension (30–60 s at 72°C). After visualization of the fragments using 1% agarose gel, we sequenced using dye terminators (BigDye™ Terminator v 3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems) purified again through ethanol

precipitation. We sequenced the samples on an ABI 3130-Genetic Analyzer (Applied Biosystems) at either Oregon State University (Corvallis, Oregon, USA) or the Universidade Estadual Paulista (Botucatu, São Paulo, Brazil).

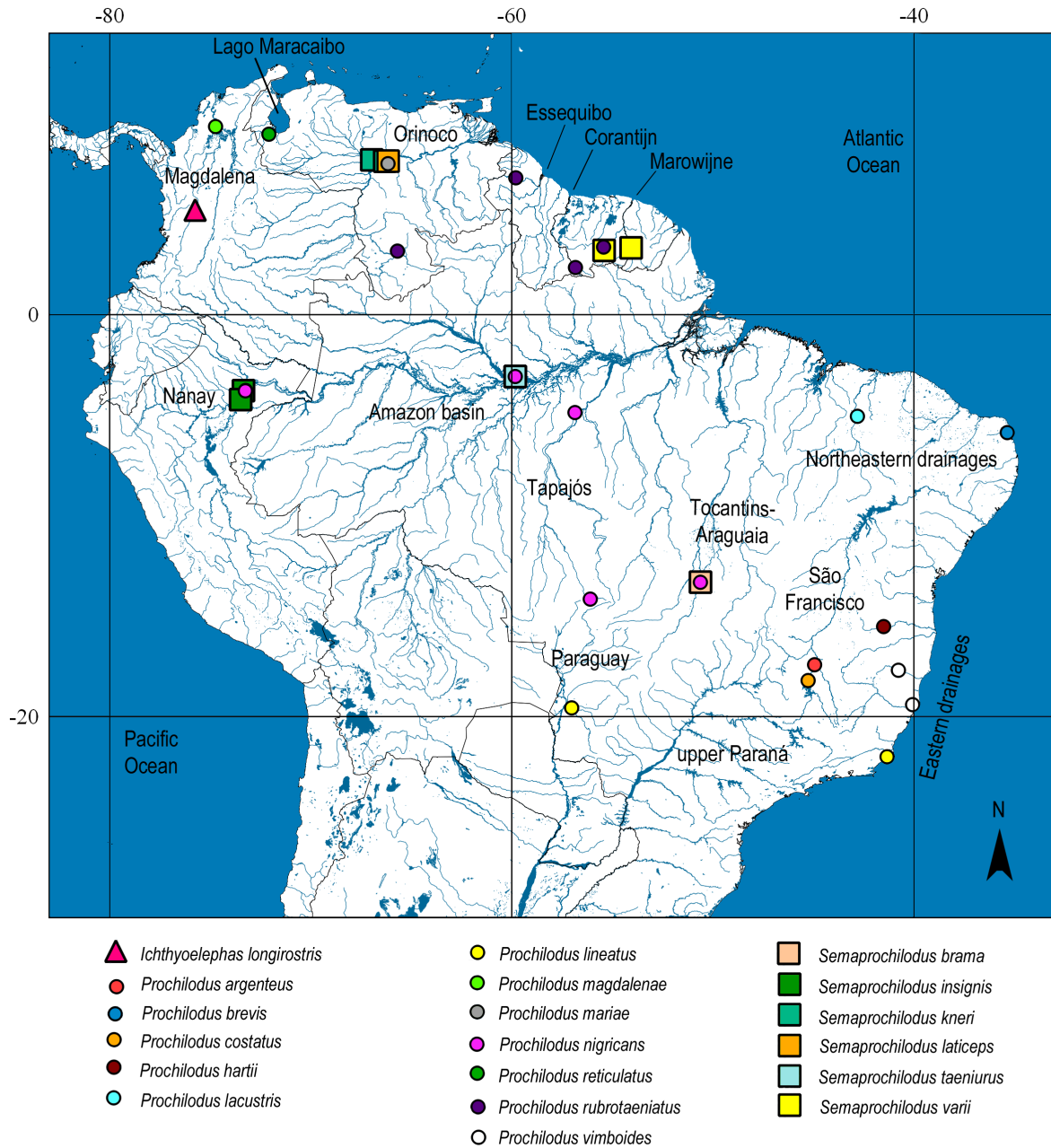


Figure 2. South American map showing collection localities. Outgroup taxa are not shown.

Table 1. Voucher specimens and locality information of prochilodontids and outgroup taxa used in this study.

Taxon	Voucher	Tissues	Locality	Coordinates	City, State	Country
<i>Ichthyoelephas longirostris</i>	ANSP 192865	6609	Rio Magdalena basin	05.2056389 -075.7471389	Honda, Tolima	Colombia
<i>Prochilodus argenteus</i>	LBP 251	4216	Rio São Francisco basin	-18.1965278 -045.2356944	Três Marias, Minas Gerais	Brazil
<i>Prochilodus argenteus</i>	LBP 251	4217	Rio São Francisco basin	-18.1965278°, -045.2356944°	Três Marias, Minas Gerais	Brazil
<i>Prochilodus brevis</i>	LBP 2496	16385	Açude Araçá, Eastern drainage	-05.8683333°, -035.3550000°	Macaíba, Rio Grande do Norte	Brazil
<i>Prochilodus costatus</i>	LBP 252	4222	Rio São Francisco basin	-18.1965278°, -045.2571389°	Três Marias, Minas Gerais	Brazil
<i>Prochilodus costatus</i>	LBP 252	4223	Rio São Francisco basin	-18.1965278°, -045.2571389°	Três Marias, Minas Gerais	Brazil
<i>Prochilodus hartii</i>	LBP 7211	33175	Rio Pardo, Eastern drainage	-15.5218350°, -041.5049500°	Machado Mineiro, Minas Gerais	Brazil
<i>Prochilodus hartii</i>	LBP 7211	33176	Rio Pardo, Eastern drainage	-15.5218350°, -041.5049500°	Machado Mineiro, Minas Gerais	Brazil
<i>Prochilodus lacustris</i>	LBP 9104	42731	Rio Poti, Parnaíba basin	-05.0574444°, -042.8084722°	Teresina, Piauí	Brazil
<i>Prochilodus lacustris</i>	LBP 9104	42732	Rio Poti, Parnaíba basin	-05.0574444°, -042.8084722°	Teresina, Piauí	Brazil
<i>Prochilodus lineatus</i>	LBP 45	3611	Rio Miranda, Paraguay basin	-19.5684167°, -057.0200833°	Corumbá, Mato Grosso do Sul	Brazil
<i>Prochilodus lineatus</i>	LBP 2348	16071	Rio Paraíba do Sul, Eastern drainage	-22.0000000°, -041.3333333°	Campos dos Goytacazes, Rio de Janeiro	Brazil
<i>Prochilodus magdalenae</i>	GR-93-1	GR207	Río Magdalena basin	09.3611100°, -074.7316600°	Magangue, Bolívar	Colombia
<i>Prochilodus magdalenae</i>	GR-93-1	GR208	Río Magdalena basin	09.3611100°, -074.7316600°	Magangue, Bolívar	Colombia
<i>Prochilodus mariae</i>	LBP 2188	15561	Laguna de Castilleros, Orinoco basin	07.5141389°, -066.1555000°	Caicara del Orinoco, Bolívar	Venezuela
<i>Prochilodus mariae</i>	LBP 2188	15562	Laguna de Castilleros, Orinoco basin	07.5141389°, -066.1555000°	Caicara del Orinoco, Bolívar	Venezuela
<i>Prochilodus</i> cf. <i>rubrotaeniatus</i>	ANSP 40692	P4313	Río Orinoco basin	03.1637222°, -065.6861389°	La Esmeralda, Amazonas	Venezuela
<i>Prochilodus nigricans</i>	LBP 7841	36858	Rio Araguaia, Amazon basin	-13.3166667°, -050.6166667°	Cocalinho, Mato Grosso	Brazil
<i>Prochilodus nigricans</i>	LBP 8589	43397	Rio Arinos/Tapajós, Amazon basin	-14.1541389°, -056.0948889°	Diamantino, Mato Grosso	Brazil
<i>Prochilodus nigricans</i>	LBP 8589	43398	Rio Arinos/Tapajós, Amazon basin	-14.1541389°, -056.0948889°	Diamantino, Mato Grosso	Brazil
<i>Prochilodus nigricans</i>	LBP 12865	53496	Rio Tapajós, Amazon basin	-04.8705833°, -056.8552778°	Itaituba, Pará	Brazil
<i>Prochilodus nigricans</i>	LBP 1690	12750	Rio Amazonas, Amazon basin	-03.0772222°, -059.8255556°	Manaus, Amazonas	Brazil
<i>Prochilodus nigricans</i>	LBP 1690	12754	Rio Amazonas, Amazon basin	-03.0772222°, -059.8255556°	Manaus, Amazonas	Brazil
<i>Prochilodus nigricans</i>	OS 18792	PE10-045	Río Itaya, Amazon basin	-03.7898610°, -073.2495830°	Maynas, Loreto	Peru
<i>Prochilodus nigricans</i>	OS 18792	PE10-058	Río Itaya, Amazon basin	-03.7898610°, -073.2495830°	Maynas, Loreto	Peru
<i>Prochilodus nigricans</i>	FMNH 113534	T54	Río Itaya, Amazon basin	-03.7719444°, -073.2377778°	Iquitos, Loreto	Peru
<i>Prochilodus reticulatus</i>	LBP 6127	29513	Rio Catatumbo, Lago Maracaibo	09.0856389°, -072.2306944°	Encontrados, Zulia	Venezuela
<i>Prochilodus reticulatus</i>	LBP 6127	29514	Rio Catatumbo, Lago Maracaibo	09.0856389°, -072.2306944°	Encontrados, Zulia	Venezuela
<i>Prochilodus rubrotaeniatus</i>	MHNG 2705.008	SU07-108	Rivière Paikali, Corantijn basin	02.3460600°, -056.8320000°	Kwamalasamutu, Sipaliwini	Suriname
<i>Prochilodus rubrotaeniatus</i>	MHNG 2717.017	SU07-776	Tapanahony river, Marowijne basin	03.3660000°, -055.4321000°	Palumeu, Sipaliwini	Suriname
<i>Prochilodus rubrotaeniatus</i>	USNM 403693	GY11.4.61	Cuyuni river, Essequibo basin	06.8098100°, -059.7986500°	Cuyuni-Mazaruni	Guyana
<i>Prochilodus vimboides</i>	LBP 2349	16011	Rio Doce, Eastern drainage	-19.4096111°, -040.0652222°	Sooretama, Espírito Santo	Brazil
<i>Prochilodus vimboides</i>	LBP 10180	47662	Rio Mucuri	-17.6951111°, -040.7698056°	Carlos Chagas,	Brazil

					Minas Gerais	
<i>Semaprochilodus brama</i>	LBP 12776	41019	Rio Araguaia, Amazon basin	-13.3103611°, -050.6132222°	Cocalinho, Mato Grosso	Brazil
<i>Semaprochilodus brama</i>	LBP 12807	41171	Rio Araguaia, Amazon basin	-13.3103611°, -050.6132222°	Cocalinho, Mato Grosso	Brazil
<i>Semaprochilodus insignis</i>	LBP 1692	12761	Rio Amazonas, Amazon basin	-03.0772222°, -059.8255556°	Manaus, Amazonas	Brazil
<i>Semaprochilodus insignis</i>	LBP 1692	12762	Rio Amazonas, Amazon basin	-03.0772222°, -059.8255556°	Manaus, Amazonas	Brazil
<i>Semaprochilodus insignis</i>	OS 18380	PE10-001	Río Itaya, Amazon basin	-04.2258000°, -073.4850000°	Maynas, Loreto	Peru
<i>Semaprochilodus insignis</i>	ANSP 180205	T43	Río Nanay, Amazon basin	-03.7800000°, -073.3426389°	Iquitos, Loreto	Peru
<i>Semaprochilodus kneri</i>	LBP 1384	12734	Río Orinoco, Orinoco basin	07.6416667°, -066.1500000°	Caicara del Orinoco, Bolivar	Venezuela
<i>Semaprochilodus kneri</i>	LBP 3041	19139	Río Orinoco, Orinoco basin	07.6365556°, -066.3178333°	Caicara del Orinoco, Bolivar	Venezuela
<i>Semaprochilodus kneri</i>	LBP 3041	19140	Río Orinoco, Orinoco basin	07.6365556°, -066.3178333°	Caicara del Orinoco, Bolivar	Venezuela
<i>Semaprochilodus kneri</i>	ANSP 187277	P4298	Río Apure, Orinoco basin	07.7027778°, -066.9611111°	San Fernando de Apure, Apure	Venezuela
<i>Semaprochilodus laticeps</i>	LBP 1383	12727	Río Orinoco, Orinoco basin	07.6416667°, -066.1500000°	Caicara del Orinoco, Bolivar	Venezuela
<i>Semaprochilodus laticeps</i>	LBP 1383	12728	Río Orinoco, Orinoco basin	07.6416667°, -066.1500000°	Caicara del Orinoco, Bolivar	Venezuela
<i>Semaprochilodus laticeps</i>	FMNH 113712	4.1	Aquarium specimen purchased in Chicago	Unknown	Unknown	Unknown
<i>Semaprochilodus taeniurus</i>	LBP 1691	12757	Rio Amazonas, Amazon basin	-03.0772222°, -059.8255556°	Manaus, Amazonas	Brazil
<i>Semaprochilodus taeniurus</i>	LBP 1691	12758	Rio Amazonas, Amazon basin	-03.0772222°, -059.8255556°	Manaus, Amazonas	Brazil
<i>Semaprochilodus taeniurus</i>	LBP 1691	12759	Rio Amazonas, Amazon basin	-03.0772222°, -059.8255556°	Manaus, Amazonas	Brazil
<i>Semaprochilodus varii</i>	MHNG uncatalogued	157.29	Tapanahony River, Marowijne basin	03.1983333°, -055.4075000°	Sipaliwini	Suriname
<i>Semaprochilodus varii</i>	ANSP 187435	6929	Lawa river, Marowijne basin	03.3252778°, -054.0633333°	Sipaliwini	Suriname
<i>Leporellus cf. vittatus</i>	AUM 54212	T09912				
<i>Schizodon scotorhabdotus</i>	AUM 53654	T09707				
<i>Chilodus fritillus</i>	AUM 51355	T10201	Río Madre de Dios	-12.2771300°, -069.1523700°	Madre de Dios	Peru
<i>Caenotropus mestomorgmatos</i>	ANSP 180516	T48	Río Nanay, Amazon basin	-03.7791667°, -076.7666667°	Iquitos, Loreto	Peru
<i>Curimatopsis macrolepis</i>	ANSP 178188	1697	Río Nanay, Amazon basin	-03.7563889°, -073.2911111°	Iquitos, Loreto	Peru
<i>Psectrogaster amazonica</i>	OS 18313	PE10-113	Río Amazonas, Amazon basin	-03.7182778°, -073.2128056°	Iquitos, Loreto	Peru
<i>Anodus elongatus</i>	OS 18724	PE10-110	Río Amazonas, Amazon basin	-03.7182778°, -073.2128056°	Iquitos, Loreto	Peru
<i>Hemiodus unimaculatus</i>	OS18345	PE10-076	Río Nanay, Amazon basin	-03.7516670°, -073.3162500°	Iquitos, Loreto	Peru
<i>Apareiodon affinis</i>	LBP 4591	24665	Rio Paranapanema, Paraná basin	-22.9042778°, -050.0010556°	Salto Grande, São Paulo	Brazil
<i>Parodon nasus</i>	LBP 1135	5635	Rio Tietê, Paraná basin	-22.8666667°, -048.3833333°	Botucatu, São Paulo	Brazil
<i>Myleus schomburgkii</i>	OS uncat	PE10-044	Río Nanay, Amazon basin		Iquitos, Loreto	Peru
<i>Brycon pesu</i>	OS 18361	PE10-072	Rio Nanay, Amazon basin	-03.7516670°, -073.3162500°	Iquitos, Loreto	Peru

Table 2. Information content and characteristics of each locus. represents nucleotide frequencies.

Locus	Bp after alignment	PCR	Primer sequence (5' – 3')	Π_A	Π_C	Π_G	Π_T	Reference
16S	510 bp	1 PCR	16Sa-L – ACGCCTGTTTATCAAAAACAT 16Sb-H – CCGGTCTGAACTCAGATCACGT	0.296	0.239	0.236	0.229	(Palumbi 1996)
COI	656 bp	1 PCR	L6252-Asn – AAGCCGGGGAAAGCCCCGGCAG H7271-COXI – TCCTATGTAGCCGAATGGTTCTTTT	0.242	0.278	0.187	0.293	(Melo <i>et al.</i> 2011)
Cytb	990 bp	1 PCR	LNF – GACTTGAAAAACCAACGTTGT H08R2 – GCTTTGGGAGTTAGDGGTGGGAGTTAGAAT C	0.269	0.310	0.146	0.275	(Oliveira <i>et al.</i> 2011)
Myh6	710 bp	1st PCR 2nd PCR	F329 – CCGCMTGGATGATCTACACA 3R1 – ATTCTCACCACCATCCAGTTGAA A3F2 – GGAGAATCARTCKGTGCTCATCA A3R2 – CTCACCACCATCCAGTTGAACAT	0.301	0.229	0.231	0.239	(Li <i>et al.</i> 2007)
Rag1	1378 bp	1st PCR 2nd PCR	Rag1CF1 – ACCCTCCGTACTGCTGAGAA Rag1CR1 – CGTCGGAAGAGCTTGTTGCC Rag1CF2 – TACCGCTGAGAAGGAGCTTC Rag1CR2 – TGTTGCCAGACTCATTGCCCTC	0.250	0.239	0.287	0.224	(Oliveira <i>et al.</i> 2011)
Rag2	1029 bp	1st PCR 2nd PCR	164F – AGCTCAAGCTGCGYGCCAT Rag2-R6 – TGRTCCARGCAGAAGTACTTG 176F – GYGCCATCTCATTCTCCAACA Rag2Ri – AGAACAAAAGATCATTGCTGGTTCGGG	0.242	0.260	0.273	0.225	(Oliveira <i>et al.</i> 2011) (Lovejoy and Collette 2001)

Sequencing alignment and phylogenetic analyses

After sequencing, we assembled and edited consensus sequences in Geneious 7.1.7 (Biomatters; www.geneious.com) and applied IUPAC ambiguity codes where we detected uncertainty of nucleotide identity. We aligned the consensus sequences of each gene in Geneious 7.1.7 and inspected alignments by eye for major misalignments. To evaluate the occurrence of substitution saturation, we estimated the index of substitution saturation (Iss) using Dambe 5.3.38 (Xia 2013). The presence of loops in 16S rRNA caused many notarial disturbances in the gene tree and we decide to exclude them and generate a reduced 16S submatrix without misalignments to use in the concatenated matrix. We then concatenated the six genes into a matrix with 16 partitions and used PartitionFinder 1.1.0 (Lanfear *et al.* 2012) to determine the best partitioning scheme and to select the best-fit model for each partition (Table 3).

We performed a Bayesian analysis of the partitioned matrix using MrBayes 3.1.2 (Ronquist *et al.* 2012) using the partitioning and models as detailed in Table 3. We performed two runs of four independent MCMC chains with 10 million replicates each, sampling one tree every five hundred generations. Additional generations were run until the standard deviation of split frequencies between the two runs was less than 0.01. The first 25% of generations were

discarded as burn-in, and the remaining trees were summarized in a phylogram with mean branch lengths and a cladogram with posterior probabilities for each split.

Table 3. Gene partitions and their best models of nucleotide evolution.

Gene	Partition	Best model for MrBayes	Best BIC models for BEAST
16S	1–510	SYM+I+G	SYM+I+G
COI 1st position	511–1167/3	SYM+I+G	TrN+G
COI 2nd position	512–1167/3	HKY+I+G	SYM+I+G
COI 3rd position	513–1167/3	GTR+G	HKY+I+G
Cytb 1st position	1168–2158/3	HKY+I+G	GTR+G
Cytb 2nd position	1169–2158/3	GTR+G	SYM+I+G
Cytb 3rd position	1170–2158/3	SYM+I+G	HKY+I+G
Myh6 1st position	2159–2869/3	SYM+G	HKY+I+G
Myh6 2nd position	2160–2869/3	GTR+I+G	TrNef+G
Myh6 3rd position	2161–2869/3	HKY+I+G	GTR+I+G
Rag1 1st position	2870–4248/3	SYM+G	HKY+I+G
Rag1 2nd position	2871–4248/3	GTR+I+G	TrNef+G
Rag1 3rd position	2872–4248/3	HKY+I+G	GTR+I+G
Rag2 1st position	4249–5278/3	GTR+I+G	HKY+I+G
Rag2 2nd position	4250–5278/3	HKY+I+G	TrNef+G
Rag2 3rd position	4251–5278/3	SYM+G	GTR+I+G

Maximum likelihood (ML) analyses were performed in RAxML HPC2 on XSEDE (Stamatakis 2006) as implemented on CIPRES Scientific Gateway v.3.3 (Miller *et al.* 2010). First, we tested running trees without partitions and then partitioned by different methods. The final ML analysis employed 16 partitions (Table 3) a random starting tree, and all other parameters at default values. One thousand bootstrap pseudoreplicates tested the support for each node in the most likely topology.

Divergence time and node-age estimations

In order to estimate divergence times, we used the Bayesian relaxed-clock method (Drummond *et al.* 2006) under the uncorrelated lognormal distribution (UCLD) rate variation models implemented in BEAST2 v. 2.1.3 (Bouckaert *et al.* 2014). We selected this model because evidence from previous empirical and simulation studies has shown that the UCLD model is

usually the most accurate and robust model (Drummond *et al.* 2006; Arroyave and Stiasny 2011). Four separated UCLN were implemented: one specifically for 16S, as this is non-coding and is fragmented due to loop removal (see above) and three additional for each of the codons of the other five genes. The clock rate of all four models was estimated with a starting rate of 0.05. The UCLD mean priors were estimated using a gamma distribution ranging from 0.0001 to 2.0 and the standard deviations were estimated using an exponential model ranging from 0.0-5.0.

Parameters and priors for the BEAST analysis were set up using BEAUTi 2 (Bouckaert *et al.* 2014). Specific substitution models were implemented for each partition in the dataset as the best models selected using jModelTest2 (Darriba *et al.* 2012). A birth-death tree prior was chosen for node time estimation, which implies a prior distribution under a birth-death stochastic branching process model (*i.e.* speciation and extinction rates can affect a lineage at anytime). This model is considered the most appropriate when extinction is known to have occurred in the group and as stated below, extinct fossil taxa are known and used for calibration (Drummond *et al.* 2006).

Three fossil calibration points were used to provide minimum ages estimates for specific lineages on the tree. Log-normally distributed priors for calibrating substitution rate estimates were chosen. Because the available fossil calibration points are generally from clades considered derived (Vari 1989a; Sidlauskas and Vari 2008), an uniform prior would be inappropriate as we know the clade must be much older than the minimum bound. The lognormal distributions were offset using the minimum age of the calibration fossil and mean and standard deviation were estimated to provide a reasonable range of potential clade ages.

For the age estimation, six independent MCMC runs, one with 166 million generations and five ranging from 274.5–278.4 million generations (due to processing time constraints on the CIPRES Scientific Gateway v.3.3), logged every 50,000 generations, were implemented in BEAST on run on a XSEDE supercomputing cluster through the CIPRES Scientific Gateway v.3.3 (Miller *et al.* 2010). Generated log data files were compared in Tracer (Rambaut *et al.* 2014) to examine effective sample size (ESS) and likelihood (-lnL) of the priors and posterior estimates. An ESS >200 is desired as this measure indicates how thoroughly an MCMC has searched the likelihood-posterior probability landscape (Drummond *et al.* 2006). However, not all ESS values met this criterion due to the time constraints of the analyses (see Discussion below). A maximum credibility tree (MCC), the tree with the highest product of posterior

probabilities for all nodes, was found using TreeAnnotator v1.8.0 (Drummond *et al.* 2012). The MCC trees were edited in FigTree v.1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>) and mean node ages, posterior probabilities and node bars representing the 95% HPD (highest posterior density) were applied to each major node.

Fossil evidence and calibration

As there are no known prochilodontid fossils, we used fossil estimations of proximal outgroups for our calibration points. Within Anostomidae, the oldest known fossils are 469 disarticulated jaw and pharyngeal teeth, putatively assigned to the genus *Leporinus* and matching the current concept of *Leporinus*, dating to the Miocene of the Cuenca basin of southern Ecuador (20–19 mya; dating based on associated invertebrate fauna (Roberts 1975)). Additional disarticulated teeth are known from the early-early middle Miocene (23–13.5 mya) and middle-late Miocene (13.5–5 mya) from the Solimões/Pebas Formation of the northwestern portion of the Amazon basin (Monsch 1998) and from 11.5 mya in the La Venta Formation of Colombia (Lundberg 1997). The only anostomid fossil known is one incomplete skull of †*Leporinus scalabrinii*, a specimen originally described as a lemur (†*Arrhinolemur scalabrinii*), from cliffs near Paraná, Entre Ríos Province, Argentina dating from 9 to 6 mya (Bogan *et al.* 2012). Since the oldest lower bound for Anostomidae is 19 mya from the disarticulated teeth from Ecuador, these are used to calibrate the node for the clade ((*Leporinus* + *Abramites*) *Schizodon*) (calibration point 1).

All known curimatid fossils are from the Tremembé Formation of the Taubaté Basin in Tremembé, São Paulo, Brazil dating to the Oligocene-Miocene boundary (23–22.5 mya) (Riccomini *et al.* 1987, 1991). From this formation, four incomplete full body specimens of †*Cyphocharax mosesi* (Travassos and Santos 1955; Malabarba 1996) and one complete full body specimen of †*Plesiocurimata alvarengai* (Figueiredo and Costa-Carvalho 1999). The potential co-occurrence of these fossils is interesting in the genus *Cyphocharax*, which is a derived clade within Curimatidae (Vari 1989a), whereas †*Plesiocurimata* was hypothesized as a basal clade sister to all other curimatid genera (Figueiredo and Costa-Carvalho 1999). †*Plesiocurimata* was placed in a basal location due to the presence of a single row of teeth on the dentary whereas all other curimatids are toothless except in larval stages. The presence of both of these lineages at this age suggests that Curimatidae was already diverse by the Oligocene-Miocene boundary and

may have originated much earlier. The †*Plesiocurimata alvarengai* fossil (Figueiredo and Costa-Carvalho 1999) is used as a node calibration point for all Anostomoidea (calibration 2), as it is the oldest known fossil from the superfamily.

The oldest identifiable characiform fossils from South America are disarticulated teeth attributed to the family Serrasalminae from the El Molino and Santa Lucia deposits of Bolivia dating to the late Cretaceous (70–65 mya) (Dahdul 2010; Malabarba and Malabarba 2010). Therefore, this calibration point was used to calibrate the clade containing four serrasalmin species on the phylogeny (calibration 3). A recent molecular phylogeny of Characiformes recovered strong support for a clade made up of Serrasalminae and Hemiodontidae sister to Anostomoidea (Oliveira *et al.* 2011). Therefore, these serrasalmin fossils are used to date the major hypothesized clade with all included families except the root *Brycon pesu*.

Species tree estimation

In order to mitigate the potential hazards of using a concatenated dataset, such as recovering a well supported but incorrect species tree (Kubatko and Degnan 2007), we utilized a Bayesian multilocus coalescent method implemented in *BEAST v.1.8 (Heled and Drummond 2010). This approach uses the coalescence of effective population size and the evolutionary histories of a subset of genes as a prior for the distribution of gene trees in a Bayesian framework, which allows the estimation of a species tree. Species groups were created based on the authors' identifications. As with the BEAST analysis, an optimal partition scheme was hypothesized using jModelTest2 (Darriba *et al.* 2012). UCLD rate models were estimated for 16S and each of the three codons of the other coding genes using a normal prior for the mean rate ranging from 0.0001 to 2. A birth-death model of speciation with uniform priors was employed and a single tree model was estimated for each gene separately. Four independent 500 million generation MCMC chains with sampling every 50,000 generations were run using *BEAST v.1.8 on the CIPRES Scientific Gateway v.3.3 (Miller *et al.* 2010). Chain convergence was analyzed by comparing these four runs with runs with prior information only. Additionally, the adequacy of the runs was assessed using ESS as described above. Results were compiled using the same method as for the BEAST analysis.

Results

Concatenated Approaches

Both maximum likelihood and Bayesian analysis of the concatenated datasets strongly supported the monophyly of Prochilodontidae (Fig. 3, clade 1) and of the two major genera, *Prochilodus* and *Semaprochilodus* (Figs. 3–4). Our results recovered *Ichthyoelephas longirostris* as sister to the clade (2) containing *Prochilodus* and *Semaprochilodus* (Figure 3), contrasting with the previous morphological hypothesis that placed *Prochilodus* sister to a clade containing *Ichthyoelephas* plus *Semaprochilodus* (Castro and Vari 2004).

Within a monophyletic *Semaprochilodus* (clade 3), we reconstructed *S. taeniurus* from the Amazon basin as sister to the clade (4) containing the remaining five congeners, although we found *S. taeniurus* as the sister to *S. insignis* and *S. kneri* in the species-tree phylogeny (Fig. 4). Within clade 4, we found two monophyletic groups: a clade (5) containing *S. brama* from the Araguaia basin appeared as sister to a clade containing *S. laticeps* from the Orinoco basin and *S. varii* from the Marowijne River of the Atlantic slope of Suriname, and a clade (6) containing *S. insignis* from the Amazon basin nested within a non-monophyletic *S. kneri* from the Orinoco basin. Overall, these results resemble strongly the morphological hypothesis (Castro and Vari 2004).

We obtained a pectinate phylogeny among the species of *Prochilodus* (clade 7) with substantial, but not complete similarity to Castro and Vari's (2004) morphological hypothesis. *Prochilodus vimboides* from the Rio Doce and Rio Mucuri in southeastern Brazil is sister to the clade (8), which contains all remaining species of *Prochilodus*. The trans-Andean *P. magdalenae* from the Río Magdalena in Colombia forms a well-supported clade with *P. reticulatus* from the geographically proximate Lago Maracaibo in Venezuela (clade 9). Clade 10 contains the remaining nine species, with the clade (11) indicating a highly supported relationship between *P. argenteus*, endemic to the Rio São Francisco, and *P. hartii* of the Rio Pardo and Rio Jequitinhonha that together are sister to the seven species of clade 12. *Prochilodus mariae* appears with weak support as sister to clade 14 in the Figure 3. Clade 13 contains *P. lineatus* of the Río Paraguai and Paraíba do Sul basins nested within a non-monophyletic *P. costatus* from the Rio São Francisco. That clade is sister to a clade (15) containing several morphologically similar species embedded within the widespread Amazonian *Prochilodus nigricans*. Within this “*Prochilodus nigricans* group” there are two distinct lineages. The first (16) is formed by *P.*

nigricans from the mainstream Amazon basin plus *P. rubrotaeniatus* (clade 17) from the Corantijn and Marowijne river basins in Suriname. The second (18) contains *P. nigricans* from the southern Amazonian tributaries (Rio Araguaia and Rio Tapajós) and a clade (19) composed of *P. cf. rubrotaeniatus* from the upper Río Orinoco, *P. rubrotaeniatus* from the Cuyuni-Essequibo river basin in Guyana, *P. brevis* and *P. lacustris*. These latter two species are both from northeastern Brazil and form the well-supported clade 20.

While this study was not designed to infer interfamilial relationship, we did recover relationships in the outgroup that largely match those found in recent molecular studies (Oliveira *et al.* 2011; Melo *et al.* 2014), particularly with respect to the close relationship among Chilodontidae and Curimatidae and Prochilodontidae (Figs. 3–5). Surprisingly, we found a highly supported relationship between Hemiodontidae and Anostomidae and this group as sister to the other three families Chilodontidae, Curimatidae and Prochilodontidae. Whether this relationship reflects biological reality, or is simply an artifact of limited taxon sampling in the outgroup, must await further study.

Time Calibration

The Maximum Clade Credibility tree from the BEAST analysis recovered the same ingroup topology as did MrBayes. ESS values for most parameters were >200, however, the mutation rates of the last three substitution models and the UCLD mean values were below 200. Most nodes in the time-calibrated phylogenies are well supported (>0.8 posterior probability) for all major clades except the Serrasalminidae sister to the Anostomoidea + Hemiodontidae (posterior probability 0.60). The best-supported time-calibrated phylogeny indicates that, based on estimated mean node ages, the origins of the superfamily Anostomoidea + Hemiodontidae dates to the Albian of the Cretaceous (91.7 mya; 95% HPD=112–72) and the cladogenetic event that originated the common ancestor of Prochilodontidae dates to the late Cretaceous (71.1 mya; 95% HPD=91.0–52.5) (Fig. 5). This age is close to the age estimation for Alestidae + Characiformes (excluding Citharinoidei) of 78 mya recovered by Arroyave and Stiassny (2011). Although the estimation of the origin of Prochilodontidae dates for the late Cretaceous, the sister genera *Prochilodus* and *Semaprochilodus* originated during the Eocene (34.5 mya; 95% HPD: 46–23) and diversified during the Miocene (20–3 mya), with the lineage leading to *Ichthyoelephas*

diverging very early in the history of Prochilodontidae, although this result should be interpreted with caution as only one *Ichthyoelephas* voucher was used in the analysis.

Species tree estimation

The most probable species tree depicts a well-supported topology that agrees closely, but not completely, with the concatenated phylogeny (Fig. 4) and that very closely matches prior morphological hypotheses of interspecific relationships. The monophyly of Prochilodontidae in the species tree is indisputable (posterior probability of 0.99) and most internal nodes receive posterior probability >0.80. Only two weakly supported relationships appear: the sister relationship of *Semaprochilodus taeniurus* to *S. insignis* + *S. kneri* (posterior probability=0.58) and that of *Prochilodus magdalenae* + *P. reticulatus* as sister to all other *Prochilodus* except *P. vimboides* (posterior probability = 0.51). The first of these conflicts with the concatenated results, in which, *S. taeniurus* is sister to all congeners, but agrees precisely with the morphological hypothesis of Castro and Vari (2004). The species tree also recovers *P. mariae* within the “*P. nigricans* group”, a placement that differs from the concatenated relationships but is also in concord with the morphological phylogeny.

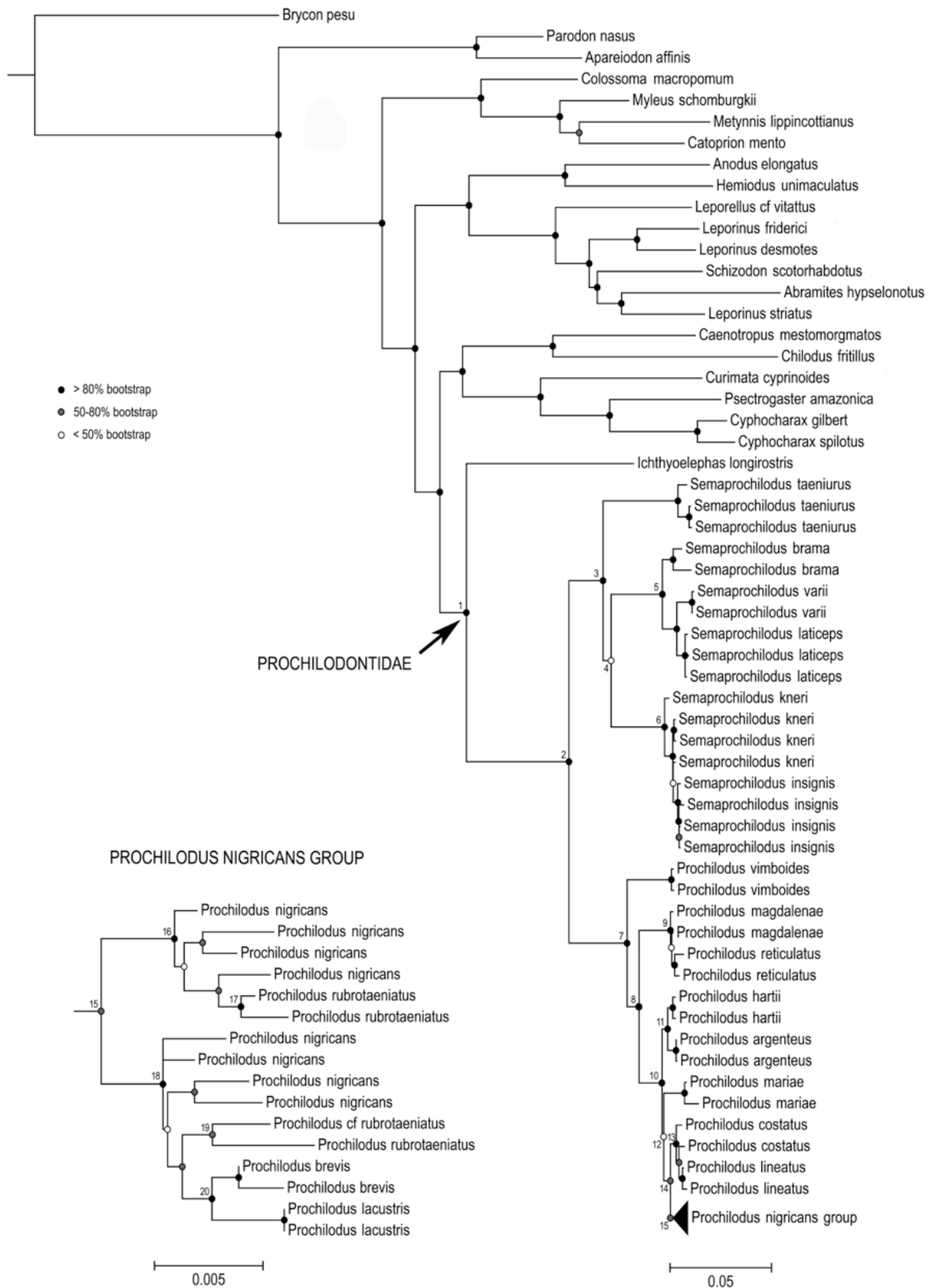


Figure 3. Best maximum likelihood tree of Prochilodontidae based on an analysis of the concatenated dataset using RAxML. Numbered clades are discussed in the text.

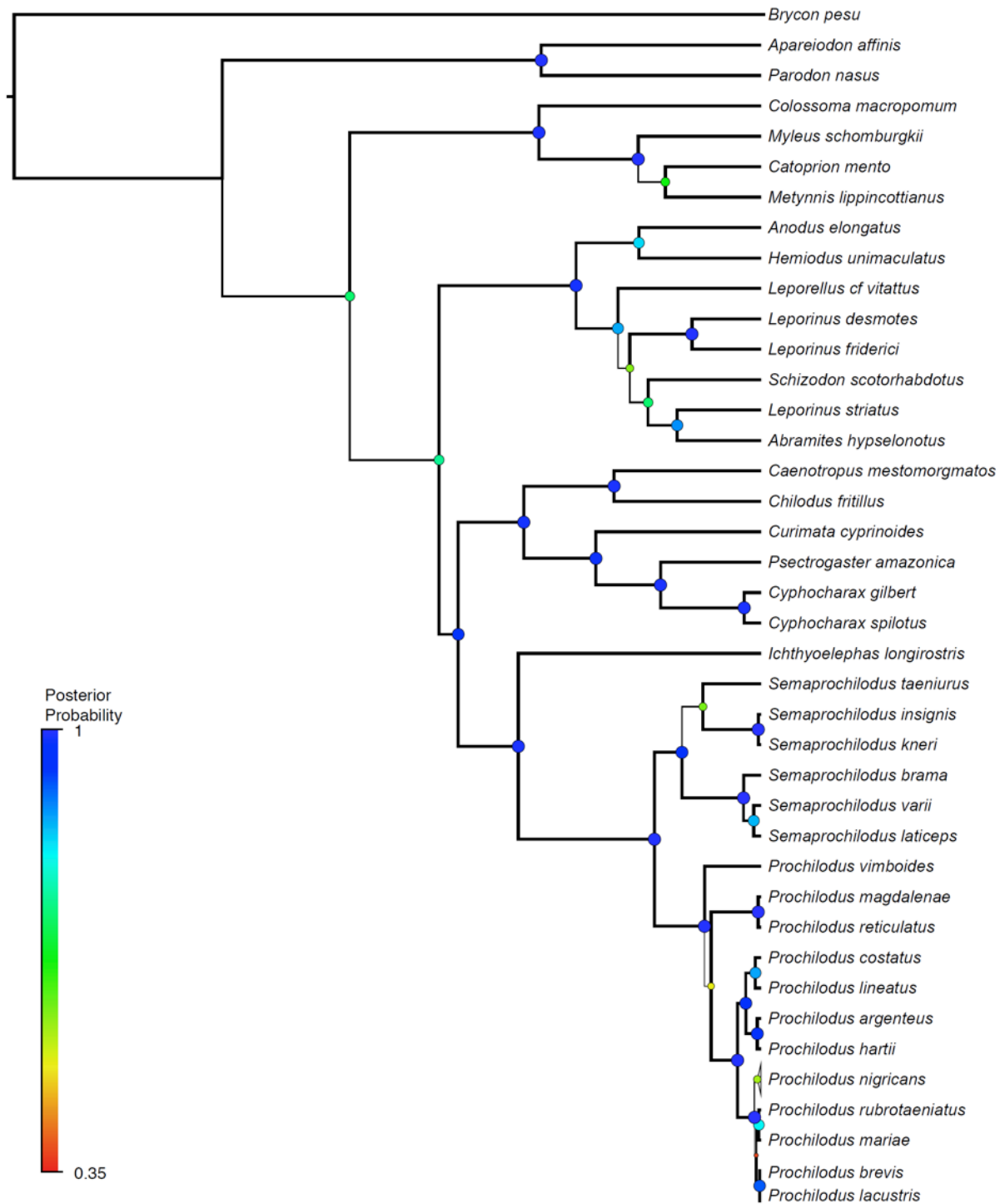


Figure 4. Species tree estimation of Prochilodontidae and other Anostomoidea based on a Bayesian multilocus coalescent method.

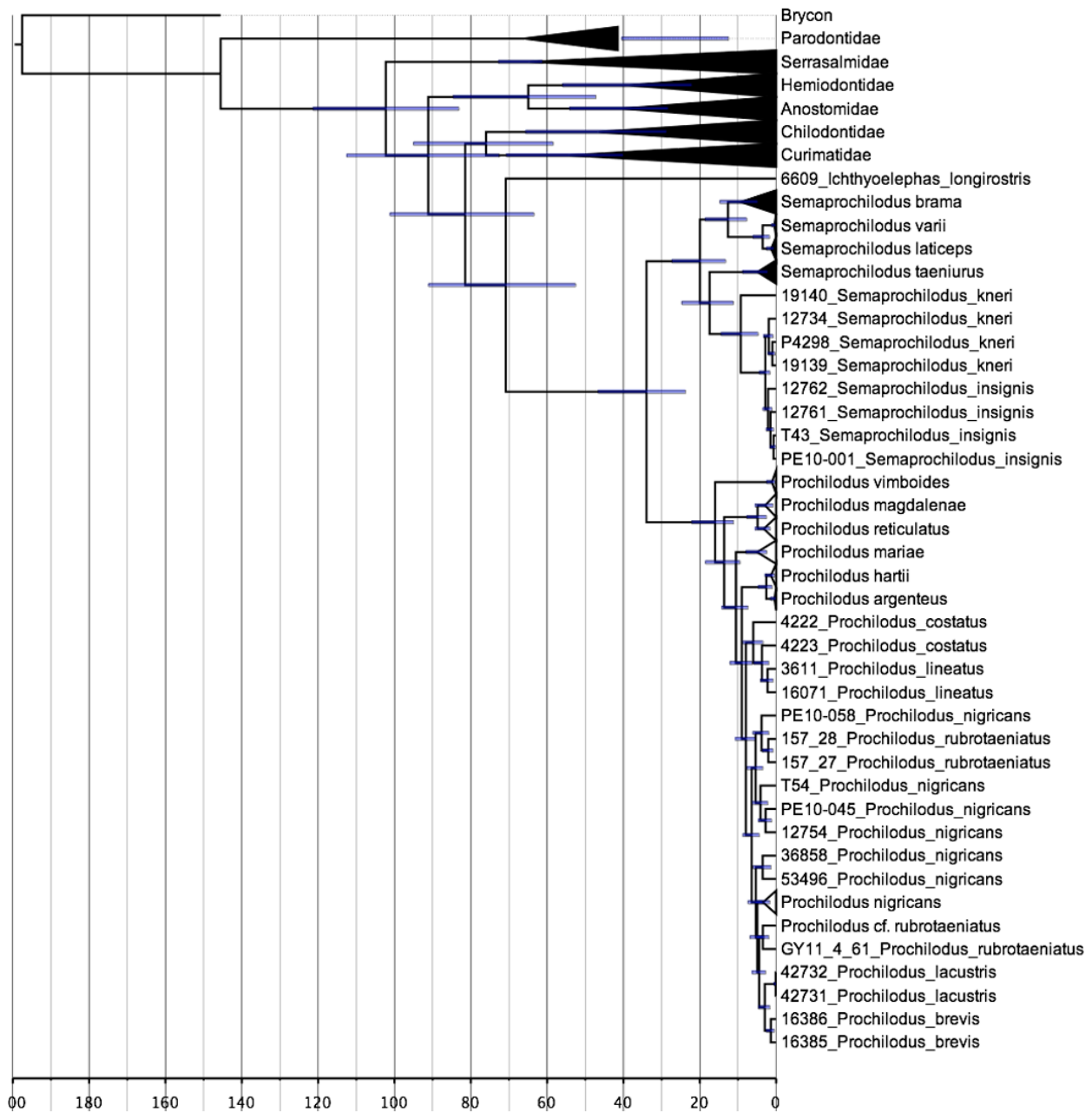


Figure 5. Time-calibrated phylogeny based on a Bayesian analysis of the concatenated dataset. Three fossil calibrations constrain the ages of nodes. Numbers at the axis correspond to million of years ago.

Discussion

Monophyly of Prochilodontidae and intergeneric relationships

Using molecular data, we tested and confirmed Vari's (1983) hypothesis of the monophyly of Prochilodontidae, which in conjunct with Castro and Vari's (2004) analysis, they supported by

an exceptional series of 58 morphological synapomorphies. We reconstructed Prochilodontidae sister to a clade composed by Chilodontidae + Curimatidae, a grouping of three families that has been recovered in other recent molecular phylogenies, such as Oliveira *et al.*'s (2011) molecular phylogeny of Characidae, and Melo *et al.*'s (2014) study of Chilodontidae, but does not appear in Vari's long-standing morphological hypothesis. All three molecular studies used the same combination of mitochondrial *loci* useful for resolving shallow clades and nuclear coding genes for resolving deep clades, and their concordance is not surprising. Our labs are working actively to test these relationships with other molecular markers.

Our results strongly support Castro and Vari's (2004) hypothesis of monophyly for each prochilodontid genus, although we could not test it for *Ichthyoelephas* because we sampled only one of the two species. Even though we could not obtain samples of *I. humeralis* from Ecuador, the 32 morphological synapomorphies defining that genus (Castro and Vari 2004) and the trans-Andean range leaves little doubt that *Ichthyoelephas* is properly conceived and monophyletic. However, we noted a very long branch for *I. longirostris*, which likely indicates an ancient separation from the common ancestor of *Prochilodus* and *Semaprochilodus*, as much as 47 mya as discussed below. We strongly corroborate this monophyly of *Prochilodus* and *Semaprochilodus* which are based on 16 and 13 morphological synapomorphies, respectively (Castro and Vari 2004).

In contrast to prior morphological work, we present herein the first hypothesis of a sister-relationship between *Ichthyoelephas* and a clade containing both *Prochilodus* and *Semaprochilodus*. Castro and Vari (2004) proposed a sister relationship between *Ichthyoelephas* and *Semaprochilodus* based on eight synapomorphies (their synapomorphies 78–85), with this clade sister to *Prochilodus*. A few of these synapomorphies for the *Ichthyoelephas* and *Semaprochilodus* clade optimized within the context of our phylogeny (synapomorphies 78, 81 and 82). Thus, the molecular hypothesis would imply either independent acquisition or secondary loss of those characters.

Interspecific relationships within Semaprochilodus

Castro and Vari (2004) proposed *Semaprochilodus taeniurus* as sister to a clade formed by *S. insignis* plus *S. kneri* based on two synapomorphies related to the epibranchials and the laterosensory canal system (their synapomorphies 100 and 101, respectively). Our Bayesian

phylogeny (Figure 5) corroborates such relationship, although with only moderate support from posterior probabilities (0.67), as well as in the species tree estimation (Figure 4) with low support (0.58), but higher support in the BEAST analysis (0.88). The ML analysis (Figure 3) recovered *S. taeniurus* as sister to the clade containing all remaining *Semaprochilodus* species, also with low bootstrap support (<50%). With this conclusion, the molecular hypothesis for the intrageneric position of *S. taeniurus* as sister to the remaining *Semaprochilodus* should be evaluated based on the concatenation of more and different *loci* in future upcoming studies.

The placement of *Semaprochilodus taeniurus* aside, all analysis (whether morphological or molecular) agrees closely on the relationships of the remaining species of *Semaprochilodus*. For example, Castro and Vari (2004) first hypothesized the close relationship between *S. kneri* from the Orinoco basin and *S. insignis* from the Amazon basin based on loss of the suprapreopercle and the elaboration of the maxilla at the location where the primordial ligament attaches (their synapomorphies 102 and 103). We confirmed the close relationship of these species and indeed, discovered very little molecular differentiation between them, with *S. insignis* actually nested within *S. kneri*. These two species are quite similar morphologically and only few meristic nuances distinguish them, thus we encourage future studies on this clade to test the taxonomic validity of the two species.

We recovered a clade composed by *Semaprochilodus brama*, *S. laticeps* and *S. varii* that was previously hypothesized by Castro and Vari (2004) due to the derived presence of “very dark pigmentation on the border of the operculum and the adjoining region of cleithrum”. Those authors were unable, however, to resolve the internal relationships. Our results show convincingly that *S. laticeps* from the Rio Orinoco basin and *S. varii* from the Marowijne river basin in the Atlantic slopes of Suriname are the closest relatives among the three, with *S. brama* from the Rio Araguaia (and possibly those from the Rio Xingu not examined here) sister to the clade composed by the other two.

Phylogenetic relationships within Prochilodus

In their morphological study of *Prochilodus*, Castro and Vari (2004) found scant phylogenetically informative variation within the genus, and were able to erect only three synapomorphies based solely on color pattern and squamation. Given that paucity of information, it was inevitable that our molecular study would return substantially greater

phylogenetic resolution than the morphological perspective, but not at all certain that the phylogenies would be congruent. Nevertheless, the two are congruent in broad strokes, with two of the three internal *Prochilodus* clades recognized by Castro and Vari (2004) appearing in our reconstruction.

We reconstructed *Prochilodus vimboides* from the Rios Doce and Mucuri in eastern Brazil as sister to all the remaining *Prochilodus* species. *Prochilodus vimboides* is a morphologically distinct species occurring in coastal rivers of eastern Brazil from São Paulo to Bahia. It has unusual characteristics such as the presence of large scales, and thus possesses the fewest lateral-line scales along the body (34–39 scales) and fewest vertebrae (36–39) of any species of *Prochilodus*.

Turning to the remaining species, we recovered the first direct evidence of the close relationship between the trans-Andean species *Prochilodus magdalenae* and *P. reticulatus*, although their similarities have long been noted. Steindachner (1879) was the first author to observe these similarities when he described *Prochilodus asper* var. *magdalenae* (= *P. magdalenae*) for what he considered a trans-Andean Colombian population of *P. asper* (the latter species is currently considered a synonym of *P. reticulatus*). Though *Prochilodus magdalenae* and *P. reticulatus* differ in the ranges and modal numbers of scales and vertebrae and have allopatric distributions (Castro and Vari 2004), the limited scale of these morphological differences plus the lack of molecular differentiation turn *P. magdalenae* paraphyletic. This combination of extreme morphological and molecular similarities suggests that these two species may represent synonyms of only one species perhaps suffering pressures to allopatric differentiation by the development of the Sierra del Perijá. Our results also are consistent with the phylogeny using the mtDNA control region (Sivasundar *et al.* 2001) which found *P. magdalenae* sister to *P. mariae*, *P. nigricans* and *P. lineatus*.

Castro and Vari (2004) considered *Prochilodus mariae* to fall within a clade also containing *P. brevis*, *P. lacustris*, *P. nigricans* and *P. rubrotaeniatus* due to the derived “presence of 2 to 8 irregular, vertical, somewhat chevron-shaped, dark bars on the caudal fin” (Castro and Vari 2004). That same set of relationship appears within the species tree reconstruction (Fig. 4), but not in the concatenated analysis (ML and BI analyses), in which the two specimens of *P. mariae* from the lower Río Orinoco in Venezuela appeared as sister to clade 14 composed by those same species together with *P. lineatus* and *P. costatus*.

Castro and Vari (2004) recognized that *Prochilodus argenteus*, *P. costatus*, *P. hartii* and *P. lineatus* (along with the *P. nigricans* group) possess a derived color pattern of wavy lines along the body and lack the derived coloration of the caudal fin that characterized the latter group, but were unable to further resolve their relationship. Here, we also found these species in close phylogenetic proximity, but were able to refine their evolutionary histories. Within lineage 11 (Fig. 3), one subclade includes *P. argenteus*, endemic to the Rio São Francisco (but subsequently introduced into several rivers in Northeastern Brazil (Castro and Vari 2004)) and *P. hartii* endemic to the Rio Jequitinhonha and Rio Pardo, coastal rivers of eastern Brazil. Another subclade unites *P. lineatus* from the La Plata system and Rio Paraíba do Sul with *P. costatus* from the Rio São Francisco. Castro and Vari (2004) recognized these as distinct species based on modal differences in scale counts and apparent allopatry, despite overlapping meristic ranges, but our results show them to be very similar genetically, with *P. lineatus* apparently nested within *P. costatus*. It is possible that these entities are still in the process of speciation, and their taxonomy clearly merits additional morphological and molecular attention.

The remaining *Prochilodus* species appear broadly within the “*Prochilodus nigricans* group” and several of them may require revision, as their species limits appear problematic. Clearly, *P. nigricans* as now delimited is not monophyletic and this species will have to be reevaluated, as will the present concept of the polyphyletic *P. rubrotaeniatus*. Two distinct lineages exist within this group (clades 16 and 18), both of which include individuals that fit the current morphological diagnosis of *P. nigricans* and *P. rubrotaeniatus*. For example, two specimens of *P. rubrotaeniatus* from the Corantijn and Marowijne river basins in Suriname (clade 17) nest within several specimens of *P. nigricans* from the mainstream of the Rio Amazonas (Iquitos-Peru and Manaus-Brazil). Similarly, two specimens of *P. rubrotaeniatus* from the Essequibo and Orinoco river basins are present in clade 18, along with *P. lacustris*, *P. brevis*, and several specimens of *P. nigricans* from the eastern Amazonian drainages of the Brazilian Shield (Rio Araguaia and Rio Tapajós). Though Turner *et al.* (2004) also reported a non-monophyletic *P. rubrotaeniatus* (with *P. mariae* nested within it), those authors did not sample *P. brevis*, *P. lacustris* or *P. nigricans*, and we are not aware of any other suggestions that *P. rubrotaeniatus* or *P. nigricans* may be polyphyletic or paraphyletic.

The proper taxonomic resolution of this situation is not at all clear. There is clearly a genetic divide between clades 16 and 18, but Castro and Vari (2004) did not remark on any

morphological differences between *Prochilodus nigricans* from the Rio Araguaia and Rio Tapajós (corresponding to *P. nigricans* of the clade 18) and samples of that species from the other parts of Amazon basin (which fall within clade 16). That fact, combined with the present of specimens that fit the morphological concept of *P. rubrotaeniatus* in both subclades implies that there is no obvious morphological diagnosis for the deepest genetic split within clade 15. The monophyletic components of this larger cluster that are morphologically diagnosable (*P. lacustris* and *P. brevis*) are also deeply nested, and their continued recognition as species would imply either recognizing a paraphyletic *P. nigricans* (including a synonymization of *P. rubrotaeniatus*), or splitting *P. nigricans* into multiple components which may not all be diagnosable. Further morphological and molecular analysis with extensive sampling from all Amazonian drainages is clearly necessary to determine which components of the *P. nigricans* complex represent valid species.

Temporal scale of diversification and biogeography

In addition to gaps in our understanding of relationships within Characiformes, very little is understood about the origins and historical diversification of the order (Albert and Reis 2011). As the order exists in both Africa and South America, the lineage is hypothesized to exist prior to the breakup of Gondwana (Malabarba and Malabarba 2010) *circa* 120–110 mya in the Early Cretaceous (Near *et al.* 2012; Arroyave *et al.* 2013). Recent time-calibrated molecular phylogenies have been estimating the origin and diversification for some families within Characiformes. For example, the origin for Alestidae is estimated to be occurred in the Eocene of Paleogene at around 54 mya (Arroyave and Stiassny 2011). The origin for Distichodontidae was estimated to have occurred during the Late Cretaceous at 67 mya. The timescale for American characiforms is relatively scarce if compared to the number of families. Late Cretaceous was the time of origin of piranhas and pacus of the family Serrasalminidae between 65 and 75 mya (Thompson *et al.* 2014). The estimation of the origin of Bryconidae is much younger than other characiform families and is dated for the Eocene-Paleocene during the Paleogene at around 46.7 mya (Abe *et al.* 2014). Our results show that the diversification of the lineage currently composed by *Ichthyoelephas* and the lineage of *Prochilodus* and *Semaprochilodus* is estimated at around 70 mya.

The relationship between *Semaprochilodus insignis* and *S. knerii* was recovered in the dated phylogeny with the diversification of the species group dating to the Early Serravallian of the Middle Miocene at 10 mya. The end of the great Pebas Lake system in the Middle Miocene rendering the establishment of modern easterly Amazon channel and the formation of the current Orinoco basin at around 11 mya (Figueiredo *et al.* 2009) coincides with the diversification of *S. insignis* and *S. knerii*.

Within *Prochilodus*, the *P. vimboides* is sister to all other congeners, which would imply that the separation of these ancient populations and the commencement of their speciation dates to or before the uplift of the Andes. It is estimated that *P. vimboides* diverged from other *Prochilodus* species in the Early Miocene, 16.2 mya, which occurred early in the estimated peak uplift of the Andes (estimate of major onset beginning approximately 23 mya (Hoorn *et al.* 2010)).

The subsequent geographical isolation perhaps resulted from the uplift events associated with the development of the Sierra del Perijá uplands along the border between Colombia and Venezuela. The ancient connection across the region encompassing what are the present Magdalena and Maracaibo drainages is well documented (see Aguilera *et al.* 2013 for references). If so, the separation of these drainages and, thus, the populations of *Prochilodus* from the two sides of the Sierra del Perijá date to the Upper Miocene *circa* 8 mya (Rodríguez-Olarte *et al.* 2011). The dated phylogeny shows a divergence of *P. magdalenae* from *P. reticulatus* at around 5 mya.

Our results show the sister relationship between *Prochilodus argenteus* and *P. hartii*. In a biogeographic study combining freshwater fish distribution and the available geological data, Ribeiro (2006) proposed three patterns of temporal diversification for freshwater fish in the Brazilian coastal drainages ranging from older diversification at familial-level to modern diversification at species-level. Our dated phylogeny shows a separation of around 2.7 mya (4.7–1 mya) between *P. argenteus* and *P. hartii* suggesting that the continuous process of stream capture transferred the hypothetical ancestors of both species from the Rio São Francisco into coastal rivers of eastern Brazil enabling events of allopatric speciation. Interestingly, geomorphological data suggest that eastern tributaries of the Rio São Francisco were captured by western tributaries of the Rio Jequitinhonha (Saadi 1995) through Neogenic reactivation of the Rio Araçuaí Fault (most recent movement on Quaternary <1.6 mya) in the Serra do Espinhaço

mountains (Saadi *et al.* 2002). Other hypothesis could be the ancient contact between the Rio São Francisco and eastern Atlantic drainages north to Abrolhos formation during cycles of sea-level fluctuations in the glacial periods as evidenced by a phylogeographic study of *Hoplias malabaricus* (Pereira *et al.* 2013).

The relationship between *Prochilodus costatus* and *P. lineatus* is consistent with biogeographic studies showing the overlapping of fish distribution between the upper Rio Paraná and the Rio São Francisco (Menezes 1972; Buckup 2011). Sivasundar *et al.* (2001) found no structure among samples of *P. lineatus* collected throughout the La Plata basin suggesting that the migratory behavior permits elevated gene flow among them. Similarly, population genetic studies showed high diversity with lack of structuring in *P. costatus* (Carvalho-Costa *et al.* 2008; Melo *et al.* 2013). Considering morphological and molecular similarities, a broader sampling using both related species using faster polymorphic markers might explain this apparent paraphyly of *P. costatus*.

Our results provide evidence for a putative connection among populations of *Prochilodus rubrotaeniatus* throughout these major watersheds likely due to stream captures and modern corridors (Lujan and Armbruster 2011). These authors pointed out that headwaters of Río Orinoco and Essequibo river (lower Río Caroni with upper Cuyuni river, and upper Río Caroni with upper Mazaruni river) would facilitate the interchange of their fish fauna. Castro and Vari (2004) did not reported any *P. rubrotaeniatus* in the upper Río Orinoco, but Turner *et al.* (2004) used a specimen identified as *P. rubrotaeniatus* from Río Paragua, a tributary of that basin. Herein, we confirmed the existence of *P. rubrotaeniatus* in the upper Río Orinoco, which is close related to populations from the Essequibo drainage (forming the clade 19), but not for populations from Suriname (clade 17). As well-stated by Winemiller and Willis (2011), the two molecular studies with *Prochilodus* (Sivasundar *et al.* 2001; Turner *et al.* 2004) suggested that the Cassiquiare portal is not important for their dispersal. Thus, our results (clade 19) suggest that headwater captures through Río Caroni promote dispersal of *Prochilodus* among major watersheds.

Author contributions

BFM, RPV, BLS and CO designed the project. BFM, KH collected data. BFM, KH, BWF, BLS and CO performed phylogenetic analyses. BWF and BLS generated the time-calibrated

phylogeny. BFM and BLS wrote most of the manuscript. RPV wrote morphological sections. All authors edited and revised the manuscript.

Acknowledgements

We thank M. Sabaj-Perez (ANSP), N. Lujan and J. Armbruster (AUM), S. Mochel and K. Swagel (FMNH), R. Covain (MHNG) and R. Reina (STRI) for curatorial assistance and/or loan of tissues and vouchers. We also thank the colleagues J. Sabino, M. L. Sarmento-Soares, M. Sabaj-Pérez and T. C. Pessali who authorized reproduction of their live prochilodontid photographs (Fig. 1), and also to R. M. C. Castro (LIRP) and J. Armbruster (AUM) for their help on the identification of some vouchers. Authors were funded by NSF (DEB-1257898) and FAPESP (proc. 2011/08374-1 and BEPE 2013/16436-2).

Capítulo 3

NOTICE

This thesis is a partial requirement for the PhD degree in Biological Sciences with emphasis in Genetics and, as such, should not be considered as a publication in the sense of the International Code of Zoological Nomenclature (although it is available without restrictions). Therefore, any new information, opinions, and hypotheses, as well as new names, taxonomic changes are not available in the zoological literature. Interested people are advised that any public reference to this study, in its current form, should only be done after previous acceptance of the author.

**The Neotropical fish family Curimatidae (Teleostei: Ostariophysi: Characiformes):
classification and phylogenetic relationships based on molecular data**

Bruno F. Melo¹, Richard P. Vari², Kendra Hoekzema³, Brian L. Sidlauskas^{2,3}, Claudio Oliveira¹

¹Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu SP, Brazil

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

³Department of Fisheries and Wildlife, Oregon State University, Corvallis OR, USA

* Corresponding author: B. F. Melo (melo@ibb.unesp.br).

Target Journal: *Molecular Phylogenetics and Evolution*

Running title: Molecular phylogeny of the detritivorous family Curimatidae

Highlights:

- First molecular phylogeny for Curimatidae.
- Previous relationships are corroborated and incongruences are discussed.
- *Curimatella*, *Cyphocharax* and *Steindachnerina* are non-monophyletic.
- Nomenclatural changes are proposed in order to recognize monophyletic clades.

Keywords: Freshwater fishes, multilocus analysis, nomenclature, phylogeny, systematics.

Abstract

The Curimatidae, a family of detritivorous Neotropical freshwater fishes, encompasses eight genera containing 105 valid species dwelling in diverse aquatic habitats from Costa Rica to Argentina. Phylogenetic relationships among the species and genera of the family were previously proposed based on evidence from extensive analyses of multiple aspects of soft anatomical and osteological systems. These hypotheses demonstrated incomplete phylogenetic resolution at various scales and remain untested. We used a large molecular dataset containing six *loci*, high taxon sampling (75% of species) and modern phylogenetic methods to reconstruct the first molecular phylogeny of the Curimatidae. The hypothesized monophyly of the Curimatidae is well supported with *Curimatopsis* sister to all congeners. The major clade sister to *Curimatopsis* contains two major subclades. The first has *Curimata* sister to *Potamorhina* and the second *Psectrogaster* sister to *Pseudocurimata*. The latter pair of genera is sister to a clade containing the non-monophyletic genera *Curimatella*, *Cyphocharax* and the monophyletic *Steindachnerina*. Interspecific relationships under the molecular phylogeny are to a significant degree similar to those obtained through morphological characters. Incongruities between our results and those of morphologically-based reconstructions are discussed. Based on the phylogenetic hypothesis arrived at herein, we synonymize *Curimatella* into *Cyphocharax*, shift *Steindachnerina corumbae* (assigned to that genus in the absence of a phylogenetic analysis) into *Cyphocharax* and recommend further analyses to resolve the generic placements of “*Cyphocharax*” *abramoides*, “*C.*” *multilineatus* and “*C.*” *nigripinnis* and refine the phylogenetic position of various species, most notably *Curimatella lepidura*.

Introduction

Detritivores of the family Curimatidae (Fig. 1) inhabit diverse freshwater ecosystems to both sides of the Andean Cordillera. East of those mountains they range from south of Buenos Aires, Argentina, through the myriad Atlantic versant rivers of cis-Andean South America to the Orinoco basin and Trinidad. Curimatid abundance and species-level diversity is greatest in major basins such as Paraná-Paraguay, Amazon, Tocantins, Orinoco and the larger drainages of the Guianas, which are home to multi-species curimatid faunas. In the trans-Andean region, a less speciose curimatid assemblage lives in rivers from northern Peru to southern Costa Rica including the Magdalena and Maracaibo basins (Bussing 1966; Ortega and Vari 1986; Vari 1989a). Few of these western systems are home to more than a single member of the family.

Many curimatid species congregate in large schools (Santos *et al.* 1985). These constitute a significant portion of the fish biomass in many major rivers (Lowe-McConnell 1975) and are exploited as a secondary resource in regional fisheries. Species of *Potamorhina*, for example contribute significantly to landings in Amazonian fish markets (Araújo-Lima and Ruffino 2003; Garcia *et al.* 2009) in addition to being important components of artisanal fisheries. Ecologically, vast schools of these and other curimatid species of moderate to mid-sizes are important contributors to carbon flow and nutrient cycling in Neotropical freshwaters and constitute a major food source for piscivorous fishes, birds and other predators.

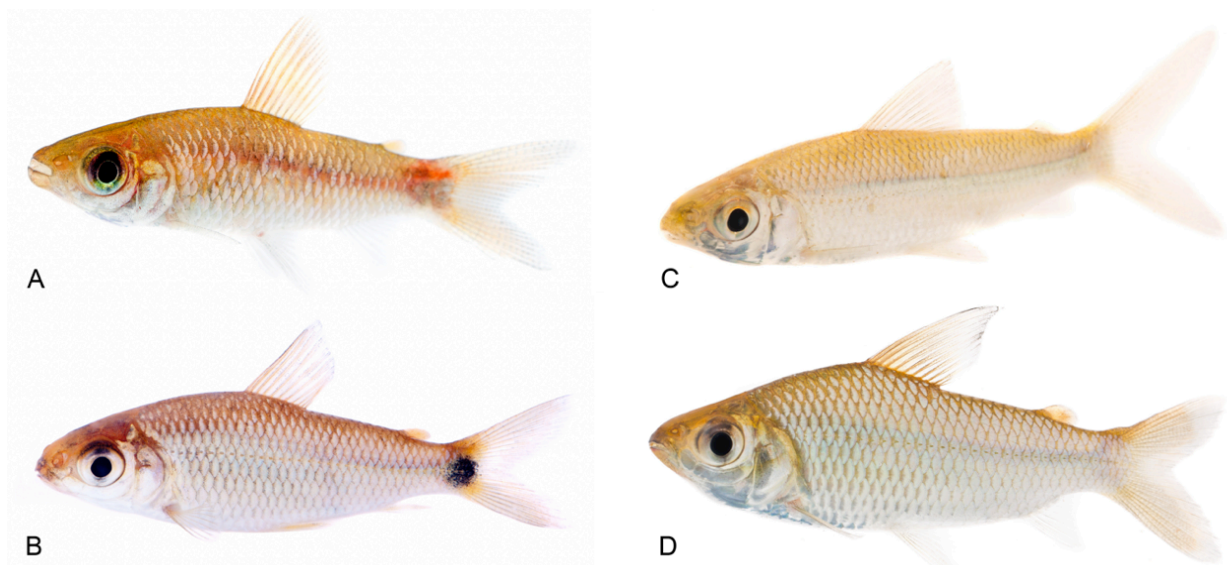


Figure 1. Representative species of the Curimatidae: A) *Curimatopsis evelynae*, B) *Cyphocharax spilurus*, C) *Cyphocharax* sp., D) *Cyphocharax immaculatus*. Photos by M. Taylor.

An absence in adults of oral dentition readily distinguishes all species of the Curimatidae within the Characiformes with exception of the edentulous *Anodus* (Hemiodontidae; Vari, 1989a). That genus differs from curimatids in numerous details (Langeani 1998). Members of the Curimatidae further share multiple unique internal modifications (Vari 1989a). The Curimatidae currently comprises eight genera and 105 species (Eschmeyer and Fong 2015) with additional species described periodically, most often from the Amazon, a region whose ichthyofauna is far from thoroughly sampled (Vari and Malabarba 1998).

Genera of the Curimatidae have been the subject of exhaustive revisions. All of these studies with the exception of that of *Cyphocharax* also included morphologically supported phylogenetic analyses. In the first of the series of revisions, Vari (1982a) redefined *Curimatopsis* and recognized four species across central and northern South America. Soon thereafter, he (Vari, 1982b) described *C. myersi* from the Río Paraguay. In the second study, Vari (1984) recognized five species of *Potamorhina*, some of which achieve the largest body sizes in the family. Third in the series was an analysis of *Curimata*, which Vari (1989b) redefined with twelve species; a total supplemented by *C. acutirostris* by Vari and Reis (1995). That same year, Vari (1989c) revised *Psectrogaster*; recognizing eight species. The fifth study in the series was the revision of the six species of the trans-Andean *Pseudocurimata* (Vari 1989d). *Steindachnerina*, the second most species-rich genus of the Curimatidae (currently with 24 species) was soon thereafter the subject of revisionary analysis (Vari, 1991). Four species of *Steindachnerina* were later described; two of which were phylogenetically evaluated in the original description and placed into Vari's phylogenetic scheme (Lucinda and Vari, 2009; Netto-Ferreira and Vari, 2011). A third proposed by Géry *et al.* (1991) without any phylogenetic comments proved to be the senior synonym of a species described (Vari 1993) and placed in the intrageneric phylogeny by Vari (1991). The fourth species was not phylogenetically evaluated (Pavanelli and Britski, 1999) prior to this analysis. The seventh study (Vari, 1992a) centered on *Cyphocharax*, the most speciose genus (33 species). Later descriptions of new species yielded a total of 39 valid *Cyphocharax* species (Vari and Blackledge 1996; Vari and Chang 2006; Vari *et al.* 2010, 2012; Wosiacki and Miranda 2013; Melo and Vari 2014, Anexo 2). The eighth and final revision involved the five species of *Curimatella* (Vari 1992b).

In the most comprehensive phylogenetic study of the Curimatidae to date, Vari (1989a) hypothesized a monophyletic Curimatidae supported by 19 morphological synapomorphies. The

vast majority of these features involved modifications of the branchial apparatus, buccopharyngeal complex, hyoid arch, jaws, palatine arch, and neurocranium. He further generated a phylogenetic hypothesis of intergeneric relationships for the family (Fig. 2). All genera are supported by one to multiple synapomorphies except for *Cyphocharax*, which remained without any derived delimiting character(s) and was of questionable monophyly. The phylogeny (Fig. 2) resolved as a lineal topology with a terminal quadritomy (*Curimatopsis* (*Potamorhina* (*Curimata* (*Psectrogaster* (*Curimatella*-*Cyphocharax*-*Pseudocurimata*-*Steindachnerina*))))).

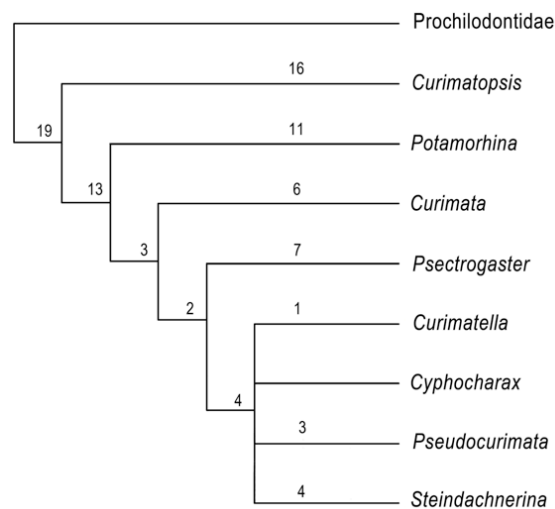


Figure 2. Phylogenetic relationships of the Curimatidae based on morphological data (modified from Vari 1989a). Numbers represent the proposed synapomorphies that support each clade.

No molecular phylogenetic analysis has been conducted to date to test Vari's (1989) hypothesis of the monophyly of the Curimatidae, the hypothesis of generic relationships or address the problematic terminal four-genus polytomy. Neither has molecular evidence been brought to bear on *Cyphocharax* for which neither generic synapomorphies nor interspecific relationships were proposed (Vari, 1992a). Molecular evidence is yet to be used to address the incompletely resolved interspecific relationships in *Curimata* (Vari 1989b), *Curimatella* (Vari, 1992b), *Curimatopsis* (Vari, 1982; Vari, 1989a), *Psectrogaster* (Vari 1989c), *Pseudocurimata* (Vari 1989d), and *Steindachnerina* (Vari 1991; Netto-Ferreira and Vari 2011).

The molecular analysis reported on herein, the first across the expanse of the Curimatidae, incorporates a large molecular dataset with high taxon sampling (75%) and modern phylogenetic methods. Our aims are: 1) to test prior morphological hypotheses of intergeneric

and interspecific relationships in the family; 2) to develop a new and more highly resolved intrafamilial phylogeny; 3) to establish a new framework for future taxonomic studies and identify problematic areas of the phylogeny requiring more intense analysis; and 4) to provide a foundation for evolutionary studies on this speciose radiation of detritivorous fishes.

Material and Methods

Taxon sampling

We used substantial in-group sampling across the Curimatidae with a total of 146 terminal taxa covering 79 species of all genera - 75% species-level coverage. In addition, we included two species of each of the Anostomidae, Chilodontidae, Prochilodontidae and members of characiform families hypothesized to be phylogenetically proximate to Anostomoidea (Hemiodontidae, Parodontidae, Serrasalminidae [only one species]) and used *Brycon pesu* (Bryconidae) to root the generated trees. Tissues were from vouchers available in various collections (Table S1; museum abbreviations follow Sabaj Perez (2013)) and preserved in 95% ethanol or a saturated DMSO/NaCl solution.

Multilocus sequencing

DNA was extracted from tissue muscles or fins with a DNeasy Tissue kit (Qiagen Inc.) following manufacturer's instructions or via a modified NaCl extraction protocol (Lopera-Barrero *et al.* 2008). We amplified partial sequences of the mitochondrial genes *16S rRNA* (16S, 604bp), *cytochrome oxidase C subunit I* (COI, 657bp) and *cytochrome B* (Cytb, 1001bp) using one round of polymerase chain reaction (PCR). Additionally, we obtained sequences of the nuclear *myosin heavy chain 6 gene* (Myh6, 715bp), *recombination activating gene 1* (Rag1, 1451bp), and *recombination activating gene 2* (Rag2, 1032bp) through nested-PCR following Oliveira *et al.* (2011). Primer sequences for each locus are those used by Melo *et al.* (2014). We used 12.5 µl as a total volume containing 9.075 µl of double-distilled water, 1.25 µl 5x reaction buffer, 0.375 MgCl₂, 0.25 µl dNTP mix at 8 mM, 0.25 µl of each primer at 10 µM (list of primers in Table 2), 0.05 µl Platinum Taq DNA polymerase enzyme (Invitrogen; www.invitrogen.com) and 1.0 µl genomic DNA (10–50 ng). The PCR consisted of an initial denaturation (4 min at 95°C) followed by 28–30 cycles of chain denaturation (30 s at 95°C), primer hybridization (30–60 s at 52–54°C), and nucleotide extension (30–60 s at 72°C). After visualization of the fragments using

1% agarose gel, we cleaned the PCR product using ExoSAP. Sequencing followed using dye terminators (BigDye™ Terminator v 3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems) purified again through ethanol precipitation. Samples were placed onto an automatic sequencer ABI 3130-Genetic Analyzer (Applied Biosystems) at Arizona State University (Tucson, Arizona, USA) or the Universidade Estadual Paulista (Botucatu, São Paulo, Brazil).

Sequencing alignment and phylogenetic analyses

After sequencing, we assembled and edited consensus sequences in Geneious 7.1.7 (Biomatters; www.geneious.com) and applied IUPAC ambiguity codes where we detected uncertainty in nucleotide identity. We aligned the consensus sequences of each gene in Geneious 7.1.7 and inspected alignments by eye for major misalignments. To evaluate the occurrence of substitution saturation, we estimated the index of substitution saturation (Iss) using Dambe 5.3.38 (Xia 2013b). We generated maximum likelihood trees for each gene to check for cross-well contamination and the identification of differences among the gene trees. We then concatenated the six genes into a matrix with 16 partitions determined using PartitionFinder 1.1.1 (Lanfear *et al.* 2012) and used ModelTest 3.6 (Darriba *et al.* 2012) to select the best-fit model of evolution. Post analyses encompassing all genera, a reduction of computational times to generate a *Cyphocharax*, *Steindachnerina* and *Curimatella* restricted phylogeny was achieved via the use of a dataset limited to representatives of this species-rich assemblage. We excluded Rag2 for this specific analysis due to detection after gene tree analysis of possible cross-contamination among samples.

Bayesian analyses were performed using a partitioned matrix in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) on the CIPRES web portal (Miller *et al.* 2010) using the 16 partitions and models detailed in Table 1. We performed two runs of four independent MCMC chains with 2 billion replicates each, sampling one tree every one thousand generations. The distributions of log likelihood scores were examined using Tracer 1.5 (Rambaut *et al.* 2014) in order to determine stationarity and decide if extra runs were required to achieve convergence. The first two thousand million generations (10%) were discarded as burn-in, and the remaining trees used to construct an 80% majority rule consensus tree in Geneious 7.1.7 and FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Table 1. Gene partitions and their best models of nucleotide evolution.

Gene	Partition	Best model for MrBayes
16S	1–497	GTR+I+G
COI 1st position	498–1256/3	GTR+I+G
COI 2nd position	499–1256/3	HKY+I+G
COI 3rd position	500–1256/3	GTR+I+G
Cytb 1st position	1257–2309/3	SYM+G
Cytb 2nd position	1258–2309/3	GTR+I+G
Cytb 3rd position	1259–2309/3	GTR+I+G
Myh6 1st position	2310–3048/3	GTR+I+G
Myh6 2nd position	2311–3048/3	SYM+G
Myh6 3rd position	2312–3048/3	GTR+I+G
Rag1 1st position	3049–4502/3	SYM+G
Rag1 2nd position	3050–4502/3	GTR+I+G
Rag1 3rd position	3051–4502/3	GTR+I+G
Rag2 1st position	4503–5501/3	SYM+I+G
Rag2 2nd position	4504–5501/3	GTR+I+G
Rag2 3rd position	4505–5501/3	SYM+G

Maximum likelihood (ML) analyses were performed in RAxML HPC2 on XSEDE (Stamatakis 2006) as implemented on CIPRES (Miller *et al.* 2010). This analysis employed a random starting tree, the GTR+G model (the only one for which RAxML applies; Stamatakis *et al.* 2008), and all other parameters at default values. One thousand bootstrap pseudoreplicates tested the support for each node in the most likely topology.

Results

The concatenated matrix resulted in a total of 5501 bp with 4639 variable sites. The Iss index revealed no saturation in transitions or transversions in either asymmetrical (Iss.cAsym) or symmetrical (Iss.sSym) topologies.

Fig. 3 shows the best maximum likelihood tree for the concatenated dataset for the Curimatidae. Our results corroborate the prior hypothesized monophyly of the Curimatidae and of *Curimata*, *Curimatopsis*, *Potamorhina*, *Psectrogaster*, and *Pseudocurimata*; thus, paralleling the morphological hypotheses of Vari (1983, 1989a). The remaining genera (*Curimatella*,

Cyphocharax and *Steindachnerina*) appeared as non-monophyletic in our results. This result was expected for *Cyphocharax* which lacks any identified synapomorphy (Vari 1989a), was only slightly less expected for *Curimatella* whose single proposed synapomorphy (Vari 1989a, 1992b) was paralleled in different manifestations in a subset of the species of *Cyphocharax*, but unexpected in *Steindachnerina* which was defined on the basis multiple synapomorphies (Vari 1991). Evaluation of the results revealed *Steindachnerina* non-monophyly was a consequence of a species assigned to the genus (*S. corumbae*; Pavanelli and Britski, 1999) post the intrageneric phylogeny (Vari 1991) without a phylogenetic analysis and whose actual relationships lie with species of *Cyphocharax*. Bayesian trees are shown in Fig. S2 and S3 and mostly agree with the ML topology.

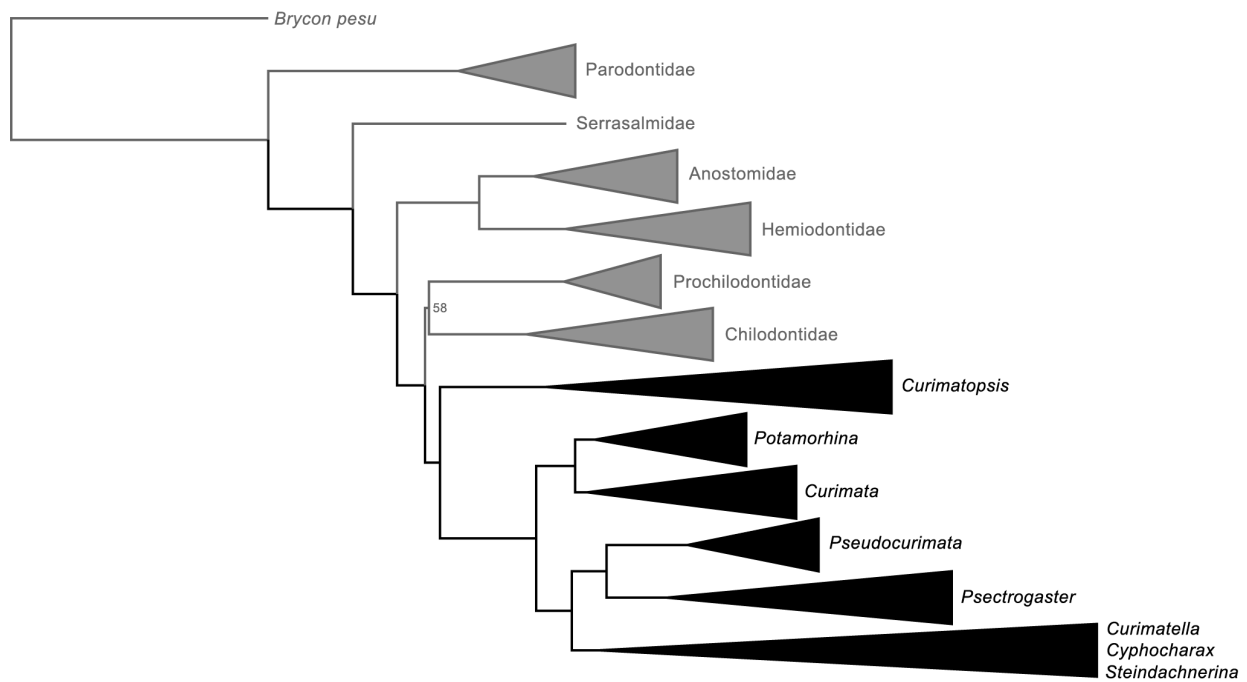


Figure 3. Intergeneric relationships of the Curimatidae based on the best maximum likelihood tree of the concatenated molecular dataset. Only values <80% of bootstrap are shown, and consequently well-supported clades are those without numbers.

Fig. 4 details the relationships among and within the four basal lineages in the Curimatidae - *Curimatopsis*, *Potamorhina*, *Curimata* and *Psectrogaster*. The highly modified *Curimatopsis* is monophyletic and sister to the seven other genera. Our phylogeny indicates that *C. macrolepis* from the Río Nanay is sister to the clade composed by the three other available

congeners. One specimen of *C. evelynae* occurred in the *C. crypticus* clade, rendering the later species paraphyletic. Samples from additional locations are prerequisite to a better determining the genetic lineages in these species. The most derived clade confirms the paraphyly of *C. crypticus* from the Amazon and Guianas, with *C. myersi* of the Río Paraguay nested within it.

In the second large clade, both *Curimata* and *Potamorhina* are monophyletic and we herein first propose their sister relationship. Nine of the 13 valid species of *Curimata* were analyzed and our phylogeny placed *C. vittata* (a specimen from each of the Río Tapajós and Río Orinoco) as sister to the remaining species of *Curimata*. The second intrageneric clade contains *C. incompta* from the Río Orinoco as sister to *C. acutirostris* plus *C. cisandina*, both from the Río Araguaia. Two specimens of *C. roseni* from the Río Nanay and Orinoco then resolved as sister to *C. inornata* from the Río Tapajós and *C. cyprinoides* from the Cuyuni River. The last portion of the phylogeny has *C. cyprinoides* from the Essequibo and Corantijn rivers as sister to *C. kneri* from the Rio Madeira and Rio Jari together with a second group of *C. roseni* from the Río Tapajós. Hence, results indicate the potential non-monophyly of both *C. cyprinoides* and *C. roseni*.

In the monophyletic *Potamorhina*, *P. laticeps*, a Lago Maracaibo endemic, is sister to the clade containing the remaining species of the genus. Maximum likelihood (Fig. 4) and Bayesian (Figs. S2–S3) analyses showed slightly distinct topologies in reference to the interspecific relationships of *Curimata* and *Potamorhina*. In that more restricted clade of the Bayesian tree (best supported), *P. latior* from the Amazon basin is sister to a clade composed of *P. squamorablevis* from the Río Paraguay plus the paraphyletic *P. altamazonica* of the Amazon basin.



Figure 4. Best maximum likelihood tree based on the concatenated dataset of the family Curimatidae. Numbers on branches represent bootstrap support.

A novel result of the analysis is a monophyletic, strongly supported clade with *Psectrogaster* the sister to *Pseudocurimata*. We incorporated all valid species of *Psectrogaster* in our dataset except for the rare northeastern *P. saguiru* and found strong support for generic monophyly. *Psectrogaster rhomboides* from the Rio Parnaíba, northeastern Brazil, is sister to all analyzed congeners. The linearized topology evidenced paraphyly for *P. curviventris* from the

Río Paraguay and *P. amazonica* from the Rio Solimões but a monophyletic *P. ciliata* from the Río Orinoco. Finally, *P. rutiloides* from the Río Nanay is sister to the clade containing *P. essequibensis* from the Essequibo that is, in turn, sister to *P. falcata* and a second specimen of *P. essequibensis*, both from the Rio Tapajós.

The monophyletic, trans-Andean genus *Pseudocurimata* includes six valid species, four of which were incorporated into the analysis. *Pseudocurimata lineopunctata* from the Rios Baudo and San Juan in Colombia appeared as sister to remaining congeners. *Pseudocurimata boelkhei* from Ecuador was then sister to the most derived clade, which has *P. boulengeri* from Ecuador as sister to *P. troschelli* from the Río Guayas in southern Ecuador and Río Tumbes in northern Peru.

Fig. 5 depicts the relationships within the most speciose lineage - named herein as the *Cyphocharax sensu lato* clade (CSLC) - that encompasses *Curimatella*, *Cyphocharax* and *Steindachnerina*. This lineage was previously hypothesized as a polytomy of these three genera plus *Pseudocurimata* (Vari, 1989a; Fig. 44). Three basal single species clades occur in CSLC: the first based on a single *Cyphocharax nigripinnis* from the Rio Negro with an autapomorphic densely pigmented adipose fin. Next is the highly modified *C. abramoides* from the upper Río Orinoco with a very deep body, a prominent predorsal spine and numerous minuscule scales over the body. Following along the phylogeny, *C. multilineatus*, a species that along with *C. pantostictos* (not analyzed) possesses derived longitudinal dark stripes on the trunk appeared as the sister to the clade which includes two large clades: the *Steindachnerina* clade and the *Cyphocharax sensu stricto* clade (CSSC; Fig. 5).

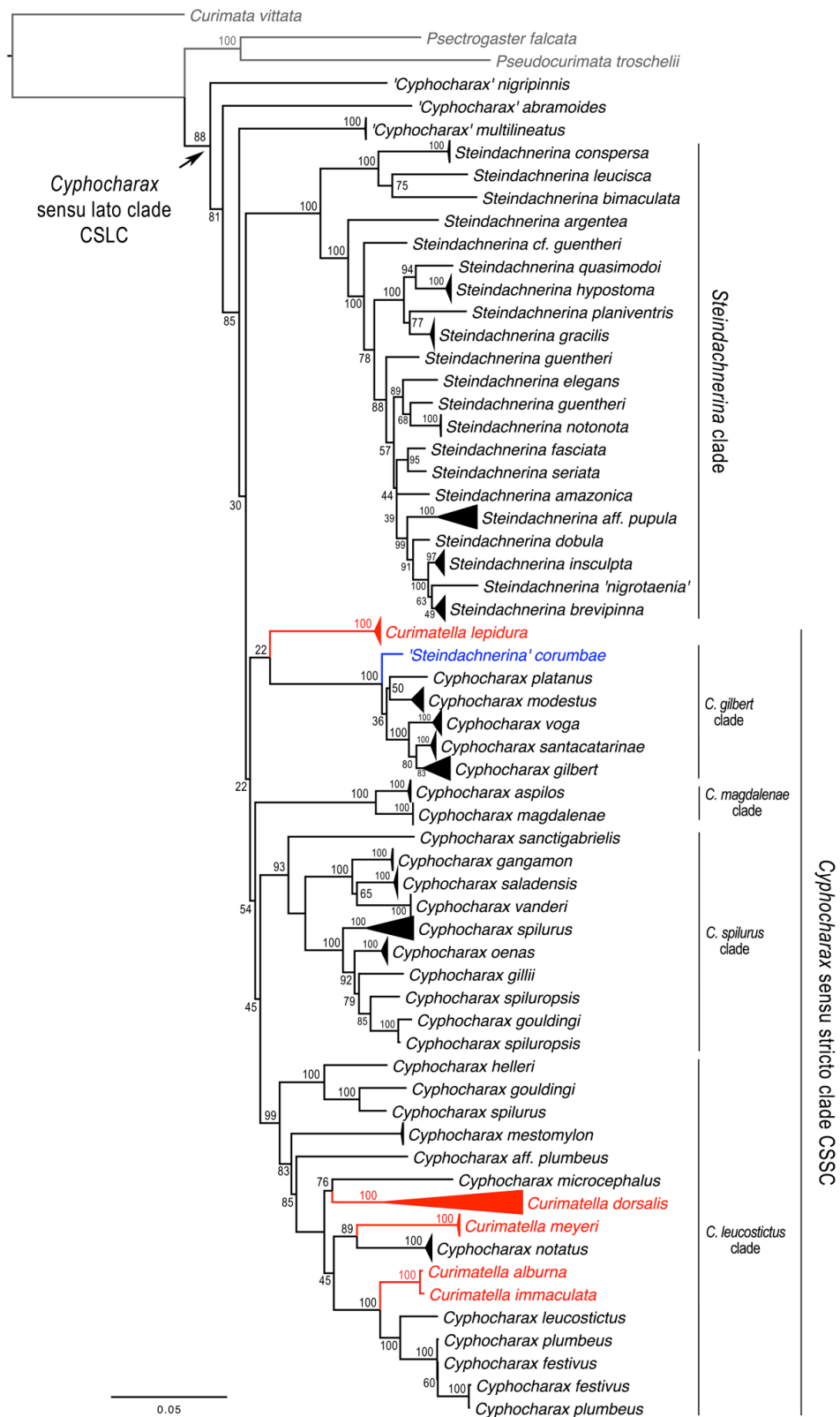


Figure 5. Best maximum likelihood tree of the species of “*Cyphocharax sensu lato* clade” (*Curimatella*, *Cyphocharax* and *Steindachnerina*) based on the reduced dataset. Numbers on branches represent percentages of the bootstrap support. Colored lineages are discussed in the text.

The well-supported *Steindachnerina* clade contains at its base a clade of *S. conspersa* from the Río Paraguay as sister to *S. bimaculata* from the Río Purus plus *S. leucisca* from the Río Napo. This clade is sister to a clade formed by the remaining species of *Steindachnerina* in which *S. argentea* from Trinidad is sister to the remaining species of *Steindachnerina*. The phylogeny then recovered a specimen identified as *S. cf. guentheri* as sister to all others in the clade; however, two other specimens of *S. guentheri* were observed within other lineages, indicating potential species polyphyly. Continuing along the phylogeny there is next a four-species clade of those species of *Steindachnerina* with transversely flattened preentral regions of the body. *Steindachnerina quasimodoi* from the Río Juruá is sister to *S. hypostoma* from the Río Napo and this two species clade is sister to *S. planiventris* from the Río Nanay plus *S. gracilis* from the Río Araguaia. Next along the phylogeny is a clade containing *S. elegans* from eastern Brazil that is sister to *S. notonota* from northeastern Brazil plus another *S. guentheri* from the Río Marañon in Peru. At this point of the phylogeny, we encounter a poorly-supported clade which includes multiple species including the two similarly spotted species, *S. fasciata* and *S. seriata* (paratype), from the Madeira and Tapajós rivers of the Brazilian Shield, respectively. These are strongly supported as sister species. The position of *Steindachnerina amazonica* within this portion of the phylogeny remains uncertain. Specimens identified herein as *S. aff. pupula* from the Río Purus are sister to a larger clade with *S. dobula* as sister to *S. insculpta* plus *S. brevipinna* and *S. nigrotaenia* (*sensu* Britski *et al.* 2007).

Two major clades remain in CSLC, the first of which, the *Cyphocharax sensu stricto* clade (CSSC), includes four monophyletic subclades termed herein the *C. gilbert* clade, the *C. magdalenae* clade, the *C. spilurus* clade and the *C. leucostictus* clade (Fig. 5). The relationships of *Curimatella lepidura* within CSSC were not well resolved in the molecular phylogeny. This species appeared closer to the *Cyphocharax gilbert* clade in the ML analysis but presented low bootstrap support (Fig. 5). Regardless, its position is distant from that of nominal congeners, which are interspersed in the *C. leucostictus* clade (red lineages).

Under the molecular hypothesis, the *Cyphocharax gilbert* clade is composed of *Steindachnerina corumbae* from the Rio Paranaíba as sister to all remaining species in this complex. This species was described post the analysis of *Steindachnerina* by Vari (1991) without a phylogenetic analysis to determine whether it shared the synapomorphies of the genus (Pavanelli and Britski 1999) and the phylogeny herein indicates that it is rather a species of

Cyphocharax. *Cyphocharax platanus* from the Rio Uruguay is sister to *C. modestus* from the upper Rio Paraná and these two species are closely related to the last portion of the lineage, a clade of three morphologically similar species in which *C. voga* is the sister to *C. santacatarinae* plus *C. gilbert*.

The second clade in CSSC (Fig. 5) is the small *Cyphocharax magdalenae* clade formed by a sister species pair: *C. magdalenae* from Costa Rica (source of our tissues, but the species ranges south to the Río Magdalena, Colombia) and *C. aspilos* from Lago Maracaibo. These two very similar species are the only trans-Andean species of *Cyphocharax*.

The third clade in CSSC is the *Cyphocharax spilurus* clade composed of nine analyzed species characterized by a dark, horizontally elongate to circular spot of caudal-peduncle pigmentation. This clade consists of *C. sanctigabrielis* (paratype) from the upper Rio Negro as sister to the remaining species. Within the latter clade is a well-supported clade with *C. gangamon* from the Rio Araguaia as sister to *C. saladensis* from the Río Uruguay plus *C. vanderi* from the upper Rio Paraná. The last major component of this clade contains *C. spilurus* from the Amazon basin (6 specimens) as sister to a clade containing *C. oenas* from the Río Orinoco which is, in turn, sister to a more restricted clade of *C. gillii* from the Río Paraguay which is sister to *C. spiluroopsis* from the Araguaia and Tapajós river basins plus *C. gouldingi* from the Araguaia. *Cyphocharax spiluroopsis* resolves as paraphyletic with *C. gouldingi* within it.

Curimatella clearly resolved as polyphyletic in our phylogeny (Fig. 5, red lineages) with *C. lepidura* particularly distant phylogenetically from its congeners. The evidence overwhelmingly justifies the synonymization of *Curimatella* into *Cyphocharax*. For purposes of clarity we continue to use that generic name in the following discussion as appropriate. The *Cyphocharax leucostictus* clade includes all species traditionally in *Curimatella* (except the morphologically much different *Curimatella* (= *Cyphocharax*) *lepidura*; see comments above on its placement), the five remaining species of *Cyphocharax* lacking caudal-peduncle pigmentation (*C. festivus*, *C. leucostictus*, *C. microcephalus*, *C. notatus* and *C. plumbeus*) plus four species with such pigmentation (*C. helleri*, *C. gouldingi*, *C. spilurus*, and *Curimatella dorsalis*) as a monophyletic unit. The basal lineage in this clade contains *Cyphocharax helleri* as sister to *C. gouldingi* plus *C. spilurus* with those two species resolving as non-monophyletic. This clade is now the subject of a more comprehensive study incorporating more *loci* and taxa. Next along the *Cyphocharax leucostictus* clade, *C. microcephalus* from the Commewijne River, Suriname,

forms a sister-species relationship with three specimens of *Curimatella dorsalis* from each of the Orinoco, Amazon and Paraguay basins. The two analyzed specimens of *Curimatella meyeri* from the Rio Madeira and Rio Juruá are most closely related to *Cyphocharax notatus* from the Rio Araguaia, rather than to traditional congeners. The most derived clade in the *C. leucostictus* clade has *Curimatella alburna* as sister to *C. immaculata*, with this species pair sister to a clade in which *Cyphocharax leucostictus* is sister to the non-monophyletic and morphologically very similar species *C. festivus* and *C. plumbeus*.

Nomenclatural changes

As a result of the phylogenetic evidence of this study, we make two revisions to the genus- and species-level nomenclature within the Curimatidae:

- Synonymization of the non-monophyletic *Curimatella* into *Cyphocharax*. As a consequence, the species previously assigned to *Curimatella* are transferred to *Cyphocharax* as new combinations: *C. alburnus*, *C. dorsalis*, *C. immaculatus*, *C. lepidurus* and *C. meyeri*.
- *Steindachnerina corumbae* is placed into the new combination - *Cyphocharax corumbae*.

Discussion

The multilocus molecular phylogeny supports the monophyly of the Curimatidae (Fig. 3) as first hypothesized based on morphological studies that yielded 19 synapomorphies in the branchial apparatus, buccopharyngeal complex, hyoid arch, jaws, palatine arch, and neurocranium (Vari 1983, 1989a) and in recent molecular studies (Oliveira *et al.* 2011; Melo *et al.* 2014). Placement of the Curimatidae as most closely related to the Chilodontidae and Prochilodontidae is recurrent in every high-level molecular phylogeny to date (*e.g.* Melo *et al.* 2014) and is undergoing further testing in an ongoing densely sampled molecular study of the Anostomoidea.

Our molecular analysis corroborated prior hypotheses (Vari 1989a) of a monophyletic *Curimatopsis* (Figs. 3–4) which is unsurprisingly since the hypothesis was based on 16 synapomorphies; Vari, 1982a; Vari, 1989a) and of its the sister group relationship to the remainder of the Curimatidae (Vari, 1989a). Three synapomorphies support the clade composed by *C. macrolepis* and *C. microlepis* (Vari, 1982a) and although we were unable to obtain samples of the rare *C. microlepis*, our results indicated monophyly for *C. macrolepis* as sister to

C. crypticus and *C. myersi*; results congruent with the morphological hypothesis (which did not include the later described *C. myersi*). Vari (1982a) did not resolve the relationships of *C. crypticus*, and *C. evelynae*. Results of this analysis indicate that *Curimatopsis crypticus*, a broadly distributed species represented in the analysis by a sample from Peru is sister to *C. myersi* from the Río Paraguay. We independently found distinct lineages of *C. crypticus* revealing a hidden diversity within the group and ongoing research indicates a need for a revised taxonomy. More broadly, a phylogeographic study of all species of *Curimatopsis* revealed several new cryptic species across the Amazon basin.

Our results revealed a sister relationship of *Potamorhina* with *Curimata*. This relationship, which is novel to this analysis, contrasts with the morphology-based hypothesis (Vari 1989a) that hypothesized the following alternative scheme of relationships (*Curimatopsis* (*Potamorhina* (*Curimata* (*Psectrogaster* (*Pseudocurimata* + *Curimatella* + *Cyphocharax* + *Steindachnerina*))))).

The largest body sizes within the Curimatidae occur in species of *Potamorhina*; a genus supported by 11 synapomorphies involving internal cartilages, infraorbitals and opercle, laterosensory canal system, anal-fin rays, and squamation (Vari, 1984, 1989a). We similarly recovered the genus as monophyletic, with *P. laticeps*, a Lago Maracaibo endemic and the only trans-Andean species in the genus, sister to the remainder of *Potamorhina*. This conclusion is congruent with the prior morphological hypothesis (Vari 1984). Our results recovered *P. latior* as sister to the clade containing *P. altamazonica* and *P. squamorablevis*. This three-species clade was also recovered by Vari (1984) on the basis of three synapomorphies but with an alternative internal configuration of *P. squamorablevis* sister to the clade of *P. altamazonica* plus *P. latior*. This later two species clade is supported by three unusual synapomorphic modifications of the gasbladder (Vari 1984). Based on our resolution, these modifications arose in the ancestor of the three species with subsequent loss in *P. squamorablevis* or independently in *P. altamazonica* and *P. latior*.

Curimata, the third largest curimatid genus, has a well-studied taxonomy and reasonably resolved morphologically based hypothesis of interrelationships (Vari 1989b). In so far as our phylogeny includes only eight of the 13 valid *Curimata* species, lineages are of necessity missing and future studies required to entirely resolve intrageneric relationships. The Bayesian topology (Fig. 4) has *C. vittata*, a distinctively pigmented species, as the sister clade of the remaining

species. Vari (1989b) did resolve the clade - proposing a trichotomy of *C. vittata*, *C. macrops* and a lineage of all remaining congeners. *Curimata vittata* possesses three autapomorphies associated with the epibranchial organ, reduced numbers of supraneurals, and patterns of pterygiophores and pigmentation, but interestingly our topology shows a deep split between the two samples of *C. vittata* (Rio Nanay in Peru and Rio Tapajós in Brazil) suggesting the presence of structured populations and, potentially undescribed species; a hypothesis to be tested by future phylogeographic studies. Following along the phylogeny of the genus, *C. incompta* appears as sister to *C. acutirostris* plus *C. cisandina*. This reconstruction differs from that of Vari (1989b) who placed *C. incompta* within a trichotomy (his Fig. 14). More recently, Vari and Reis (1995) described *C. acutirostris* from the Rio Araguaia and using Vari's (1989b) phylogeny found that species the sister of the clade containing *C. cisandina*, *C. inornata* and *C. roseni*; a result which differs from our reconstruction. Our results do corroborate the clade supported by 13 synapomorphies (Vari, 1989b) of *C. inornata* plus *C. cyprinoides roseni* as sister to *C. roseni* with, however, one specimen identified as *C. kneri* nested within *C. roseni* and neither *C. cyprinoides* nor *C. roseni* retrieved as monophyletic. Interestingly, *C. cyprinoides* and *C. kneri* although separated in our phylogeny are the most similar allopatric species in the genus; differing externally only in lateral-line scale counts.

Psectrogaster and *Pseudocurimata* appeared as sister genera in our molecular phylogeny. This novel hypothesis contrasts with Vari's (1989a) hypothesis that has *Psectrogaster* as sister to the four-genus polytomy of *Curimatella*, *Cyphocharax*, *Pseudocurimata* and *Steindachnerina*. This latter clade is supported by four synapomorphies: loss of the ventral process on the fourth ceratobranchial; presence of a basihyal tooth plate; reorientation of the canals in the infraorbitals; and reduction of the laterosensory canal system in the sixth infraorbital. Recognition of our molecular topology of *Pseudocurimata* as sister to *Psectrogaster* implies the independent acquisition of these derived conditions in *Pseudocurimata* and the ancestor of *Curimatella*, *Cyphocharax* and *Steindachnerina* (the *Cyphocharax sensu stricto* clade [CSSC] below).

Our results corroborate the monophyly of *Psectrogaster* as proposed by Vari (1989a, 1989c) with, however, an alternative, albeit incompletely resolved, scheme of species relationships. We found *P. rhomboides* as sister to all remaining species of the genus but with only moderate support, contrary to Vari's proposal of *P. rhomboides* as a member of the most derived intrageneric clade together with *P. amazonica* and *P. curviventris*. In our results *P.*

curviventris appears as paraphyletic and sister to *P. ciliata*, *P. amazonica*, *P. rutiloides*, *P. essequibensis* and *P. falcata*. The clade containing *P. essequibensis* appears paraphyletic with *P. falcata* nested within it. Such a relationship is not surprising given their external similarities, and may indicate a complex *P. essequibensis* including undescribed species.

Molecular results support the monophyly of the trans-Andean *Pseudocurimata*, which is supported by three synapomorphies related to the expansion of the second hypobranchial, the position of the first pterygiophore and the reduction or loss of the second pair of uroneurals. Internal resolution for *Pseudocurimata* similarly agrees with that advanced by Vari (1989d). The distinctively spotted *P. lineopunctata* is sister to all remaining species, followed by *P. boelkhei* and the clade containing *P. boulengeri* plus *P. troschelli*. Interestingly, the geographical distribution of the species (*P. lineopunctata* occurs at the northern most portion of the range for the genus and *P. troschelli* in the southern most extent) is concordant with the phylogenetic relationships and congruent with Vari's (1989d) hypothesis. Thus, both morphological and molecular data confirm initial diversification of *Pseudocurimata* at the north of its range followed by southward colonization through various central trans-Andean drainages and then diversification along their allopatric distributions.

Relationships within the Cyphocharax sensu lato clade (CSLC)

The *Cyphocharax sensu lato* clade (CSLC) encompasses those species previously assigned to *Cyphocharax*, *Curimatella* and *Steindachnerina*. Three taxonomic questions involving the basal portions of the CSLS phylogeny are of note.

The three basal clades in CSLC each consist of a single species, all now assigned to *Cyphocharax* (*C. nigripinnis*, *C. abramoides* and *C. multilineatus*, respectively). Given the topology of the tree, these sequential basal sister lineages result in the non-monophyly of *Cyphocharax* as now delimited. Two options exist as to how to address this problem. The first is retaining these three species in *Cyphocharax* via the expansion of the concept of the genus to encompass all species in CSLC. The major complication with this option is that under the topology of the overall familial phylogeny that course of action would also necessitate the synonymy into *Cyphocharax* of both *Steindachnerina* and *Curimatella* so as to achieve monophyly of *Cyphocharax*. This would result in an out-sized genus containing a disproportionate number (60 or 57%) of the 105 valid species in the Curimatidae (see, also

comments below concerning *Curimatella*). The seemingly preferable alternative to avoid such an outsized and unwieldy *Cyphocharax* is the description of new genus for each of these clades (with *Cyphocharax nigripinnis*, *C. abramoides* and *C. multilineatus* as type species, respectively). Although autapomorphies per se admittedly do not justify new genera, it is striking that each of these species is quite distinctive in the Curimatidae. We appreciate the advantages of this second option in avoiding an out-sized *Cyphocharax*; however, in our opinion proposal of the requisite new genera is premature. Both *Cyphocharax nigripinnis* and *C. abramoides* are incorporated into the phylogenetic analysis on the basis of a single sequenced specimen and in the case of *C. multilineatus*, the resolution of the phylogeny is unsatisfactory because it is poorly-supported by bootstrap pseudoreplicates. Given these problematic factors, we defer the proposal of the three new genera and generically identify each of the species as ‘*Cyphocharax*’. A need for additional morphological and molecular comparisons to test the phylogenetic hypothesis among basal portions of the CSLC clade is critical.

Relationships within the Steindachnerina clade

Vari (1989a) proposed four synapomorphies associated with restructurings of the branchial and hyoid arches that support monophyly of *Steindachnerina*. After the taxonomic revision and proposal of interspecific relationships (Vari 1991), four new species were described and assigned to the genus. Three of these were directly or indirectly phylogenetically analyzed. The first of these, *S. notograptos* of Lucinda and Vari (2009), was determined to be a relatively basal lineage sister to the majority of its congeners. The second *S. seriata* by Netto-Ferreira and Vari (2011) was hypothesized to be a member of an unresolved clade containing *S. dobula*, *S. pupula*, *S. quasimodoi*, *S. gracilis*, *S. hypostoma*, and *S. planiventris*. Although Géry *et al.* (1991) did not specifically phylogenetically analyze their *S. varii*, that species was barely a senior synonym of *S. runa* described, and phylogenetically analyzed, by Vari (1991) and as such assumes the phylogenetic placement of *S. runa*.

The fourth species, *Steindachnerina corumbae*, was described by Pavanelli and Britski (1999) who assigned it to *Steindachnerina* without a phylogenetic analysis. Thus, its placement remained untested until now. Our analysis included a specimen of the species from the Rio Corumbá proximate to the type-locality and revealed contra to its aphyletic assignment to *Steindachnerina* by Pavanelli and Britski (1997), that *S. corumbae* is instead a member of the

well-supported *Cyphocharax gilbert* clade as the sister to a group of *Cyphocharax* species. In order to resolve its erroneous original generic assignment problem, we herein transfer *S. corumbae* of those authors to *Cyphocharax* as a new combination, *Cyphocharax corumbae*.

Within the strongly supported *Steindachnerina* clade (without the mistakenly assigned *S. corumbae*), *S. conspersa* is sister to *S. bimaculata* plus *S. leucisca* in a basal subclade of this lineage. Vari (1991) proposed a clade formed by *S. argentea*, *S. conspersa*, *S. bimaculata* and *S. binotata* on the basis of two synapomorphies associated to the buccopharyngeal complex and pigmentation pattern. Alternatively, our phylogeny placed *S. argentea* as sister to all remaining congeners other than *S. bimaculata*, *S. conspersa*, and *S. leucisca*. Indeed, Vari (1991) recognized these three later species along with *S. binotata* as a distinct lineage supported by six synapomorphies. Evidence thereby suggests that the weakly developed flaps of the buccopharyngeal process and the small spot of pigmentation near the base of the middle caudal-fin rays (spot absent in *S. leucisca* and *S. binotata*) are plesiomorphic. Vari (1991) similarly found *S. conspersa* to be sister to the other species in the subclade based on one synapomorphy (one or more longitudinal series of small body spots). Within this later clade, *S. bimaculata* was hypothesized to be sister to *S. leucisca* plus *S. binotata* on the basis of five synapomorphies. Although *S. binotata* was unavailable for inclusion in this analysis, the topology herein strongly corroborates Vari (1991)'s hypothesis of interspecific relationships within this clade.

We corroborate the monophyly previously hypothesized by the morphological study (Vari 1991) of a large clade containing the remaining species of *Steindachnerina*. This lineage of fifteen species shared three synapomorphies, including the presence of remarkable, multiple, lobulated fleshy bodies on the buccopharyngeal complex. Within this lineage, Vari (1991) did not propose phylogenetic relationships among the various species, with *S. amazonica*, *S. fasciata*, *S. guentheri*, and *S. notonota* placed into a polytomy together with a multi-species lineage. Herein, we present the first hypothesis of relationships for these species, albeit with some lack of species-level resolution.

Vari (1991) recognized a distinctive clade with *Steindachnerina quasimodoi* as sister to a polytomy of *S. gracilis*, *S. hypostoma*, and *S. planiventris* that is supported by cranial modifications, elongation of the distance between the anus and the first anal-fin ray, and the possession of a distinctly flattened prepelvic region of the body. Herein, we recovered this clade

as monophyletic but with a limited resolution: ((*S. quasimodoi* + *S. hypostoma*)(*S. planiventris* + *S. gracilis*)).

Steindachnerina guentheri appeared three times along our phylogeny and its position remains uncertain; a situation potentially indicative of a species complex and/or misidentification. Although some species appeared as unresolved phylogenetically, the scheme of relationships in our analysis revealed various associations including a clade of *S. elegans* as sister to *S. guentheri* plus *S. notonota*. Vari (1991) did not find monophyly or close relationship among these species but they possess overall similar pigmentation patterns. Further morphological studies should test our molecular hypothesis.

The most derived clade in the *Steindachnerina* clade includes some well-supported subclades such as *Steindachnerina fasciata* plus *S. seriata*; species sharing a very distinctive pigmentation pattern of dark stripes along the lateral surface of the body. In their description of *S. seriata*, Netto-Ferreira and Vari (2011) placed *S. seriata* in the clade composed by *S. dobula*, *S. gracilis*, *S. hypostoma*, *S. planiventris*, *S. pupula* and *S. quasimodoi* contra to its association herein with *S. fasciata*.

Our results unite *Steindachnerina* aff. *pupula* as sister to the multi-species lineage that has *S. dobula* as sister to the most derived clade of *S. insculpta* which is, in turn, sister to *S. brevipinna* plus a single specimen identified here as *S. nigrotaenia sensu* Britski *et al.* (2007). This placement requires more critical analysis.

Relationships within the Cyphocharax sensu stricto clade (CSSC)

The *Cyphocharax sensu stricto* clade (CSSC) as herein delimited is well supported and includes all members traditionally assigned to *Cyphocharax* with the exception of '*Cyphocharax*' *abramoides*, '*Cyphocharax*' *multilineatus*, and '*Cyphocharax*' *nigripinnis* (see comments above concerning CSLC) and the addition of what was originally *Steindachnerina corumbae* (comments above) plus the species traditionally in *Curimatella*. Vari (1992a) revised *Cyphocharax* without arriving at a hypothesis of intrageneric phylogenetic relationships. Hence, our molecular phylogeny is the first hypothesis of interspecific relationships for the genus.

Curimatella lepidura (= *Cyphocharax lepidurus*) is included within the *Cyphocharax gilbert* clade under the molecular phylogeny albeit with low support (see discussion on *Curimatella* below). There is support for the group containing *Steindachnerina corumbae* (=

Cyphocharax corumbae) as sister to the clade of *Cyphocharax platanus* and *C. modestus*, from the lower and upper Rio Paraná, respectively. Although we did not analyze *Cyphocharax nagelii*, based on morphological evidence that species is expected to be a member of this clade. The last lineage is composed by three species from proximate geographic regions (*C. voga* (*C. santacatarinae* + *C. gilbert*)). Analyzed *C. voga* are from coastal southern Brazilian rivers, *C. santacatarinae* the Ribeira de Iguape in southeastern Brazil and *C. gilbert* of eastern basins north of the Ribeira de Iguape. This south to north progression parallels the phylogenetic relationships in the clade proposed in our analysis.

The *Cyphocharax magdalenae* group, the next clade in CSSC, is composed of two trans-Andean *Cyphocharax* species. *Cyphocharax aspilos* is a Lago Maracaibo endemic and *C. magdalenae* ranges from the Río Magdalena in Colombia through Pacific versant drainages to Costa Rica. These distinctive forms possess a deeper caudal peduncle without any dark pigmentation; a combination unique to them in *Cyphocharax* (Vari 1992a).

The well-supported *Cyphocharax spilurus* clade (Fig. 5) is the third in the CSSC and one of the most complex groups within the genus. All included taxa possess a dark midlateral patch of caudal-peduncle pigmentation; however, such a coloration pattern is not exclusive to this group in the genus. Species of *Cyphocharax* outside the clade with such pigmentation are *C. helleri*, *C. mestomylon* and members of the *C. gilbert* clade (*C. gilbert*, *C. modestus*, *C. platanus*, *C. santacatarinae* and *C. voga*). Unsourced species potentially of the *C. spilurus* clade are *C. aninha*, *C. biocellatus*, *C. meniscraporus*, *C. punctatus*, *C. signatus* and *C. spilotus*.

The first species diverging in the *Cyphocharax spilurus* clade is *C. sanctigabrielis*, which has distinct, broad, dark caudal-peduncle pigmentation (Melo and Vari 2014, Anexo 2). A reduction in the degree of caudal-peduncle pigmentation in the remaining species of this clade is a potential synapomorphy for that assemblage (Fig. 5). Following in the *C. spilurus* clade is a monophyletic three species clade with *C. gangamon* the sister to the species pair of *C. saladensis* plus *C. vanderi*. *Cyphocharax gangamon* occurs in the Amazon basin, *C. saladensis* in coastal rivers of southeastern Brazil and portions of the La Plata basin, with *C. vanderi* allopatrically endemic to the upper Rio Paraná in the La Plata system. This scheme relationship indicating possible species-pair cladogenesis in the La Plata basin should be tested in a broad morphologically based phylogenetic analysis.

Next along the *Cyphocharax spilurus* clade is the following lineage: (*C. spilurus* (*C. oenas* (*C. gillii* (*C. spiluroopsis* (*C. gouldingi* + *C. spiluroopsis*))))). These species demonstrate progressive increases in overall morphological similarity, albeit differing in details of the dark caudal-peduncle spot and multiple meristic features (Vari 1992a). This is the first hypothesis proposing monophyly for this clade and we suggest that the evolution of the caudal-fin pigmentation pattern had substantial influence on species diversification in at least this portion of *Cyphocharax*.

The last monophyletic assemblage in CSSC is the *Cyphocharax leucostictus* clade that contains the remaining species of the *Cyphocharax*, plus all species of *Curimatella* other than for *C. lepidura*. The next clade has *Cyphocharax helleri* united to a specimen of *C. gouldingi* and a specimen of *C. spilurus*. This demonstrates that some of the species could not be fully resolved based on our molecular dataset and/or paraphyly of *C. gouldingi* and *C. spilurus*.

Our results strongly demonstrate the polyphyly of *Curimatella*. Vari (1989a) supported the monophyly of the genus on the basis of one synapomorphy: the presence of scales extending across the lobes of the caudal fin. This character has been shown to have originated independently in distinct clades across the Characiformes (e.g. Anostomidae, Characidae, and Distichodontidae). More significantly, various species of *Cyphocharax* even under the traditional definition of the genus have diverse expanses of caudal-fin scaling, albeit never to a degree or of the exact form as that present in species of *Curimatella*. Interestingly the highly developed caudal fin scale pattern in species assigned to *Curimatella* does not delimit a monophyletic assemblage.

Although the position of *Curimatella lepidura* (= *Cyphocharax lepidurus*), a species that differs in many aspects from its traditional congeners remains uncertain in the CLSC, its congeners are closer phylogenetically to diverse species of *Cyphocharax* (Fig. 5) than to that species. The widely distributed *Curimatella dorsalis* (= *Cyphocharax dorsalis*) is sister to *Cyphocharax microcephalus* (a species with variable degrees of limited caudal-fin squamation), whereas *Curimatella meyeri* (= *Cyphocharax meyeri*) is sister to *Cyphocharax notatus*. Finally, *Curimatella alburna* (= *Cyphocharax alburnus*) is sister to *Curimatella immaculata* (= *Cyphocharax immaculatus*) with that two species clade, in turn, sister to the clade containing *Cyphocharax leucostictus*, *C. plumbeus* and *C. festivus*.

The phylogenetic evidence of our study more than amply demonstrates that *Curimatella* is definitely polyphyletic. Only one option exists to resolve this problem within the context of the overall familial phylogeny, the synonymy of *Curimatella* into *Cyphocharax*; an action we formally take herein.

Interesting all these species of *Cyphocharax* within the CSSC lack caudal-peduncle pigmentation with the exception of *C. mestomylon*, which appears sister to all members of this less-inclusive clade and *C. dorsalis* (see Fig. 5). *Cyphocharax plumbeus* is polyphyletic, appearing in different places across the *Cyphocharax leucostictus* clade and *C. festivus* presents as paraphyletic. This species-pair need taxonomic revision, since their morphological characters are quite variable across the Amazon basin.

Using high taxon sampling we present the first interspecific relationship for *Cyphocharax*, the most speciose curimatid genus (including species of the polyphyletic *Curimatella*). Future directions involve efforts to determine whether the description of new genera is needed to address the generic level assignments of “*Cyphocharax*” *abramoides*, “*C.*” *nigripinnis* and “*C.*” *multilineatus* (and possibly *C. pantostictus*) and to resolve questions of the placement of various species, in particular *Curimatella lepidura* (= *Cyphocharax lepidurus*).

Author contributions

BFM, RPV, BLS and CO designed the project. BFM, KH collected data. BFM, KH, BWF, BLS and CO performed phylogenetic analyses. BFM and RPV wrote most of the manuscript. All authors edited and revised the manuscript.

Acknowledgements

We thank M. Sabaj-Pérez (ANSP), N. Lujan and J. Armbruster (AUM), S. Mochel and K. Swagel (FMNH), R. Covain (MHNG), M. C. C. de Pinna and M. Gianeti (MZUSP), C. Lucena (MCP) and R. Reina (STRI) for curatorial assistance and/or loan of tissues and vouchers and M. Taylor for life coloration photos. Authors were funded by NSF (DEB-1257898) and FAPESP (proc. 2011/08374-1 and BEPE 2013/16436-2).

Table S1. Voucher specimens and locality information of curimatids and outgroups used in this study.

Taxon	Voucher	Tissues	Locality	Coordinates	City, State	Country
<i>Curimata acutirostris</i>	LBP 7744	36724	Rio Araguaia, Amazon basin	13°19'S 50° 37'W	Cocalinho, Mato Grosso	Brazil
<i>Curimata cisandina</i>	LBP 1802	13168	Rio Araguaia, Amazon basin	15°32'S 52°12'W	Barra do Garças, Mato Grosso	Brazil
<i>Curimata cisandina</i>	LBP 9260	41319	Rio Araguaia, Amazon basin	13°19'22.8"S 50°37'20.7"W	Cocalinho, Mato Grosso	Brazil
<i>Curimata cyprinoides</i>	MHNG 2705,006	157.7 / SU07-217	Cuyuni River, Corantijn basin	2,40614 -56,9268	Sipaliwini	Suriname
<i>Curimata cyprinoides</i>	USNM 402473	GY11-1-03	Cuyuni River, Essequibo basin	6.39592 -58.72884	Cuyuni-Mazaruni	Guyana
<i>Curimata cyprinoides</i>	USNM 402471	GY11-2-53	Cuyuni River, Essequibo basin	6.84722 -60.13631	Cuyuni-Mazaruni	Guyana
<i>Curimata incompta</i>	ANSP 191354	BO-6125	Río Ventuari, Orinoco basin			Venezuela
<i>Curimata inornata</i>	LBP 13842	57293	Rio Tapajós, Amazon basin	4°16'49.5"S 59°59'26.1"W	Itaituba, Pará	Brazil
<i>Curimata kneri</i>	UFRO uncatalogued	67020	Rio Madeira, Amazon basin		Porto Velho, Rondônia	Brazil
<i>Curimata roseni</i>	LBP 5430	27169	Rio Jari, Amazon basin	0°56'00"S 52°32'30"W	Almeirim, Pará	Brazil
<i>Curimata roseni</i>	LBP 12864	53467	Rio Tapajós, Amazon basin	04°16'49.5"S 59°59'26.1"W	Itaituba, Pará	Brazil
<i>Curimata roseni</i>	LBP 12864	53468	Rio Tapajós, Amazon basin	04°16'49.5"S 59°59'26.1"W	Itaituba, Pará	Brazil
<i>Curimata roseni</i>	ANSP 189094	V071	Río Ventuari, Orinoco basin		San Fernando Atabapo, Amazonas	Venezuela
<i>Curimata roseni</i>	ANSP 180389	T38	Río Nanay, Amazon basin		Maynas, Loreto	Peru
<i>Curimata vittata</i>	LBP 13846	57302	Rio Tapajós, Amazon basin	04°16'49.5"S 59°59'26.1"S	Itaituba, Pará	Brazil
<i>Curimata vittata</i>	ANSP 182217	V070	Río Ventuari, Orinoco basin		San Fernando Atabapo, Amazonas	Venezuela
<i>Curimatella alburna</i>	ANSP 191246	B06119	Río Ventuari, Orinoco basin		Manapiare, Amazonas	Venezuela
<i>Curimatella alburna</i>	ANSP 182223	T2177	Río Rupununi, Essequibo basin		Rupununi	Guyana
<i>Curimatella dorsalis</i>	LBP 3054	19167	Río Orinoco basin	07°38'11.6"S 66°19'04.2"W	Caicara del Orinoco, Bolívar	Venezuela
<i>Curimatella dorsalis</i>	LBP 3759	22034	Rio Negro, Paraguay basin	19°34'33.7"S 56°14'49.5"W	Aquidauana, Mato Grosso do Sul	Brazil
<i>Curimatella dorsalis</i>	OS 18376	PE10-069	Río Nanay, Amazon basin	-3.7516670000 - 73.3162500000	Maynas, Loreto	Peru
<i>Curimatella immaculata</i>	LBP 5606	27341	Rio Balsas, Rio Parnaíba basin	07°30'55"S 46°04'53"W	Balsas, Maranhão	Brazil
<i>Curimatella immaculata</i>	LBP 15269	63217	Rio Branco, Amazon basin	03°20'38.8"N 59°50'04.3"W	Bonfim, Roraima	Brazil
<i>Curimatella lepidura</i>	LBP 11352	45506	Rio São Francisco basin	17°13'02.7"S 44°48'14.7"W	Pirapora, Minas Gerais	Brazil
<i>Curimatella lepidura</i>	LBP 11352	45507	Rio São Francisco basin	17°13'02.7"S 44°48'14.7"W	Pirapora, Minas Gerais	Brazil
<i>Curimatella meyeri</i>	LBP 4253	22748	Rio Jurua, Amazon basin	07°09'49.6"S 73°43'29.7"W	Cruzeiro do Sul, Acre	Brazil
<i>Curimatella meyeri</i>	UFRO uncat	67037	Rio Madeira, Amazon basin		Amazonas	Brazil
<i>Curimatopsis crypticus</i>		7057				
<i>Curimatopsis crypticus</i>	LBP 4454	24361	Rio Negro, Amazon basin	00°40'03.1"S 62°58'23.5"W	Barcelos, Amazonas	Brazil
<i>Curimatopsis evelynae</i>		T09855				
<i>Curimatopsis macrolepis</i>	ANSP 178188	1697	Río Nanay, Amazon basin	03°45'23"S 73°17'28"W	Maynas, Loreto	Brazil

<i>Curimatopsis macrolepis</i>	OS 18337	PE10-086	Río Nanay, Amazon basin	-3.7516670 -73.2872220	Maynas, Loreto	Brazil
<i>Curimatopsis macrolepis</i>	OS 18767	PE10-087	Río Nanay, Amazon basin	-3.7516670 -73.2872220	Maynas, Loreto	Brazil
<i>Curimatopsis myersi</i>	LBP 14006	58310	Río Paraguay basin	17°49'26.7"S 57°31'03.0"W	Poconé, Mato Grosso	Brazil
<i>Curimatopsis myersi</i>	LBP 14006	58311	Ro Paraguay basin	17°49'26.7"S 57°31'03.0"W	Poconé, Mato Grosso	Brazil
<i>Cyphocharax abramoides</i>	ANSP 189092	V5411	Río Orinoco basin		Amazonas	Venezuela
<i>Cyphocharax aspilos</i>	LBP 6109	29560	Lago Maracaibo	09°38'53.8"N 72°34'56.4"W	Machiques del Perijá, Zulia	Venezuela
<i>Cyphocharax aspilos</i>	LBP 6109	29561	Lago Maracaibo	09°38'53.8"N 72°34'56.4"W	Machiques del Perijá, Zulia	Venezuela
<i>Cyphocharax festivus</i>	OS 18326	PE10-145	Río Nanay, Amazon basin	-3.7809720 -73.3638890	Maynas, Loreto	Peru
<i>Cyphocharax festivus</i>	OS 18326	PE10-149	Río Nanay, Amazon basin	-3.7809720 -73.3638890	Maynas, Loreto	Peru
<i>Cyphocharax gangamon</i>	LBP 1446	12595	Río Araguaia, Amazon basin	17°19'19.4"S 53°14'25.9"W	Alto Araguaia, Mato Grosso	Brazil
<i>Cyphocharax gangamon</i>	LBP 1446	12596	Río Araguaia, Amazon basin	17°19'19.4"S 53°14'25.9"W	Alto Araguaia, Mato Grosso	Brazil
<i>Cyphocharax gilbert</i>	LBP 8343	40130	Río Mucuri	17°41'42.4"S 40°46'11.3"W	Carlos Chagas, Minas Gerais	Brazil
<i>Cyphocharax gilbert</i>	LBP 10734	49733	Río Paraíba do Sul	22°04'7.8"S 41°54'36.2"W	Conceição do Macabu, Rio de Janeiro	Brazil
<i>Cyphocharax gillii</i>	LBP 10789	49895	Río Paraguay, La Plata basin	18°25'24.4"S 54°50'05.9"W	Coxim, Mato Grosso do Sul	Brazil
<i>Cyphocharax gouldingi</i>	LBP 1537	11889	Río Araguaia, Amazon basin	15°53'53.4"S 52°13'00.6"W	Aragarças, Goiás	Brazil
<i>Cyphocharax gouldingi</i>	LBP 2432	16291	Río Araguaia, Amazon basin	15°40'57.7"S 52°13'24.8"W	Barra do Garças, Mato Grosso	Brazil
<i>Cyphocharax helleri</i>	MHNG 2718.046	157.24	Tapanahony River, Marowijne River basin			Suriname
<i>Cyphocharax leucostictus</i>	LBP 8815	44165	Río Araguaia, Amazon basin	13°26'19.4"S 50°39'46.4"W	Cocalinho, Mato Grosso	Brazil
<i>Cyphocharax leucostictus</i>	LBP 15951	66102	Río Xingu, Amazon basin	13°29'41.8"S 53°04'57.7"W	Canarana, Mato Grosso	Brazil
<i>Cyphocharax magdalenae</i>	STRI 1144	BFD 1642	Río Coto, Río Coloradita		Puntarenas	Costa Rica
<i>Cyphocharax magdalenae</i>	STRI 1145	BFD 1631	Río Coto, Río Coloradita		Puntarenas	Costa Rica
<i>Cyphocharax mestomylon</i>	LBP 16372	67271	Río Tapajós, Amazon basin	04°52'14.1"S 56°51'19"W	Itaituba, Pará	Brazil
<i>Cyphocharax mestomylon</i>	LBP 16372	67272	Río Tapajós, Amazon basin	04°52'14.1"S 56°51'19"W	Itaituba, Pará	Brazil
<i>Cyphocharax microcephalus</i>	MHNG 2723.019	157.25	Commewijne River			Suriname
<i>Cyphocharax modestus</i>	LBP 8362	40165	Río Paraná, La Plata basin	22°22'42.4"S 47°25'37.9"W	Araras, São Paulo	Brazil
<i>Cyphocharax modestus</i>	LBP 9654	45695	Río Paraná, La Plata basin	22°13'59.9"S 53°47'02.6"W	Ivinhema, Mato Grosso do Sul	Brazil
<i>Cyphocharax multilineatus</i>	LBP 6964	34001	Río Negro, Amazon basin	00°00.321'N 66°55.357"W	São Gabriel da Cachoeira, Amazonas	Brazil
<i>Cyphocharax multilineatus</i>	LBP 6964	34002	Río Negro, Amazon basin	00°00.321'N 66°55.357"W	São Gabriel da Cachoeira, Amazonas	Brazil
<i>Cyphocharax nigripinnis</i>	LBP 4490	24472	Río Negro, Amazon basin	00°53'18.6"S 62°40'36.1"W	Barcelos, Amazonas	Brazil
<i>Cyphocharax notatus</i>	LBP 5734	26865	Río Araguaia, Amazon basin	15°19'57.6"S 52°12'10.4"W	Barra do Garças, Mato Grosso	Brazil
<i>Cyphocharax notatus</i>	LBP 5734	26867	Río Araguaia, Amazon basin	15°19'57.6"S 52°12'10.4"W	Barra do Garças, Mato Grosso	Brazil
<i>Cyphocharax oenas</i>	LBP 9970	46801	Laguna Castellero, Orinoco	7°37'53.2"N 66°09'14.6"W	Caicara del Orinoco,	Venezuela

			basin		Bolivar	
<i>Cyphocharax oenas</i>	LBP 9970	46802	Laguna Castillero, Orinoco basin	7°37'53.2"N 66°09'14.6"W	Caicara del Orinoco, Bolivar	Venezuela
<i>Cyphocharax platanus</i>	MCP 21655	T21655	Rio Uruguay basin	29°29'54"S 56°49'12"W	Uruguaiana, Rio Grande do Sul	Brazil
<i>Cyphocharax plumbeus</i>	OS 18373	PE10-071	Rio Nanay, Amazon basin	-3.7516670 -73.316250	Maynas, Loreto	Peru
<i>Cyphocharax plumbeus</i>	OS 18769	PE10-099	Rio Nanay, Amazon basin	-3.7516670 -73.287222	Maynas, Loreto	Peru
<i>Cyphocharax saladensis</i>	LBP 13164	55055	Rio Uruguay basin	29°30'42.8"S 56°43'09.9"W	Uruguaiana, Rio Grande do Sul	Brazil
<i>Cyphocharax saladensis</i>	LBP 13164	55062	Rio Uruguay basin	29°30'42.8"S 56°43'09.9"W	Uruguaiana, Rio Grande do Sul	Brazil
<i>Cyphocharax sanctigabrielis</i>	LBP 6993	33399	Rio Negro, Amazon basin	00°00.321'N 66°55.357'W	São Gabriel da Cachoeira, Amazonas	Brazil
<i>Cyphocharax santacatarinae</i>	LBP 7390	35379	Rio Ribeira de Iguape	24°35'41.1"S 48°12'53.3"W	Itapeúna, São Paulo	Brazil
<i>Cyphocharax santacatarinae</i>	LBP 7447	35788	Rio Ribeira de Iguape	24°15'06.3"S 47°14'53.8"W	Registro, São Paulo	Brazil
<i>Cyphocharax spilolus</i>	LBP 4747	25521	Rio Guaíba, Atlantic drainage	30°17'07.0"S 51°18'01.1"W	Barra do Ribeiro, Rio Grande do Sul	Brazil
<i>Cyphocharax spiluroopsis</i>	LBP 8876	44345	Rio Araguaia, Amazon basin	13°22'36.1"S 50°40'08.4"W	Cocalinho, Mato Grosso	Brazil
<i>Cyphocharax spiluroopsis</i>	LBP 14163	59220	Rio Tapajós, Amazon basin	04°33'45.4"S 56°15'36.9"W	Itaituba, Pará	Brazil
<i>Cyphocharax</i> cf. <i>spilurus</i>	MHNG uncat	157.16	Comté			Suriname
<i>Cyphocharax</i> gr. <i>spilurus</i>	MHNG uncat	157.19	Corantijne River basin			Suriname
<i>Cyphocharax</i> gr. <i>spilurus</i>	MHNG uncat	157.20	Sipaliwini River basin			Suriname
<i>Cyphocharax spilurus</i>	ANSP 189157	6877	Lawa River, Marowijne basin		Sipaliwini	Suriname
<i>Cyphocharax spilurus</i>		GY11-2-74	Cuyuni River, Essequibo basin		Cuyuni-Mazaruni	Guyana
<i>Cyphocharax spilurus</i>	USNM 403679	GY11-4-43	Cuyuni River, Essequibo basin	6.79711 -59.9928	Cuyuni-Mazaruni	Guyana
<i>Cyphocharax vanderi</i>	LBP 3871	22449	Rio Paranapanema, La Plata basin	22°59'25.3"S 48°25'37.9"W	Botucatu, São Paulo	Brazil
<i>Cyphocharax vanderi</i>	LBP 3871	22450	Rio Paranapanema, La Plata basin	22°59'25.3"S 48°25'37.9"W	Botucatu, São Paulo	Brazil
<i>Cyphocharax voga</i>	LBP 3340	20479	Small Atlantic draining river	32°09'06.9"S 52°06'24.2"W	Rio Grande, Rio Grande do Sul	Brazil
<i>Cyphocharax voga</i>	LBP 17002	68367	Laguna dos Patos	32°052'2.8"S 52°15'14.4"W	Rio Grande, Rio Grande do Sul	Brazil
<i>Potamorhina altamazonica</i>	LBP 2571	17020	Rio Purus, Amazon basin	08°51'21.5"S 68°42'22.6"W	Boca do Acre, Amazonas	Brazil
<i>Potamorhina altamazonica</i>	OS 18312	PE10-118	Rio Nanay, Amazon basin		Maynas, Loreto	Peru
<i>Potamorhina altamazonica</i>	OS 18312	PE10-119	Rio Nanay, Amazon basin		Iquitos, Loreto	Peru
<i>Potamorhina laticeps</i>	LBP 6133	29516	Lago Maracaibo	09°38'53.8"N 72°34'56.4"W	Machiques de Perijá, Zulia	Venezuela
<i>Potamorhina latior</i>	LBP 4252	22717	Rio Juruá, Amazon basin	07°09'49.6"S 73°43'29.7"W	Cruzeiro do Sul, Acre	Brazil
<i>Potamorhina latior</i>	LBP 14931	61544	Rio Branco, Amazon basin	01°50'05.2"N 61°06'53.2"W	Caracará, Roraima	Brazil
<i>Potamorhina</i>	LBP 3768	22067	Rio Paraguay basin	19°34'33.7"S 56°14'49.5"W	Aquidauana, Mato Grosso	Brazil

<i>squamoralevis</i>					do Sul	
<i>Potamorhina squamoralevis</i>	LBP 3768	22070	Río Paraguay basin	19°34'33.7"S 56°14'49.5"W	Aquidauana, Mato Grosso do Sul	Brazil
<i>Psectrogaster amazonica</i>	OS 18313	PE10-011	Río Amazonas, Amazon basin	03°43'05.8"S/73°12'46.1"W	Iquitos, Loreto	Peru
<i>Psectrogaster amazonica</i>	OS 18313	PE10-013	Río Amazonas, Amazon basin	03°43'05.8"S/73°12'46.1"W	Iquitos, Loreto	Peru
<i>Psectrogaster ciliata</i>	LBP 3057	19164	Río Orinoco basin	07°38'11.6"N 66°19'04.2"W	Caicara del Orinoco, Bolivar	Venezuela
<i>Psectrogaster ciliata</i>	LBP 3057	19165	Río Orinoco basin	07°38'11.6"N 66°19'04.2"W	Caicara del Orinoco, Bolivar	Venezuela
<i>Psectrogaster curviventris</i>	LBP 657	8076	Río Paraguay basin	16°25.680"S 56°25.143"W	Poconé, Mato Grosso	Brazil
<i>Psectrogaster curviventris</i>	LBP 3850	22280	Río Paraguay basin	19°34'02.3"S 56°14'09.1"W	Aquidauana, Mato Grosso do Sul	Brazil
<i>Psectrogaster essequibensis</i>	LBP 14272	59531	Río Tapajós, Amazon basin	05°14'08.1"S 56°25'55.1"W	Itaituba, Pará	Brazil
<i>Psectrogaster essequibensis</i>	USNM 401563	GY11-1-22	Cuyuni River, Essequibo basin	6.87464 -60.2485	Cuyuni-Mazaruni	Guyana
<i>Psectrogaster falcata</i>	LBP 13777	57112	Río Tapajós, Amazon basin	04°27'29.5"S 56°16'03.0"W	Itaituba, Pará	Brazil
<i>Psectrogaster rhomboides</i>	LBP 5533	27204	Río Parnaíba basin	07°32'26"S 46°02'21"W	Balsas, Maranhão	Brazil
<i>Psectrogaster rutiloides</i>	OS 18315	PE10-114	Río Nanay, Amazon basin		Iquitos, Loreto	Peru
<i>Psectrogaster rutiloides</i>	OS 18315	PE10-116	Río Nanay, Amazon basin		Iquitos, Loreto	Peru
<i>Pseudocurimata boelkhei</i>		T13562				Ecuador
<i>Pseudocurimata boelkhei</i>		T13563				Ecuador
<i>Pseudocurimata boulengeri</i>		T13773				Ecuador
<i>Pseudocurimata boulengeri</i>		T13774				Ecuador
<i>Pseudocurimata lineopunctata</i>	BFD01628	STRI-1389	Río Baudó, Pacific versant river			Colombia
<i>Pseudocurimata lineopunctata</i>	BFD01630	STRI-881	Río San Juan, Pacific versant river			Colombia
<i>Pseudocurimata troschelii</i>	LBP 9371	43968	Río Tumbes, Pacific versant river	03°36'37.7"S 80°26'23.9"W	Tumbes, Tumbes	Peru
<i>Pseudocurimata troschelii</i>	Univ. Guayaquil 1542	PSB201	Río Guayas basin	02°24'39.2"S 79°37'25.4"W	Churute, Guayas	Ecuador
<i>Pseudocurimata troschelii</i>	Univ. Guayaquil 1542	PSB202	Río Guayas basin	02°24'39.2"S 79°37'25.4"W	Churute, Guayas	Ecuador
<i>Steindachnerina amazonica</i>	LBP 2463	16313	Río Araguaia, Amazon basin	15°52'40.4"S 52°18'15.5"W		
<i>Steindachnerina argentea</i>	BFD01625	STRI-4270	Turure River, Trinidad			Trinidad and Tobago
<i>Steindachnerina bimaculata</i>	LBP 173	4023	Río Purus, Amazon basin	10°03.320'W 67°51.450'W	Rio Branco, Acre	Brazil
<i>Steindachnerina</i>	LBP 5185	26336	Río Paraná, La Plata basin	22°47'29"S 53°20'58"W	Porto Rico, Paraná	Brazil

<i>brevipinna</i>						
<i>Steindachnerina brevipinna</i>	LBP 8448	42357	Río Paraguay, La Plata basin	16°03'13.6"S 57°48'31.8"W	Cáceres, Mato Grosso	Brazil
<i>Steindachnerina conspersa</i>	LBP 14055	58401	Río Paraguay, La Plata basin	17°49'51.7"S 57°23'42.8"W	Poconé, Mato Grosso	Brazil
<i>Steindachnerina conspersa</i>	LBP 14055	58402	Río Paraguay, La Plata basin	17°49'51.7"S 57°23'42.8"W	Poconé, Mato Grosso	Brazil
<i>Steindachnerina corumbae</i>	LBP 17244	68908	Río Corumbá, La Plata basin	17°42'03.4"S 48°33'18.4"W	Caldas Novas, Goiás	Brazil
<i>Steindachnerina dobula</i>	ANSP 180798	4123	Río Madre de Diós, Amazon basin		Madre de Dios	Peru
<i>Steindachnerina dobula</i>	LBP 16820	69256	Río Purus, Amazon basin	10°03.320'S 67°51.450'W	Rio Branco, Acre	Brazil
<i>Steindachnerina elegans</i>	LBP 8272	38329	Rio Verde Grande, São Francisco basin	15°19'24.2"S 43°39'53.5"W	Jaíba, Minas Gerais	Brazil
<i>Steindachnerina elegans</i>	LBP 8272	38330	Rio Verde Grande, São Francisco basin	15°19'24.2"S 43°39'53.5"W	Jaíba, Minas Gerais	Brazil
<i>Steindachnerina fasciata</i>	MZUSP 96802	6167	Rio Tapajós, Amazon basin	10°23'10.0"S 54°18'22.0"W	Peixoto de Azevedo, Mato Grosso	Brazil
<i>Steindachnerina gracilis</i>	LBP 8855	44286	Rio Araguaia, Amazon basin	13°22'36.1"S 50°40'08.4"W	Cocalinho, Mato Grosso	Brazil
<i>Steindachnerina gracilis</i>	LBP 8855	44287	Rio Araguaia, Amazon basin	13°22'36.1"S 50°40'08.4"W	Cocalinho, Mato Grosso	Brazil
<i>Steindachnerina guentheri</i>	LBP 12560	54206	Río Marañón, Amazon basin	04°28'28.2"S 73°35'46.0"W	Nauta, Loreto	Peru
<i>Steindachnerina cf. guentheri</i>	AUM 54441	T09661				
<i>Steindachnerina guentheri</i>	AUM uncat	T09791				
<i>Steindachnerina hypostoma</i>	ANSP 178124	1736	Río Napo, Amazon basin		Maynas, Loreto	Peru
<i>Steindachnerina hypostoma</i>	ANSP 178124	1742	Río Napo, Amazon basin		Maynas, Loreto	Peru
<i>Steindachnerina insculpta</i>	LBP 3192	19389	Rio Tietê, La Plata basin	23°00'46.0"S 47°59'51.9"W	Conchas, São Paulo	Brazil
<i>Steindachnerina insculpta</i>	LBP 12311	40021	Rio Araras, La Plata basin	22°22'42.4"S 47°25'37.9"W	Araras, São Paulo	Brazil
<i>Steindachnerina leucisca</i>	ANSP 178125	1744	Río Napo, Amazon basin		Maynas, Loreto	Peru
<i>Steindachnerina notonota</i>	LBP 2354	16488	Rio Ceará-Mirim	05°37'47"S 35°37'09"W	Taipu, Rio Grande do Norte	Brazil
<i>Steindachnerina notonota</i>	LBP 2354	16489	Rio Ceará-Mirim	05°37'47"S 35°37'09"W	Taipu, Rio Grande do Norte	Brazil
<i>Steindachnerina planiventris</i>	OS 18324	PE10-144	Río Nanay, Amazon basin	-3.7890720 -73.3638890	Maynas, Loreto	Peru
<i>Steindachnerina planiventris</i>	OS 18324	PE10-146	Río Nanay, Amazon basin	-3.7890720 -73.3638890	Maynas, Loreto	Peru
<i>Steindachnerina quasimodoi</i>	LBP 4125	23621	Rio Juruá, Amazon basin	07°26'35.5"S 73°03'33.5"W	Mâncio Lima, Acre	Brazil
<i>Steindachnerina seriata</i>	MZUSP 97569	7272	Rio Tapajós, Amazon basin	07°51'47.0"S 55°10'47.0"W	Novo Progresso, Pará	Brazil
<i>Ichthyoelephas longirostris</i>	ANSP 192865	6609	Rio Magdalena basin	05°12'20.3"N/75°44'49.7"W	Honda, Tolima	Colombia
<i>Prochilodus nigricans</i>	OS 18792	PE10-045	Río Itaya, Amazon basin	-3.7898610000/-73.2495830000	Maynas, Loreto	Peru
<i>Semaprochilodus taeniurus</i>	LBP 1691	12757	Rio Amazonas, Amazon basin	03°04'38"S/59°49'32"W	Manaus, Amazonas	Brazil
<i>Leporellus cf. vittatus</i>	AUM 54212	T09912				

<i>Schizodon scotorhabdotus</i>	AUM 53654	T09707				
<i>Chilodus fritillus</i>	AUM 51355	T10201	Río Madre de Dios, Amazon basin	12.27713S/69.15237W	Madre de Dios	Peru
<i>Caenotropus mestomorgmatos</i>	ANSP 180516	T48	Río Nanay, Amazon basin	3°46'45"S/73°226"W	Iquitos, Loreto	Peru
<i>Anodus elongatus</i>	OS 18724	PE10-110	Río Amazonas, Amazon basin	03°43'05.8"S/73°12'46.1"W	Iquitos, Loreto	Peru
<i>Hemiodus unimaculatus</i>	OS18345	PE10-076	Río Nanay, Amazon basin	-3.7516670000/-73.3162500000	Iquitos, Loreto	Peru
<i>Apareiodon affinis</i>	LBP 4591	24665	Río Paranapanema, Paraná basin	22°54'15.4"S/50°00'03.8"W	Salto Grande, São Paulo	Brazil
<i>Parodon nasus</i>	LBP 1135	5635	Rio Tietê, Paraná basin	22°52'S/48°23'W	Botucatu, São Paulo	Brazil
<i>Myleus schomburgkii</i>	OS uncat	PE10-044				
<i>Brycon pesu</i>	OS 18361	PE10-072	Rio Nanay, Amazon basin	-3.7516670000/-73.3162500000	Iquitos, Loreto	Peru

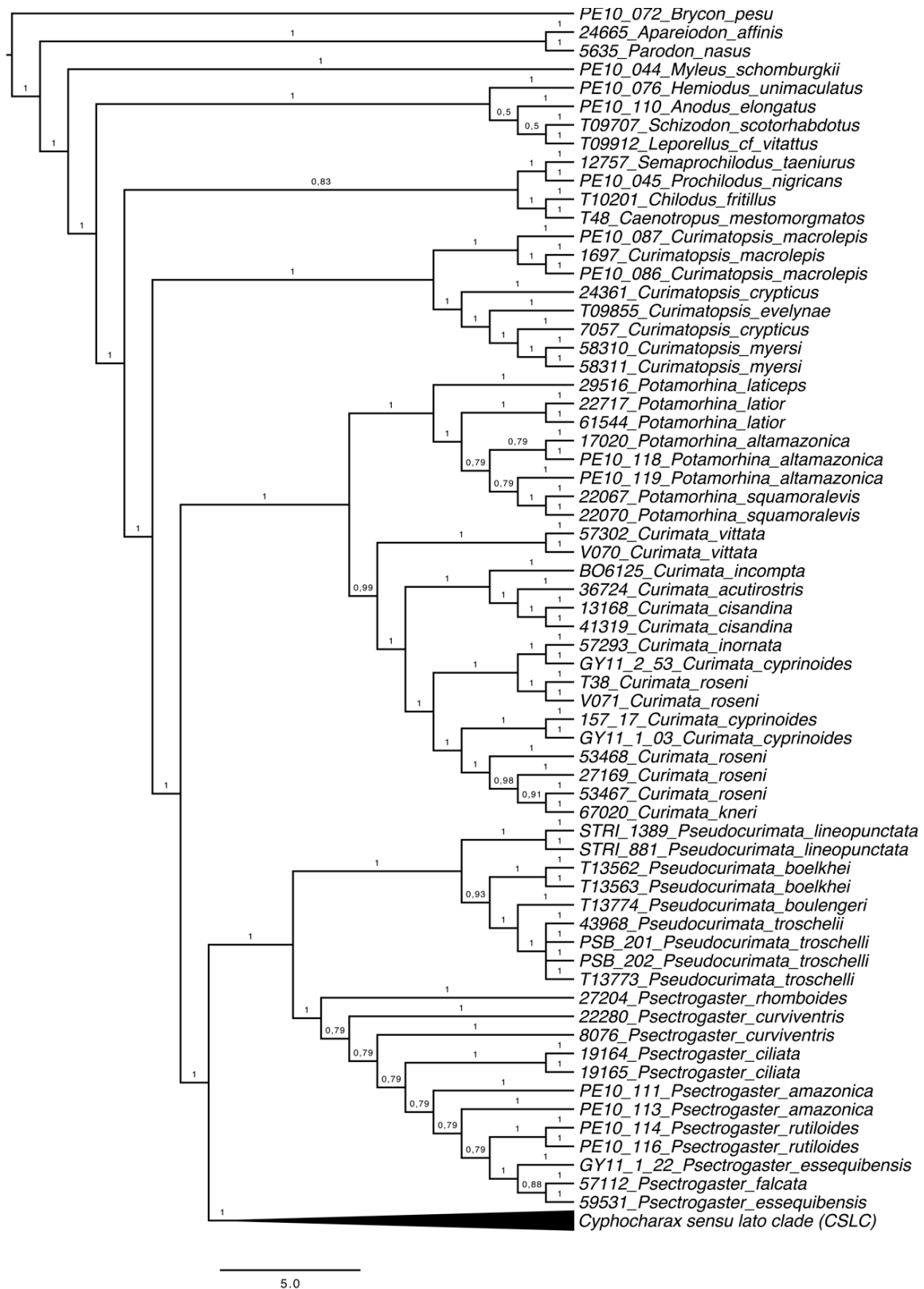


Figure S2. Bayesian analysis of the concatenated dataset of the family Curimatidae. Numbers on branches represent posterior probabilities.

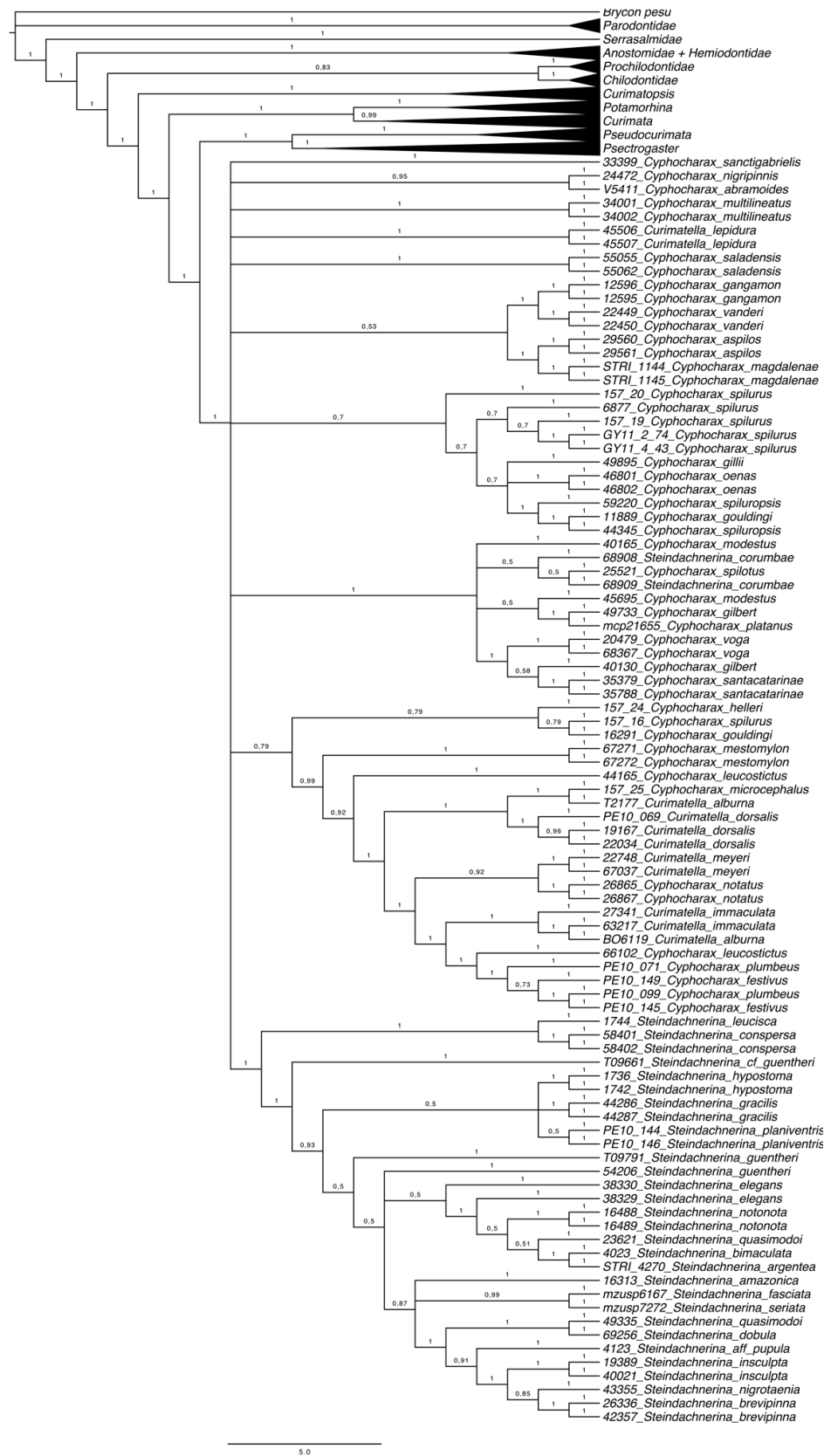


Figure S3. Bayesian analysis of the concatenated dataset of the Curimatidae showing the resolution within the *Cyphocharax sensu lato* clade (CSLC). Numbers on branches represent posterior probabilities.

Capítulo 4

Molecular phylogenetics of the family Anostomidae (Teleostei: Characiformes)

Brian L. Sidlauskas^{1,2*}, Bruno F. Melo³, Kendra Hoekzema¹, Michael Burns¹, Benjamin W. Frable¹, Mark Sabaj Perez⁴, Claudio Oliveira³, Richard P. Vari²

¹Department of Fisheries and Wildlife, Oregon State University, Corvallis OR, USA

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

³Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu SP, Brazil

⁴Department of Ichthyology, Academy of Natural Sciences, Philadelphia, PA, USA

* Corresponding author: B. L. Sidlauskas (brian.sidlauskas@oregonstate.edu)

Target Journal: *Molecular Phylogenetics and Evolution*

Running title: Molecular phylogeny of the headstanders of the family Anostomidae

Highlights:

- First molecular phylogeny for Anostomidae.
- Anostominae sister to *Leporellus* rather than *Laemolyta*.
- *Anostomus* paraphyletic with *A. brevior* sister to *Petulanos*.
- *Leporinus* paraphyletic with several monophyletic clades.

Keywords: Freshwater fishes, *Leporinus*, multilocus analysis, phylogeny, systematics.

Abstract

Within the hyperdiverse fish order Characiformes, the Neotropical Anostomidae is one of the most ecomorphologically diverse families with approximately 150 species distributed in 14 genera, mostly within the paraphyletic *Leporinus*. Despite the prevalence of these fishes throughout South America, their phylogenetic relationships have only recently been reconstructed using a comprehensive morphological dataset. Until now, no study has treated the family's relationships in any depth using molecular data. We used a multilocus dataset of three mitochondrial and three nuclear genes spanning 62% of known species and all fourteen genera. Our results corroborate the monophyly of the family, and place it as sister to a clade containing the other three anostomoid families: Prochilodontidae, Curimatidae and Chilodontidae. Within the family, our reconstruction of intrageneric relationships agrees largely with the earlier morphological study, but differs in the placement of Anostominae as sister to *Leporellus* rather than *Laemolyta*, implying the convergent evolution of the retention of a superior mouth throughout ontogeny. We also returned a non-monophyletic *Anostomus*. Within the paraphyletic genus *Leporinus*, which earlier provided impossible to resolve with morphological data, we reconstructed strong support for several novel clades that can serve to improve the classification of species of the genus in an eventual revision. Aspects of body coloration unify several of these clades suggesting that pigmentation may hold substantial phylogenetic signal within Anostomidae. Aside from providing a new look at the relationships and classification of these important fishes, the phylogeny will serve as framework for future evolutionary studies of pigmentation, osteology, morphometrics, dentition and ecological variation.

Introduction

The Neotropical family Anostomidae is one of the most diverse groups of characiform fishes, comprising approximately 150 species (Eschmeyer and Fong, 2015; Sidlauskas and Birindelli, in press). They inhabit many environments ranging from deep lakes and large reservoirs to very fast rapids, and occur in all major Neotropical drainages and small coastal rivers from southern Central America to portions of Argentina (Garavello and Britski 2003). Due to their huge variety of head shapes, dentition, coloration, and dietary preferences, anostomids form one of the most interesting cases of ecomorphologically diversification among fishes (Sidlauskas 2008; Sidlauskas and Vari 2008).

Vari (1983) treated the phylogenetic relationships of Anostomidae and proposed its monophyly and close relationship with members of Chilodontidae. Winterbottom (1980) also studied morphological features of members of the subfamily Anostominae that possesses the most strongly superior mouths, and proposed the first species-level phylogeny for any portion of the family. In the most comprehensive phylogenetic study to date, Sidlauskas and Vari (2008) used osteological and anatomical characters for the entire family and corroborated Vari (1983)'s hypothesis of familial monophyly. They also confirmed the monophyly of most genera (with the notable exception of *Leporinus*) erected a new distinctive genus *Petulanos*, and elevated the subgenus *Hypomasticus* to generic status (Sidlauskas and Vari 2008). The morphological phylogeny of Sidlauskas and Vari (2008) placed *Leporellus* as sister to all remaining genera and *Leporinus* as paraphyletic with *Abramites* sister to *Leporinus striatus*. Their hypothesis also embeds most other anostomid genera in a single clade nested within *Leporinus*, in which *Rhytiodus* is sister to *Schizodon* and *Laemolyta* is sister to the Anostominae *sensu* Winterbottom (1980).

Despite an increasing number of molecular phylogenetic studies focusing on components of Characiformes (Oliveira *et al.* 2011; Abe *et al.* 2014; Melo *et al.* 2014; Thompson *et al.* 2014), none until now have focused on the Anostomidae. Our aim was test the morphological hypothesis of intergeneric and interspecific relationships of the Anostomidae (Sidlauskas and Vari 2008) and to propose the first molecular phylogeny for the family, which will in turn serve as a framework for future evolutionary studies.

Material and methods

Taxon sampling

The anostomid taxa used for the molecular analyses are listed in Table S1. We sampled 181 terminal taxa covering 91 of approximately 150 known species (62% taxon coverage) and all fourteen genera. The outgroup taxa included species from each of the other three anostomoid families (Chilodontidae, Curimatidae and Prochilodontidae), representatives of three families (Hemiodontidae, Parodontidae and Serrasalminidae) previously hypothesized to be closely related to Anostomoidea, and one specimen of *Brycon pesu*, which was used to root the trees. We used tissues preserved in 95% ethanol or a saturated DMSO/NaCl solution, primarily from specimens deposited in museum and university collections (Table S1; abbreviations follow Sabaj Pérez (2013)).

Multilocus sequencing

We extracted DNA from tissue muscles or fins with a DNeasy Tissue kit (Qiagen Inc.) following manufacturer's instructions or a modified NaCl extraction protocol adapted from Lopera-Barrero *et al.* (2008). Following recent phylogenetic studies with characiforms (Oliveira *et al.* 2011; Abe *et al.* 2014; Melo *et al.* 2014), we amplified partial sequences of the mitochondrial genes *16S rRNA* (16S, 510 bp), *cytochrome oxidase C subunit 1* (COI, 656 bp) and *cytochrome B* (Cytb, 990 bp) using one round of polymerase chain reaction (PCR). Additionally, we obtained sequences of the nuclear *myosin heavy chain 6 gene* (Myh6, 710 bp), *recombination activating gene 1* (Rag1, 1378 bp), and *recombination activating gene 2* (Rag2, 1029 bp) through nested-PCR following Oliveira *et al.* (2011). Primers used in this study are listed on Table 2 of Capítulo 2. We did 12.5 µl reactions containing 9.075 µl of double-distilled water, 1.25 µl 5x reaction buffer, 0.375 MgCl₂, 0.25 µl dNTP mix at 8 mM, 0.25 µl of each primer at 10 µM (list of primers in Table 2), 0.05 µl Platinum Taq DNA polymerase enzyme (Invitrogen; www.invitrogen.com) and 1.0 µl genomic DNA (10–50 ng). The PCR consisted of an initial denaturation (4 min at 95°C) followed by 28–30 cycles of chain denaturation (30 s at 95°C), primer hybridization (30–60 s at 52–54°C), and nucleotide extension (30–60 s at 72°C). After visualization of the fragments on a 1% agarose gel, we sequenced using dye terminators (BigDye™ Terminator v 3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems) purified again through ethanol precipitation. We sequenced the samples on an ABI 3130-Genetic Analyzer (Applied

Biosystems) at either Arizona State University (Tucson, Arizona, USA) or the Universidade Estadual Paulista (Botucatu, São Paulo, Brazil).

Sequencing alignment and phylogenetic analyses

After sequencing, we assembled and edited consensus sequences in Geneious 6.1.8 (Biomatters; www.geneious.com) and we applied IUPAC ambiguity codes where we detected uncertainty of nucleotide identity. We aligned the consensus sequences of each gene in Geneious 6.1.8, trimmed sequences to a uniform length, and we inspected alignments by eye for major misalignments. The presence of loops in 16S rRNA caused substantial alignment ambiguity and low support for the resulting gene tree in preliminary trials, so we excised the hypervariable loop regions and generated a reduced and easily alignable 16S submatrix for use in the concatenated matrix. We then concatenated the six genes into a matrix with 7 partitions and used a greedy search in PartitionFinder 1.1.1 (Lanfear *et al.* 2012) (raxml modelset, linked branch lengths, selection criteria based on BIC), to determine the best partitioning scheme and to select the best-fit model for each partition.

Maximum likelihood (ML) analyses were performed in RAxML HPC2 on XSEDE (Stamatakis 2006) as implemented on CIPRES Scientific Gateway v.3.3 (Miller *et al.* 2010). First, we tested running trees without partitions and then partitioned by different methods. The final ML analysis employed seven partitions a random starting tree, and all other parameters at default values. One thousand bootstrap pseudoreplicates tested the support for each node in the most likely topology.

We ultrametricized and dated the maximum-likelihood phylogeny using penalized likelihood in R8S version 1.8 (Sanderson 2012) using a smoothing parameter of 1 and a minimum rate parameter of 0.01. The analysis included three minimum age calibrations based on known anostomoid fossils: a minimum age of 22.5 million years for Curimatidae based on †*Cyphocharax mosesi* (Malabarba 1996a); 19 million years for the node representing the most recent common ancestor of all species currently assigned to *Leporinus*, based on a large series of fossil teeth assignable to that genus (Roberts 1975); and a minimum age of 6 million years for the most recent common ancestor of *Abramites hypselonotus* and *Leporinus striatus*, based on the complete fossilized skull of †*Leporinus scalabrinii* (Bogan *et al.* 2012), which possesses the synapomorphies of that clade. R8S also requires a constraint near the root of the phylogeny to

return informative results; we fixed the age of the most recent common ancestor of the four anostomoid families to 72.6 million years, based on a date returned from a similar analysis of an unpublished dataset of ultraconserved elements. This date is slightly older than the most recent published estimates of the anostomoid crown clade age (Chen *et al.* 2013), estimated at about 45 mya.

Results and Discussion

Monophyly of Anostomidae

Our molecular phylogeny (Fig. 1) strongly supports the monophyly of Anostomidae, thereby corroborating previous morphological hypotheses (Vari 1983; Sidlauskas and Vari 2008). We also reconstructed Anostomidae as sister to the other three anostomoid families, a result that contradicts the previous morphological relationships (Vari 1983; Sidlauskas and Vari 2008) but agrees with molecular phylogenies (Calcagnotto *et al.* 2005; Oliveira *et al.* 2011; Melo *et al.* 2014). The well-supported time-calibrated phylogeny (Fig. 1) indicates that, based on estimated mean node ages, the origins of the Anostomidae dates to the Lower Eocene at around 55.2 mya. This timescale is consistent with previous dated phylogenies within the Characiformes. For example, Arroyave and Stiassny (2011) found the origin for Alestidae in the Eocene of Paleogene at around 54 mya. The Distichodontidae was estimated to have originated during the Late Cretaceous at 67 mya. Thompson *et al.* (2014) found the origin for the family Serrasalminidae between 65 and 75 mya. The estimation of the origin of Bryconidae is much younger and is dated for the Eocene-Paleocene during the Paleogene at around 46.7 mya (Abe *et al.* 2014).

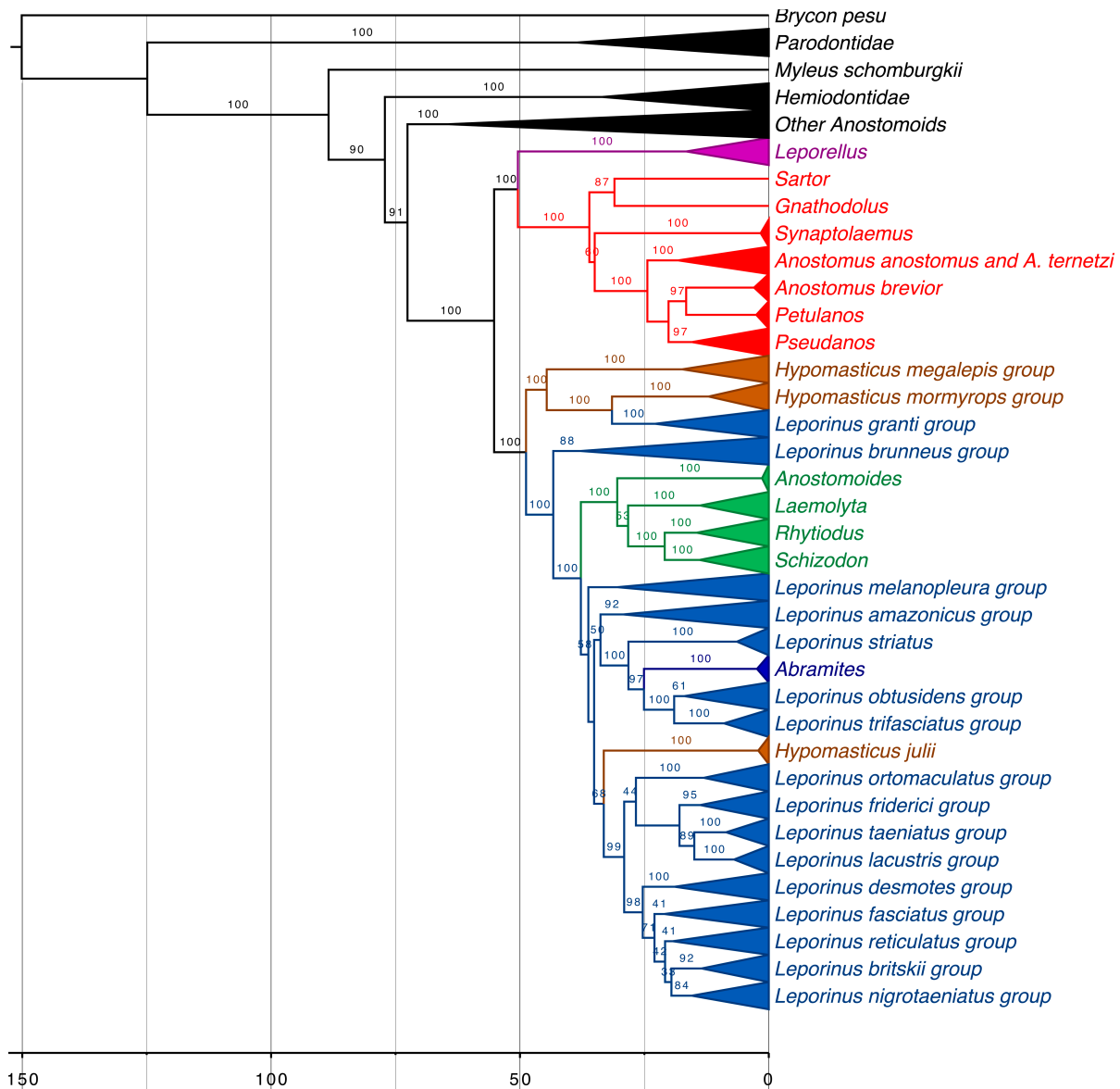


Figure 1. Time-calibrated molecular phylogeny of the Anostomidae based on the concatenated dataset. Numbers on branches represent bootstrap support.

***Leporellus* sister to Anostominae**

Leporellus appeared as sister to the subfamily Anostominae (*sensu* Winterbottom 1980) (Fig. 1), a surprising relationship given the strong support for placement of Anostominae as sister to *Laemolyta* in the osteological hypothesis (Sidlauskas and Vari 2008). The taxonomy of *Leporellus* is currently in need of substantial revision, as the single species currently considered valid (*L. vittatus*) ranges throughout the Orinoco, Amazon, São Francisco, Magdalena and La Plata basins, as well as the coastal drainages of the Guyana Shield. We analyzed six specimens

of *Leporellus* including one (11255) from the upper Rio Paraná, which includes the type locality (Orisanga) of the currently synonymized species *L. pictus*. The substantial genetic divergence among these six specimens (Fig. 2), and the marked pigmentation variation across the nominal range of *L. vittatus* suggest strongly that this species is actually a complex of several distinct specific entities. This hypothesis should be tested by a taxonomic revision of the genus with addition of molecular data.



Figure 2. Partial maximum likelihood tree showing the interspecific relationships within *Leporellus* and subfamily Anostominae. Number on branches represent bootstrap support.

We found monophyly for the Anostominae (Figs. 1–2), the subfamily containing six highly modified genera with upturned mouths as previously hypothesized by Winterbottom (1980) but phylogenetically tested by Sidlauskas and Vari (2008). *Sartor respectus* appeared as sister to *Gnathodolus bidens* and this clade closely related to that containing the remaining four genera. Such relationship slightly differs from that of Sidlauskas and Vari (2008) in which *Sartor* and *Gnathodolus* are also sister genera but placed in a derived clade sister to *Synaptolaemus*. A second large clade contains *S. latofasciatus* sister to all anostomine genera although with low bootstrap support. Our phylogeny shows paraphyly of *Anostomus*, in which *A. anostomus* is sister to *A. ternetzi* but *A. brevior* (with one specimen of *A. anostomus* from the Tapanahony river of Suriname, Marowijne drainage, that need reidentification) appears as sister to two species of *Petulanos*. All three species of *Anostomus* resemble each other greatly in coloration, and *A. anostomus* and *A. brevior* were once considered conspecific (Géry 1961b), so the proposition that they are actually members of separate genus-level lineages is quite novel.

Sidlauskas and Vari (2008) proposed the new genus *Petulanos* to accommodate *P. intermedius*, *P. plicatus* and *P. spiloclistron*, distinct spotted species previously recognized as *Anostomus*, but possessing numerous morphological synapomorphies separating them from that genus. If corroborated in future studies, the reconstruction of *A. brevior* as sister to *Petulanos* suggests that genus' concept may need to be expanded to include two morphologically distinct subclades, with substantial redefinition of the the synapomorphies for *Petulanos*.

The last reconstructed clade within Anostominae (Fig. 2) includes the five analyzed species of *Pseudanos*. We diagnosed *P. irinae* although Birindelli *et al.* (2012) placed that species in synonymy of *P. trimaculatus*, and indeed found a close genetic match, with the two analyzed *P. trimaculatus* are nested within the clade of *P. irinae* corroborating Birindelli *et al.* (2012)'s hypothesis that the two nominal species are better considered to as regional color morphs. *Pseudanos gracilis* is sister to *P. winterbottomi* with a single specimen identified as *P. varii* sister to *P. winterbottomi*, albeit nearly genetically identical and separated by a very short branch. This relationship matches with the placement A of Sidlauskas and Santos (2010) who hypothesized *P. winterbottomi* as sister to *P. gracilis* within the Winterbottom's (1980) phylogeny.

***Hypomasticus* and *Leporinus granti* group**

We reconstructed a paraphyletic *Hypomasticus*, with five or six *Leporinus* species embedded within it (Fig. 3). Within the first clade composed entirely by *Hypomasticus* from the Guiana Shield, we found *H. megalepis* from the Essequibo basin sister to the clade of *H. despaxi* from Guianas plus the newly described *H. lineomaculatus* from the Rio Jari of the Amazon basin. Our results are consistent with the osteological phylogeny that proposed *H. megalepis* sister to *H. despaxi* (Sidlauskas and Vari 2008) and with the hypothesis of Birindelli *et al.* (2013c) who described *H. lineomaculatus* and discussed a putative relationship with *H. despaxi*. The next well-supported clade is the *Leporinus granti* group, formed by species from eastern Brazil with the following relationships (*H. mormyrops* (*L. steindachneri* (*L. copelandii* + *H. thayeri*))). This clade is sister to the clade (*L. lebaili* (*L. cf. granti* (*L. granti* + *L. melanostictus*))). The phylogeny shows that *L. copelandii*, *L. steindachneri*, *L. lebaili*, *L. granti* and *L. melanostictus* should perhaps be included within *Hypomasticus*. This nomenclatural change would preserve the monophyly of *Hypomasticus*, but would also redefine the morphological concept of that genus, since the species currently in *Leporinus* do not possess the subterminal or inferior mouths, and associated restructuring of the bones of the oral jaw, that currently define *Hypomasticus* (Sidlauskas and Vari 2008).

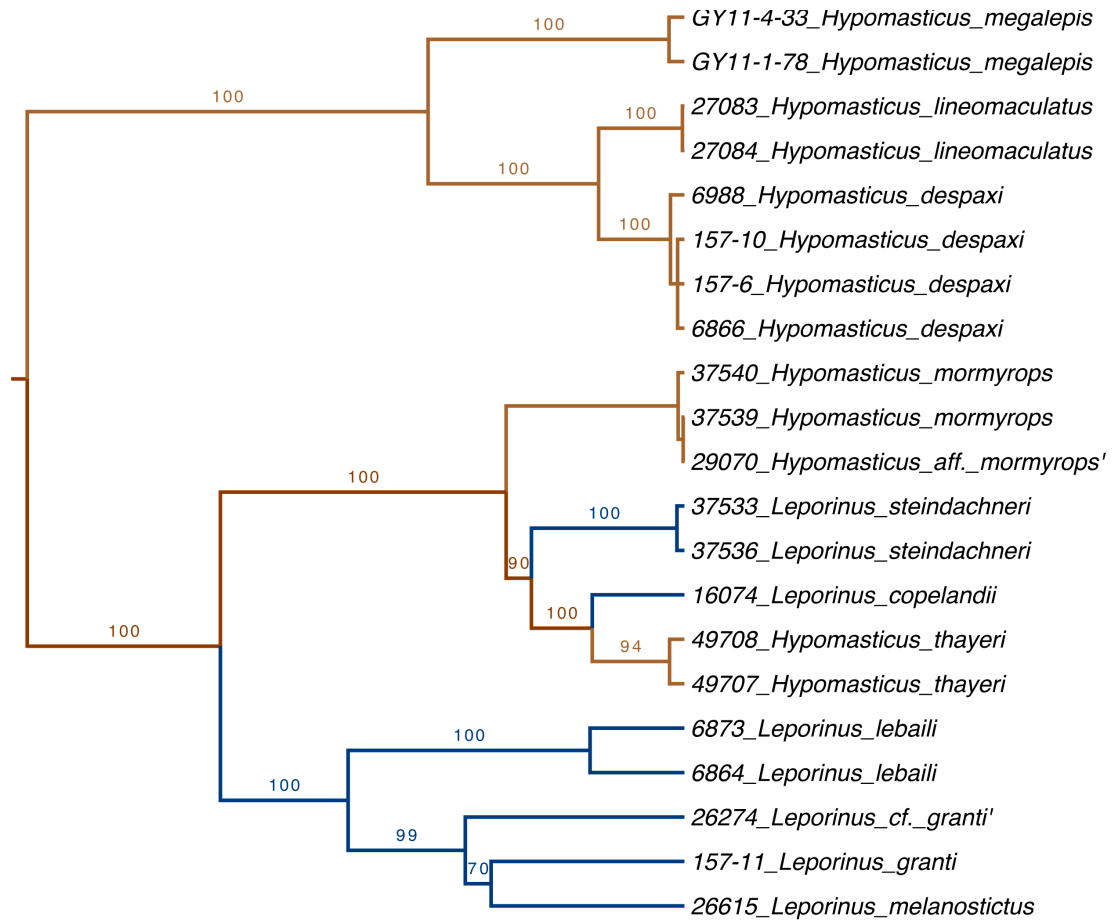


Figure 3. Partial maximum likelihood tree showing the relationships within *Hypomasticus* and *Leporinus granti* group. Numbers on branches represent bootstrap support.

Leporinus brunneus* + *L. ecuadorensis

We found monophyly for a small clade of two *Leporinus* whose geographic distributions interestingly crosses the Andes, including *L. brunneus* from the Orinoco basin as sister to *L. ecuadorensis* from the trans-Andean coastal rivers of Ecuador (Fig. 4). This clade is reciprocally monophyletic with a much larger clade containing the remainder of the species currently recognized in *Leporinus*, as well as members of *Abramites*, *Anostomoides*, *Laemolyta*, *Rhytiodus* and *Schizodon*. The eventual recognition of a monophyletic *Leporinus* will likely require the creation of a new genus to accommodate *L. brunneus* and *L. ecuadorensis*, as well as potentially other species not sampled in this study.

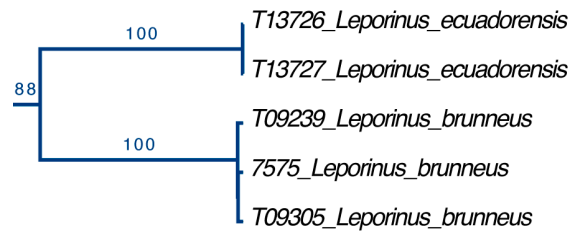


Figure 4. Partial maximum likelihood tree showing interspecific relationships within the *Leporinus brunneus* clade.

Anostomoides*, *Laemolyta*, *Rhytioidus* and *Schizodon

We recovered a well-supported clade containing (*Anostomoides* (*Laemolyta* (*Rhytioidus* + *Schizodon*))) (Fig. 5). This configuration is consistent with the morphological phylogeny (Sidlauskas and Vari 2008) except for the placement of Anostominae as sister to *Leporellus* rather than *Laemolyta*. Interspecific relationships within *Laemolyta* have *L. garmani* sister to all other species, corroborating the relationship found by Sidlauskas and Vari (2008). *Laemolyta taeniata* appeared polyphyletic with one specimen sister to *L. fernandezi* and another one within the clade of *L. proxima*, albeit some of these vouchers need reidentification. *Rhytioidus* is sister to *Schizodon* as first proposed by Sidlauskas and Vari (2008). Within *Rhytioidus*, *R. argenteofuscus* is sister to a single clade composed by three specimens that are nearly genetically identical, despite being identified as two *R. microlepis* and one *R. lauzannei*, rendering doubt about the validity of the latter, later-described species. Within *Schizodon*, there is a well supported clade containing (*S. knerii* (*S. nasutus* + *S. isognathus*)). Sidlauskas and Vari (2008) also found *S. isognathus* as sister to *S. nasutus* but obtained equivocal resolution for the placement of *S. knerii*, so the molecular phylogeny refines and is consistent with the morphological result. Another strongly supported clade includes (*S. scotorhabdotus* (*S. vittatus* (*S. fasciatus* (*S. intermedius* + *S. borellii*))))). Sidlauskas and Vari (2008) recovered *S. fasciatus* sister to *S. vittatus* but did not include *S. intermedius* or *S. borellii* in their analysis. Inclusion of more species of *Schizodon*, including those from more southerly river systems, will be necessary to obtain a full resolution of its phylogenetic relationships.

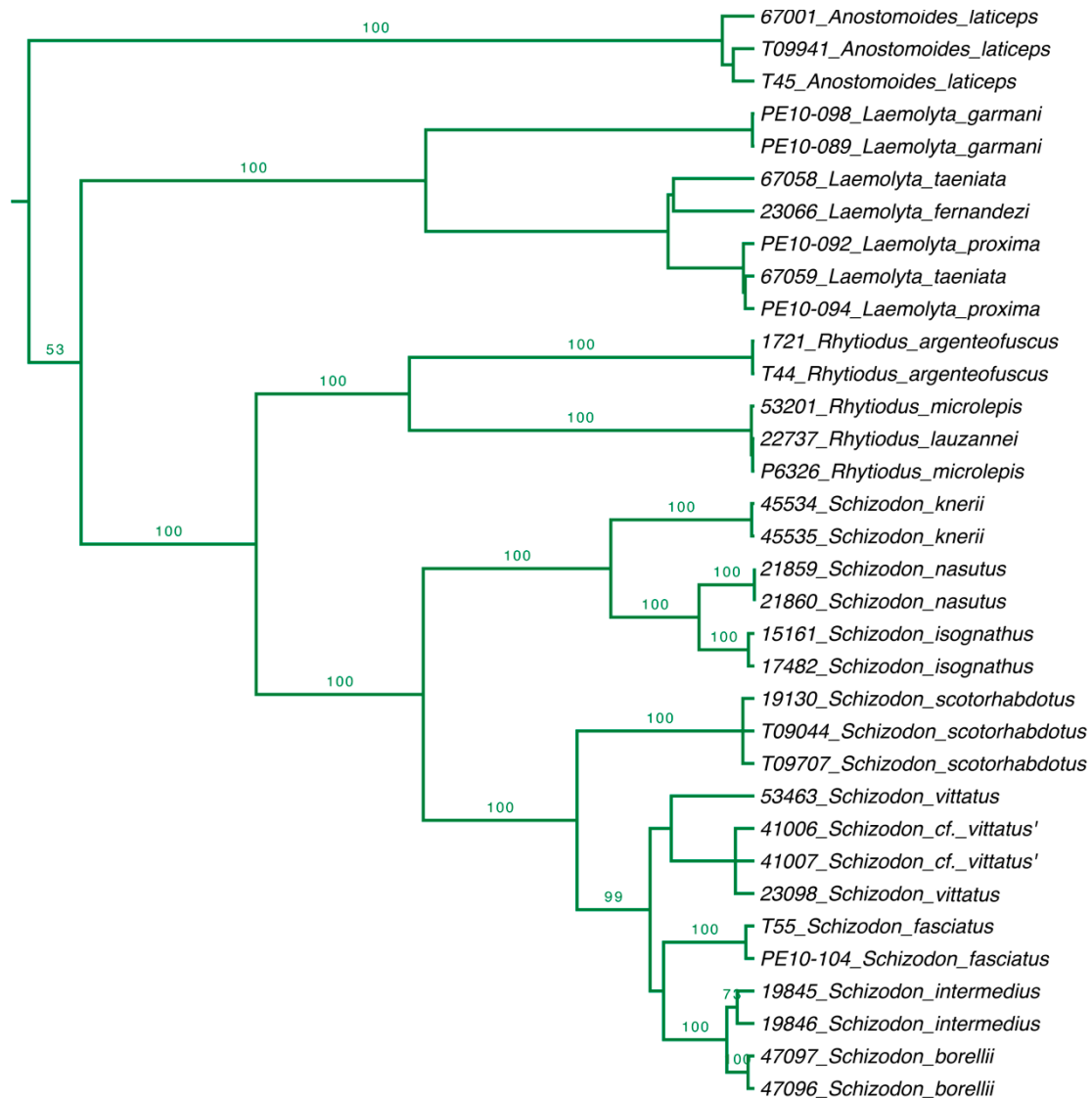


Figure 5. Partial maximum likelihood tree showing intergeneric and interspecific relationships of *Anostomoides*, *Laemolyta*, *Rhytidodus* and *Schizodon*. Numbers on branches represent bootstrap support.

Subgroups of *Leporinus*

The remaining species of *Leporinus* sort into distinct clades, most of which represent novel hypotheses returned by this molecular phylogeny (Fig. 1). Most have not yet been defined with a morphological diagnosis, in part because of conflicting signal in the observed skeletal variation (Sidlauskas and Vari 2008), but the frequent grouping herein of species with similar color patterns suggests that pigmentation may eventually provide a practical diagnosis for a series of new genera. The first lineage to diverge is the *L. melanopleura* group (Fig. 6) containing (*L. marcgravii* (*L. amblyrhinchus* (*L. melanopleura* + *L. melanopleurodes*))). Sidlauskas and Vari

(2008) did not analyze any of these species, and thus these hypotheses of relationship are completely novel. Birindelli *et al.* (2013b) proposed that *L. melanopleura* and *L. melanopleurodes* could represent sister taxa based on overall coloration and by having the fourth and fifth infraorbitals fused, a condition that admittedly occurs in several other species of *Leporinus* and *Hypomasticus*. Future morphological phylogenies should test the molecular hypothesis presented herein.

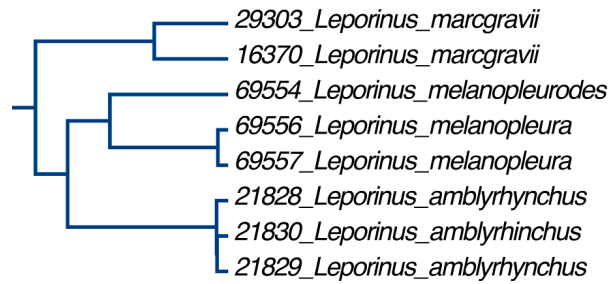


Figure 6. Partial maximum likelihood tree showing interspecific relationships within the *Leporinus melanopleura* group.

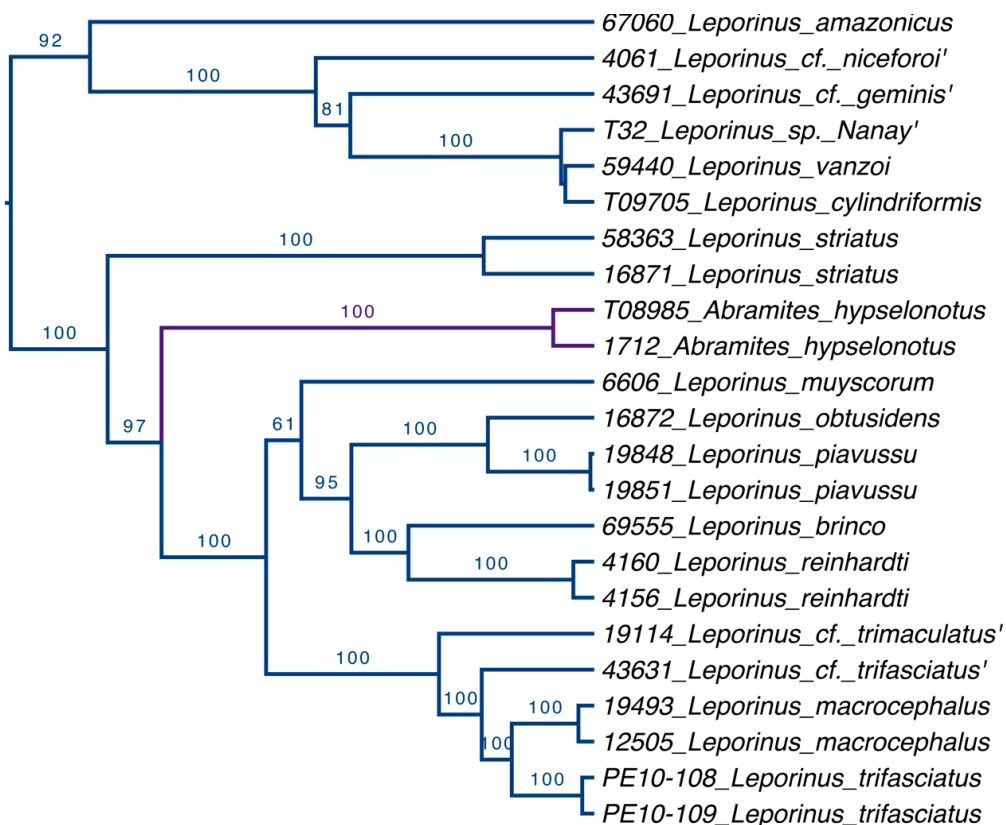


Figure 7. Partial maximum likelihood tree showing interspecific relationships within the *Leporinus amazonicus* group, *L. striatus*, *Abramites*, *L. obtisidens* group and *L. trifasciatus* group. Numbers on branches represent bootstrap support.

The second well-supported clade is the *Leporinus amazonicus* group (Fig. 7) that appears, with moderate support, as sister to *L. striatus*, *Abramites*, and the *L. obtusidens* and *L. trifasciatus* groups as defined below. The *L. amazonicus* group includes (*L. amazonicus* (*L. cf. niceforoi* (*L. cf. geminis* (*L. sp. Nanay* (*L. vanzoi* + *L. cylindriiformis*))))), many of which may represent new slender-bodied species similar to the enigmatic *L. cylindriiformis* (Sidlauskas *et al.* 2011). The specimen labeled as *L. cf. geminis* has shorter snout and deeper body than do the other two analyzed specimens of *L. geminis* and might represent a distinct species. Other specimens identified as *L. cf. cylindriiformis*, *L. geminis*, and *L. vanzoi* appeared closely related to the *L. britskii* and *L. reticulatus* groups (discussed below), though the precise taxonomic assignment of some of these species requires reexamination.

The well-supported clade (*Leporinus striatus* (*Abramites hypselonotus* (*L. obtusidens* group + *L. trifasciatus* group))) is sister to the *L. amazonicus* group. Sidlauskas and Vari (2008) previously advanced a close relationship between *Abramites* and *L. striatus* using osteological characters, a result which we corroborate here. However, they refrained from synonymizing *Abramites* into *Leporinus* or expanding the definition of *Abramites* to include some *Leporinus* species due the low analyzed taxon sampling. Our phylogeny indicates that the latter option is likely warranted, and that *Abramites* should expand to include the *L. striatus*, *L. obtusidens* and the *L. trifasciatus* groups. This option will require redefining the diagnostic characters for the genus *Abramites*, including the deep body and greater number of anal-fin rays, as well as numerous osteological features (Vari and Williams 1987; Sidlauskas and Vari 2008). Such change should only be made after a thorough morphological investigation of the taxa forming this newly recognized clade.

The well-supported clade sister to *Abramites* contains species of the *Leporinus obtusidens* group and *L. trifasciatus* group. Within the *L. obtusidens* group, we found the clade (*L. muyscorum* (*L. obtusidens* + *L. piavussu*)(*L. brinco* + *L. reinhardti*)). Sidlauskas and Vari (2008) did not analyze any of these species. Britski *et al.* (2012) recently described *L. piavussu*, a very similar species to *L. obtusidens* but did not analyze its phylogenetic placement. In the recent description of *L. brinco*, Birindelli *et al.* (2013b) stated that the new species could be closely related to *L. elongatus* and *L. conirostris*, based on squamation, pigmentation, mouth position and dentition. We do not corroborate such hypothesis and, instead, proposed that *L. brinco* (paratype sequenced) is strongly supported as sister to *L. reinhardti* from the Rio São Francisco.

Within the *Leporinus trifasciatus* group, we resolved relationships as (*L. cf. trimaculatus* (*L. cf. trifasciatus* (*L. macrocephalus* + *L. trifasciatus*))). This group comprises species of large body sizes and more robust body shapes than are typical for anostomids. The *L. cf. trimaculatus* is a morphologically unusual specimen from the Río Orinoco with no obvious color markings, a big head, and very short, ramified caudal fin. It may represent an entirely new taxon, or perhaps just a developmentally anomalous specimen. *Leporinus cf. trifasciatus* is from the Araguaia basin (probably a new species) and appeared separated from others *L. trifasciatus* from Peru. The monophyly of this group is first proposed herein.

Another section of the phylogeny has *Hypomasticus jullii* from Tapajós and Xingu basins as sister to the remaining species, rather than near the other species of *Hypomasticus* with which it shares numerous morphological features, including an unusual trough-like form of the anguloarticular's ascending process, and an inferior mouth. This phylogenetic placement may represent an error in labeling the tissues during extraction or during the loan from MZUSP, rather than biological reality. We are currently sequencing two new samples of this species and this surprising placement certainly needs further reexamination.

The most derived clade has strong bootstrap support and contains two well-defined groups: the *Leporinus friderici* group *sensu lato* and the clade containing the remaining species groups (Fig. 1). Within the *L. friderici* group *sensu lato* (Fig. 8), we found four subclades. First is the *L. ortomaculatus* group with *L. ortomaculatus* sister to *L. maculatus*. This is the first hypothesis of close relationship between these two species. Second is the more strictly defined *L. friderici* group that includes four species with similar body shape and coloration: *L. friderici*, *L. agassizi*, *L. punctatus*, and *L. piau*. The specimens of *L. friderici* (157-7 and 7015) are from the Marowijne basin (Suriname-French Guyana boundary), near to locality from which of *Salmo friderici* (Bloch, 1794) was described (an unspecified locality in Suriname). One specimen of *L. punctatus* from the Orinoco basin is sister to *L. agassizi* from the Takutu/Branco system of the Amazon basin. Another *L. friderici* from Sipaliwini river is sister to *L. agassizi* from the Essequibo basin. The complexity increases in another group containing *L. agassizi* from the Río Nanay in Peru sister to *L. piau* from the Rio São Francisco plus another *L. friderici* from the upper Rio Tapajós, although this later relationship receives only moderate support. Despite having sampled many specimens within this group, a phylogenetic study involving still more

taxa from other distinct drainages will be necessary to understand completely the phylogenetic relationships within the complex of species surrounding *L. friderici*.

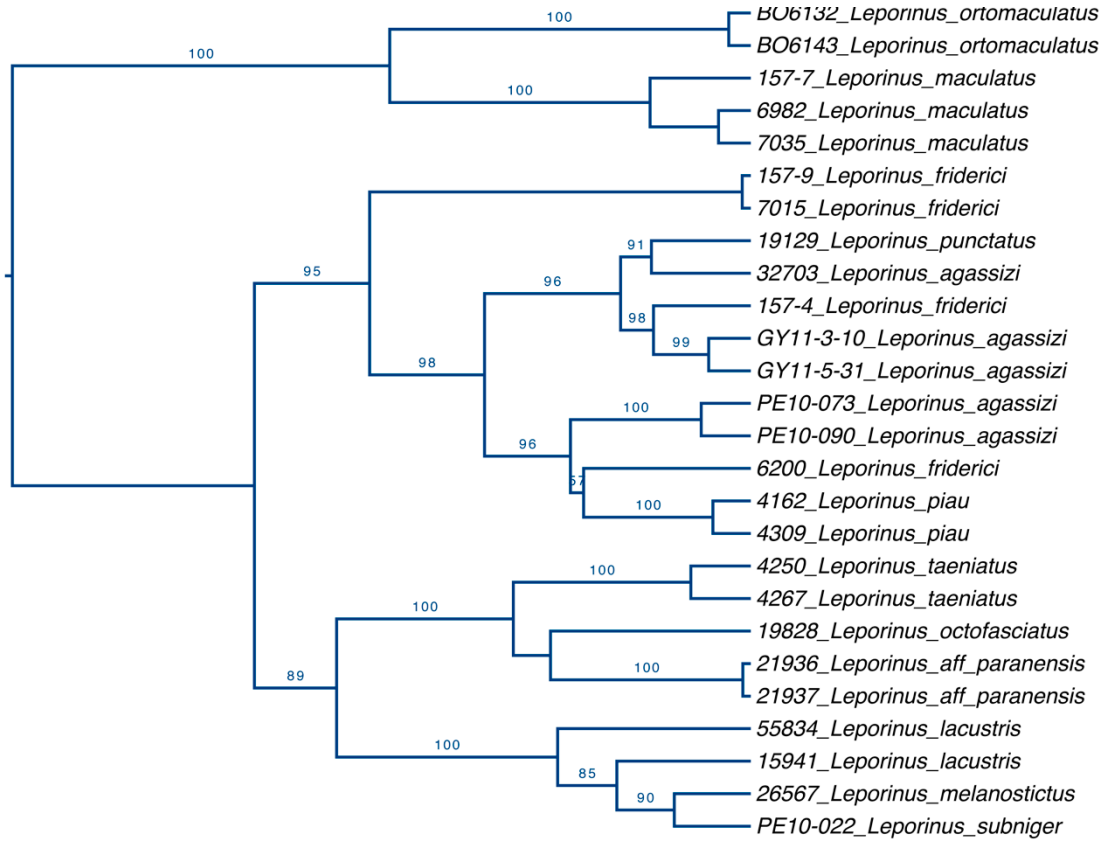


Figure 8. Partial maximum likelihood tree showing interspecific relationships within the *Leporinus friderici* group *sensu lato*. Numbers on branches represent bootstrap support.

Our results show *Leporinus taeniatus* closely related with *L. aff. paranensis* and *L. octofasciatus*, both from the upper Rio Paraná, and together these form a distinct lineage with the relationships (*L. taeniatus* (*L. aff. paranensis* + *L. octofasciatus*)), proposed herein for the first time. Sister to *L. taeniatus* group is the *L. lacustris* group (*L. lacustris* (*L. melanostictus* + *L. subniger*)), with the first of those species possibly paraphyletic due to the presence of two distinct lineages, one from the Paraguay and another from the Paraná river basins.

Of the five remaining major clades, reconstructed with the relationships (*Leporinus desmotes* group (*L. fasciatus* group (*L. reticulatus* group (*L. britskii* group + *L. nigrotaeniatus* group))))), two demonstrate distinctive pigmentation patterns consisting of prominent dark vertical bands (Fig. 1). The first of these is the *Leporinus desmotes* group, containing (*L.*

pearsoni (*L. desmotes* (*L. desmotes* + *L. jatuncochi*))) (Fig. 9). The *L. desmotes* specimen (V5274) is from the Río Casiquiare and the 2068 specimen is from Rupununi river in Guyana and apparently closely related to *L. jatuncochi* from Río Nanay in Peru. Sidlauskas and Vari (2008) stated that *L. jatuncochi* might be a junior synonym of *L. desmotes* based on the fact that the latter was described with no explicit diagnosis separating the species, other than the geographic separation. We used *L. jatuncochi* as a valid name for the Peruvian specimens due the proximity of their collection locality to the type locality in Amazonian Ecuador, but the taxonomy of this cluster of species requires revision to clarify the morphological boundaries of the included species.

The *Leporinus fasciatus* group includes the type-species of *Leporinus* (*L. fasciatus*) and any nomenclatural revisions will need to maintain the name *Leporinus* for at least this subclade. It includes *L. altipinnis* as the sister to a clade containing several *L. fasciatus* and *L. affinis* placed in two distinct clades along the *L. fasciatus* group. The *L. fasciatus* (157-8) from the Marowijne basin (closest to type-locality) is sister to *L. cf. fasciatus* (66817) from the Tapajós (possibly new species with dark spots at center of scales) plus *L. affinis* from the Rio Araguaia. The *L. fasciatus* (157-2) from the Nickerie river in Suriname is sister to other *L. fasciatus* from the Amazon basin and one specimen of *L. affinis* from the Rio Juruá. Further taxonomic studies should focus on this interesting *L. fasciatus* group and re-evaluate the taxonomic limits of the included species.

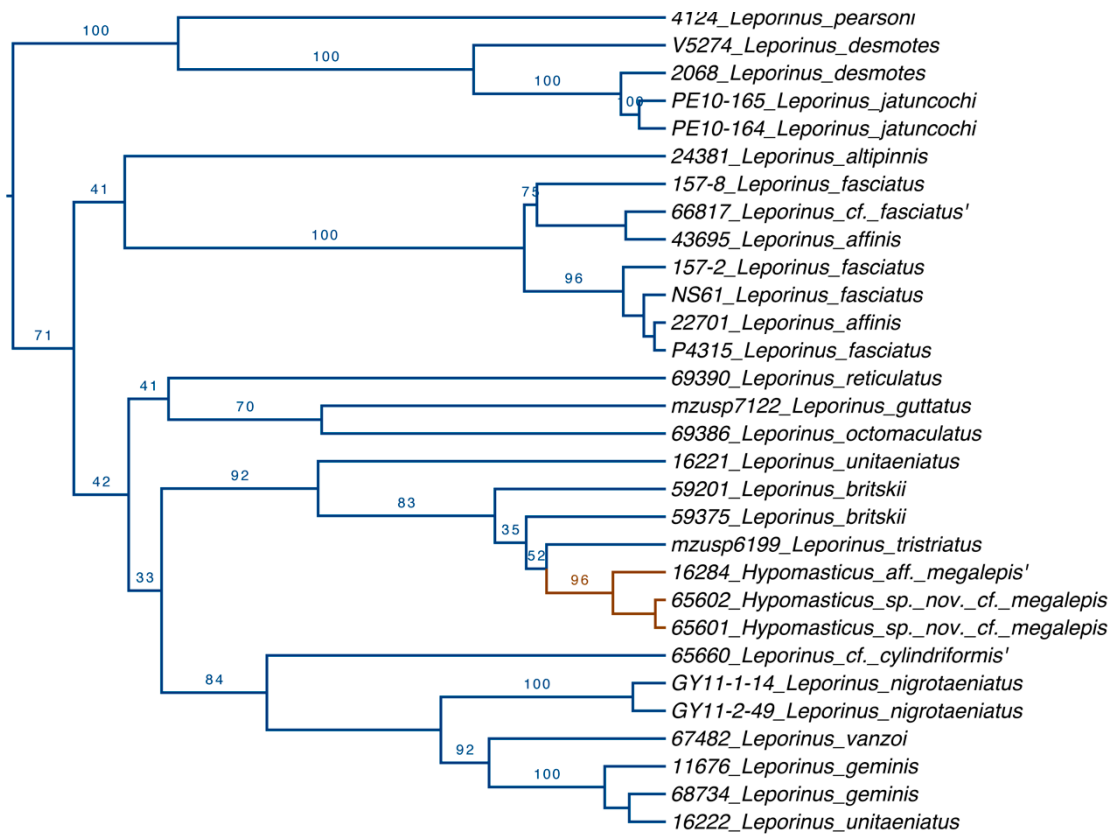


Figure 9. Partial maximum likelihood tree showing interspecific relationships within the *Leporinus desmotes* group, *L. fasciatus* group, *L. britskii* group, and the *L. nigrotaeniatus* group. Numbers on branches represent bootstrap support.

The *Leporinus reticulatus* group contains, with moderate support, *L. reticulatus* sister to *L. guttatus* plus *L. octomaculatus*. The putative relationship among these three species was mentioned by Birindelli and Britski (2009) who found a fusion at the fourth and fifth infraorbitals shared among these species. We confirm such relationship but could not confidentially propose resolution due moderate statistical support for these clades.

The *Leporinus britskii* group (Fig. 9) includes (*L. unitaeniatus* (*L. britskii* (*L. britskii* (*L. tristriatus* (*H. aff. megalepis* + *H. cf. megalepis*))))). Several species in this clade and in the *L. nigrotaeniatus* group appear as paraphyletic or polyphyletic (including *L. unitaeniatus*, *L. britskii* and *L. geminis*), but this may be an artifact caused by the incomplete data matrix underlying the reconstruction. We sequenced a paratype of *L. tristriatus* (mzusp6199) that appeared as sister to several enigmatic specimens from the Rio Araguaia and Rio Xingu that are superficially similar to (but apparently unrelated to) *Hypomasticus megalepis* from the Guyanas. As these may

represent new species, the generic assignment need to be revised and should probably be placed in *Leporinus* rather than *Hypomasticus*.

The remaining species are included within the *Leporinus nigrotaeniatus* group (Fig. 9) that contains (*L. cf. cylindriformis* (*L. nigrotaeniatus* (*L. vanzoi* (*L. geminis* (*L. geminis* + *L. unitaeniatus*))))). *Leporinus cf. cylindriformis* from the Rio Xingu is sister to the clade containing *L. nigrotaeniatus* from the upper Essequibo basin, which in turn clustered with a well-supported clade of the second analyzed specimen of *L. vanzoi* (the first appeared within the *L. amazonicus* group). *Leporinus vanzoi* is sister to *L. geminis* from the Rio Araguaia with an additional specimen of *L. unitaeniatus*.

Author contributions

BLS, BFM, RPV and CO designed the project. BLS, BFM, CO, RPV identified vouchers. BFM, KH and MB collected data. BLS, BFM, KH, MB and BWF performed phylogenetic analyses. BLS and BFM wrote most of the manuscript. All authors edited and revised the manuscript.

Acknowledgements

We thank N. Lujan and J. Armbruster (AUM), W. Aguirre (DePaul University), S. Mochel and K. Swagel (FMNH), M. de Pinna, A. Datovo, O. Oyakawa, J. Birindelli and M. Gianeti (MZUSP), R. Covain (MHNG), C. Cramer (UNIR), and R. Reina (STRI) for curatorial assistance and/or loan of tissues and vouchers. NSF (DEB-1257898) and FAPESP (proc. 2011/08374-1 and proc. BEPE 2013/16436-2) funded this project.

Table S1. Voucher specimens and locality information of anostomids and outgroups used in this study.

Taxon	Voucher	Tissue	Locality	City, State	Country
<i>Abramites hypselonotus</i>	ANSP 178126	1712	Rio Napo, Amazon basin	Maynas, Loreto	Peru
<i>Abramites hypselonotus</i>	AUM 53775	T08985			
<i>Anodus elongatus</i>	OS 18724	PE10-110	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Anostomoides laticeps</i>	UNIR 8702	67001	Rio Aripuanã, Amazon basin	Rondônia	Brazil
<i>Anostomoides laticeps</i>	AUM 53813	T09941			
<i>Anostomoides laticeps</i>	FMNH uncat	T45			
<i>Anostomus anostomus</i>	MHNG uncat	157.12	Tapanahony river, Marowijne basin		Suriname
<i>Anostomus anostomus</i>	USNM 402897	GY11-2-08	Cuyuni river, Essequibo basin	Cuyuni, Mazaruni	Guyana
<i>Anostomus anostomus</i>	USNM 402905	GY11-3-52	Cuyuni river, Essequibo basin	Cuyuni, Mazaruni	Guyana
<i>Anostomus brevior</i>	FMNH ex ANSP189141	6879	Lawa river, Marowijne basin		Suriname
<i>Anostomus brevior</i>	ANSP 189141	6912	Lawa river, Marowijne basin		Suriname
<i>Anostomus ternetzi</i>	LBP 4375	24146	Rio Branco, Amazon basin	Mucajá, Roraima	Brazil
<i>Anostomus ternetzi</i>	MZUSP 97271	mz7163	Rio Jamanxim/Tapajós , Amazon basin	Novo Progresso, Pará	Brazil
<i>Anostomus ternetzi</i>	AUM 43551	P4696			
<i>Anostomus ternetzi</i>	AUM 53596	T9714			
<i>Apareiodon affinis</i>	LBP 4591	24665	Rio Paraná, La Plata basin	Salto Grande, São Paulo	Brazil
<i>Brycon pesu</i>	OS 18361	PE10-072	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Caenotropus mestomorgmatos</i>	ANSP 180516	T48	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Chilodus fritillus</i>	AUM 51355	T10201	Rio Madre de Dios, Amazon basin	Madre de Dios	Peru
<i>Curimatopsis macrolepis</i>	ANSP 178188	1697	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Gnathodolus bidens</i>	AUM 53991	T09892			Venezuela
<i>Hemiodus unimaculatus</i>	OS 18345	PE10-076	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Hypomasticus despaxi</i>	MHNG uncat	157.6	Tapanahony river, Marowijne basin	Sipaliwini	Suriname
<i>Hypomasticus despaxi</i>	MHNG uncat	157.10	Tapanahony river, Amazon basin	Sipaliwini	Suriname
<i>Hypomasticus despaxi</i>	ANSP 189010	6866	Lawa river, Marowijne basin	Sipaliwini	Suriname
<i>Hypomasticus despaxi</i>	ANSP 189010	6988	Lawa river, Marowijne basin	Sipaliwini	Suriname
<i>Hypomasticus jullii</i>	MZUSP 96386	mz7177	Rio Jamanxim/Tapajós, Amazon basin	Novo Progresso, Pará	Brazil
<i>Hypomasticus jullii</i>	MZUSP 97206	Mz7275	Rio Iriri/Xingu, Amazon basin	Altamira, Pará	Brazil
<i>Hypomasticus lineomaculatus</i> (paratype)	LBP 5391	27083	Rio Jari, Amazon basin	Laranjal do Jari, Pará	Brazil
<i>Hypomasticus lineomaculatus</i> (paratype)	LBP 5391	27084	Rio Jari, Amazon basin	Laranjal do Jari, Pará	Brazil
<i>Hypomasticus megalepis</i>	USNM 402923	GY11-1-78	Cuyuni river, Essequibo basin	Cuyuni, Mazaruni	Guyana
<i>Hypomasticus megalepis</i>	USNM 402921	GY11-4-33	Cuyuni river, Essequibo basin	Cuyuni, Mazaruni	Guyana
<i>Hypomasticus</i> aff. <i>megalepis</i>	LBP 2455	16284	Rio Araguaia, Amazon basin	Barra do Garças, Mato Grosso	Brazil
<i>Hypomasticus mormyrops</i>	LBP 8103	37539	Rio Paraíba do Sul basin	St. Bárbara Tugurio, São Paulo	Brazil
<i>Hypomasticus mormyrops</i>	LBP 8103	37540	Rio Paraíba do Sul basin	St. Bárbara Tugurio, São Paulo	Brazil
<i>Hypomasticus</i> aff. <i>mormyrops</i>	LBP 6448	29070	Rio Paraíba do Sul basin	Guararema, São Paulo	Brazil
<i>Hypomasticus</i> sp. nov. cf.	LBP 15911	65601	Rio Xingu, Amazon basin	Canarana, Mato Grosso	Brazil

<i>megalepis</i>					
<i>Hypomasticus</i> sp. nov. cf. <i>megalepis</i>	LBP 15911	65602	Rio Xingu, Amazon basin	Canarana, Mato Grosso	Brazil
<i>Hypomasticus thayeri</i>	LBP 10833	49707	Rio Paraíba do Sul basin	Conceição do Macabu, Rio de Janeiro	Brazil
<i>Hypomasticus thayeri</i>	LBP 18618	49708	Rio Paraíba do Sul basin	Conceição do Macabu, Rio de Janeiro	Brazil
<i>Laemolyta fernandezi</i>	LBP 4003	23066	Rio Araguaia, Amazon basin	São Félix do Araguaia, Mato Grosso	Brazil
<i>Laemolyta garmani</i>	OS 18777	PE10-089	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Laemolyta garmani</i>	OS 18777	PE10-098	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Laemolyta proxima</i>	OS 18778	PE10-092	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Laemolyta proxima</i>	OS 18778	PE10-094	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Laemolyta taeniata</i>	UNIR uncat	67058	Rio Madeira, Amazon basin	Rondônia	Brazil
<i>Laemolyta taeniata</i>	UNIR uncat	67059	Rio Madeira, Amazon basin	Rondônia	Brazil
<i>Leporellus vittatus</i>	LBP 1669	11255	Rio Paraná, La Plata basin	Andirá, Paraná	Brazil
<i>Leporellus vittatus</i>	LBP 10418	48957	Rio São Francisco	Buritizero, Minas Gerais	Brazil
<i>Leporellus vittatus</i>	ANSP 182218	P6295	Rio Amazonas	Maynas, Loreto	Peru
<i>Leporellus vittatus</i>	ANSP 182609	P6322	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Leporellus</i> cf. <i>vittatus</i>	AUM 54212	T09912			
<i>Leporellus</i> cf. <i>vittatus</i>	AUM 54212	T09913			
<i>Leporinus</i> aff. <i>paranensis</i>	LBP 3808	21936	Rio Paranapanema, upper Rio Paraná	Avaré, São Paulo	Brazil
<i>Leporinus</i> aff. <i>paranensis</i>	LBP 3808	21937	Rio Paranapanema, upper Rio Paraná	Avaré, São Paulo	Brazil
<i>Leporinus affinis</i>	LBP 4248	22701	Rio Juruá, Amazon basin	Cruzeiro do Sul, Acre	Brazil
<i>Leporinus affinis</i>	LBP 12699	43695	Rio Araguaia, Amazon basin	Cocalinho, Mato Grosso	Brazil
<i>Leporinus agassizi</i>	LBP 6857	32703	Rio Negro, Amazon basin	Barcelos, Amazonas	Brazil
<i>Leporinus agassizi</i>	USNM 403520	GY11-3-10	Cuyuni river, Essequibo basin	Cuyuni, Mazaruni	Guyana
<i>Leporinus agassizi</i>	USNM 402629	GY11-5-31	Cuyuni river, Essequibo basin	Cuyuni, Mazaruni	Guyana
<i>Leporinus agassizi</i>	OS 18369	PE10-073	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Leporinus agassizi</i>	OS 18775	PE10-090	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Leporinus altipinnis</i>	LBP 4459	24381	Rio Negro, Amazon basin	Barcelos, Amazonas	Brazil
<i>Leporinus amazonicus</i>	UNIR uncat	67060	Rio Madeira, Amazon basin	Rondônia	Brazil
<i>Leporinus amblyrhynchus</i>	LBP 3917	21828	Rio Paranapanema, upper Rio Paraná basin	Chavantes, São Paulo	Brazil
<i>Leporinus amblyrhynchus</i>	LBP 3917	21829	Rio Paranapanema, upper Rio Paraná basin	Chavantes, São Paulo	Brazil
<i>Leporinus brinco</i> (paratype)	MZUSP 111257	69555	Rio Gongogi, Eastern drainage	Itajibá, Bahia	Brazil
<i>Leporinus britskii</i>	LBP 14158	59201	Rio Tapajós, Amazon basin	Itaituba, Pará	Brazil
<i>Leporinus britskii</i>	LBP 14214	59375	Rio Tapajós, Amazon basin	Itaituba, Pará	Brazil
<i>Leporinus brunneus</i>	ANSP 192149	7275	Rio Orinoco basin		
<i>Leporinus brunneus</i>	AUM 53512	T09239			
<i>Leporinus brunneus</i>	AUM 54394	T09305	Rio Ventuari, Orinoco basin		Venezuela
<i>Leporinus</i> cf. <i>cylindriformis</i>	LBP 15940	65660	Rio Xingu, Amazon basin	Canarana, Mato Grosso	Brazil
<i>Leporinus</i> cf. <i>cylindriformis</i>	AUM 53659	T09705	Rio Maniapiare/Ventuari, Orinoco basin		
<i>Leporinus</i> cf. <i>fasciatus</i>	LBP 16116	66817	Rio Tapajós, Amazon basin	Itaituba, Pará	Brazil
<i>Leporinus</i> cf. <i>geminis</i>	LBP 12702	43691	Rio Araguaia, Amazon basin	Cocalinho, Mato Grosso	Brazil
<i>Leporinus</i> cf. <i>granti</i>	LBP 5147	26274	Rio Verde/Tapajós, Amazon basin	Campo Novo dos Parecis,	Brazil

					Mato Grosso
<i>Leporinus cf. niceforoi</i>		4061			
<i>Leporinus cf. trifasciatus</i>	LBP 12722	43631	Rio Araguaia, Amazon basin	Cocalinho, Mato Grosso	Brazil
<i>Leporinus cf. trimaculatus</i>	LBP 3030	19114	Rio Orinoco basin	Caicara del Orinoco, Bolívar	Venezuela
<i>Leporinus copelandii</i>	LBP 2380	16074	Rio Paraíba do Sul	Campos dos Goytacazes, Rio de Janeiro	Brazil
<i>Leporinus desmotes</i>	ANSP 179650	2068	Rupununi river, Essequibo basin	Rupununi	Guyana
<i>Leporinus desmotes</i>	AUM 43770	V5274	Rio Cassiquiare		Venezuela
<i>Leporinus ecuadorensis</i>		T13726			Ecuador
<i>Leporinus ecuadorensis</i>		T13727			Ecuador
<i>Leporinus fasciatus</i>	MHNG uncat	157.2	Nickerie river		Suriname
<i>Leporinus fasciatus</i>	MHNG uncat	157.8	Tapanahony river, Marowijne basin	Sipaliwini	Suriname
<i>Leporinus fasciatus</i>	ANSP 180321	NS61	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Leporinus fasciatus</i>	AUM 43300	P4315			
<i>Leporinus friderici</i>	MHNG uncat	157.4	Sipaliwini river	Sipaliwini	Suriname
<i>Leporinus friderici</i>	MHNG uncat	157.9	Tapanahony river, Marowijne basin	Sipaliwini	Suriname
<i>Leporinus friderici</i>	MZUSP 96786	mz6200	Rio Teles Pires/Tapajós, Amazon basin	Peixoto de Azevedo, Mato Grosso	Brazil
<i>Leporinus friderici</i>	ANSP 189264	7015	Lawa river, Marowijne basin	Sipaliwini	Suriname
<i>Leporinus geminis</i>	LBP 1621	11676	Rio Araguaia, Amazon basin	Aragarças, Goiás	Brazil
<i>Leporinus geminis</i>	LBP 17190	68734	Rio Araguaia, Amazon basin	Britânia, Goiás	Brazil
<i>Leporinus granti</i>	MHNG uncat	157.11	Tapanahony river, Marowijne basin	Sipaliwini	Suriname
<i>Leporinus guttatus</i> (paratype)	MZUSP 96865	mzusp7122	Rio Iriri/Xingu, Amazon basin	Altamira, Pará	Brazil
<i>Leporinus jatuncochi</i>	OS 18325	PE10-164	Aquarium trade of Iquitos	Maynas, Loreto	Peru
<i>Leporinus jatuncochi</i>	OS 18325	PE10-165	Aquarium trade of Iquitos	Maynas, Loreto	Peru
<i>Leporinus lacustris</i>	LBP 2340	15941	Rio Tietê, upper Rio Paraná	Bofete, São Paulo	Brazil
<i>Leporinus lacustris</i>	LBP 13411	55834	Rio Paraguay	Poconé, Mato Grosso	Brazil
<i>Leporinus lebaili</i>	ANSP 189043	6864	Lawa river, Marowijne basin	Sipaliwini	Suriname
<i>Leporinus lebaili</i>	ANSP 189043	6873	Lawa river, Marowijne basin	Sipaliwini	Suriname
<i>Leporinus macrocephalus</i>	LBP 1422	12505	Rio Taquari, Paraguay basin	Coxim, Mato Grosso do Sul	Brazil
<i>Leporinus macrocephalus</i>	LBP 1422	19493	Rio Taquari, Paraguay basin	Coxim, Mato Grosso do Sul	Brazil
<i>Leporinus maculatus</i>	MHNG uncat	157.7	Tapanahony river, Marowijne basin	Sipaliwini	Suriname
<i>Leporinus maculatus</i>	ANSP 189041	6982	Lawa river, Marowijne basin	Sipaliwini	Suriname
<i>Leporinus maculatus</i>	FMNH uncat	7035	Lawa river, Marowijne basin	Sipaliwini	Suriname
<i>Leporinus marcgravii</i>	LBP 2491	16370	Rio Mondividiu, upper Rio Paraná	Montividiu, Goiás	Brazil
<i>Leporinus marcgravii</i>	LBP 6222	29303	Rio Grande, upper Rio Paraná	Muzambinho, Minas Gerais	Brazil
<i>Leporinus melanopleura</i>	MZUSP 111246	69556	Rio Una, Eastern drainage	São José da Vitória, Bahia	Brazil
<i>Leporinus melanopleura</i>	MZUSP 111246	69557	Rio Una, Eastern drainage	São José da Vitória, Bahia	Brazil
<i>Leporinus melanopleurodes</i>	MZUSP 111242	69554	Rio das Almas, Eastern drainage	Jequiçá, Bahia	Brazil
<i>Leporinus melanostictus</i>	LBP 5487	26567	Rio Jari, Amazon basin	Laranjal do Jari, Amapá	Brazil
<i>Leporinus melanostictus</i>	LBP 5179	26615	Rio Jari, Amazon basin	Almeirim, Pará	Brazil
<i>Leporinus muyscorum</i>	ANSP 192864	6606	Rio Magdalena basin		Colombia
<i>Leporinus nigrotaeniatus</i>	USNM 403524	GY11-1-14	Cuyuni river, Essequibo basin	Cuyuni, Mazaruni	Guyana
<i>Leporinus nigrotaeniatus</i>	USNM 403536	GY11-2-49	Cuyuni river, Essequibo basin	Cuyuni, Mazaruni	Guyana
<i>Leporinus obtusidens</i>	LBP 3912	16872	Rio Paranapanema, upper Rio Paraná	Itatinga, São Paulo	Brazil

<i>Leporinus octofasciatus</i>	LBP 3939	19828	Rio Paranapanema, upper Rio Paraná	Chavantes, São Paulo	Brazil
<i>Leporinus octomaculatus</i>	LBP 13249	69386	Rio Tapajós, Amazon basin	Diamantino, Mato Grosso	Brazil
<i>Leporinus ortomaculatus</i>	ANSP 191182	BO6132	Rio Ventuari, Orinoco basin		Venezuela
<i>Leporinus ortomaculatus</i>	ANSP 191200	BO6143	Rio Ventuari, Orinoco basin		Venezuela
<i>Leporinus pearsoni</i>	ANSP 180841	4124	Rio Tahuamanu, Madre de Dios	San Lorenzo, Madre de Dios	Peru
<i>Leporinus piau</i>	LBP 260	4162	Rio São Francisco basin	Três Marias, Minas Gerais	Brazil
<i>Leporinus piau</i>	LBP 340	4309	Rio São Francisco basin	Abaeté, Minas Gerais	Brazil
<i>Leporinus piavussu</i>	LBP 17722	19848	Rio Tietê, upper Rio Paraná	Botucatu, São Paulo	Brazil
<i>Leporinus piavussu</i>	LBP 3303	19851	Rio Tietê, upper Rio Paraná	Botucatu, São Paulo	Brazil
<i>Leporinus punctatus</i>	LBP 3047	19129	Rio Orinoco basin	Caicara del Orinoco, Bolívar	Venezuela
<i>Leporinus reinhardti</i>	LBP 259	4156	Rio São Francisco basin	Três Marias, Minas Gerais	Brazil
<i>Leporinus reinhardti</i>	LBP 259	4160	Rio São Francisco basin	Três Marias, Minas Gerais	Brazil
<i>Leporinus reticulatus</i>	LBP 13250	69390	Rio Tapajós, Amazon basin	Diamantino, Mato Grosso	Brazil
<i>Leporinus</i> sp. cf. <i>cylindriformis</i>	ANSP 180317	T32	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Leporinus steindachneri</i>	LBP 8098	37533	Rio Mucuri	Carlos Chagas, Minas Gerais	Brazil
<i>Leporinus steindachneri</i>	LBP 8098	37536	Rio Mucuri	Carlos Chagas, Minas Gerais	Brazil
<i>Leporinus striatus</i>	LBP 3180	16871	Rio Paranapanema, upper Rio Paraná	Itatinga, São Paulo	Brazil
<i>Leporinus striatus</i>	LBP 14028	58363	Rio Cuiabá, upper Rio Paraguay	Poconé, Mato Grosso	Brazil
<i>Leporinus subniger</i>	OS 18726	PE10-022	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Leporinus taeniatus</i>	LBP 329	4250	Rio São Francisco basin	Moema, Minas Gerais	Brazil
<i>Leporinus taeniatus</i>	LBP 329	4267	Rio São Francisco basin	Moema, Minas Gerais	Brazil
<i>Leporinus trifasciatus</i>	OS 18311	PE10-108	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Leporinus trifasciatus</i>	OS 18311	PE10-109	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Leporinus tristriatus</i> (paratype)	MZUSP 110987	mzusp6199	Rio Teles Pires/Tapajós, Amazon basin	Peixoto de Azevedo, Mato Grosso	Brazil
<i>Leporinus unitaeniatus</i>	LBP 2387	16221	Rio Araguaia, Amazon basin	Barra do Garças, Mato Grosso	Brazil
<i>Leporinus unitaeniatus</i>	LBP 2387	16222	Rio Araguaia, Amazon basin	Barra do Garças, Mato Grosso	Brazil
<i>Leporinus vanzoi</i>	LBP 14247	59440	Rio Tapajós, Amazon basin	Itaituba, Pará	Brazil
<i>Leporinus vanzoi</i>	LBP 16476	67482	Rio Tapajós, Amazon basin	Itaituba, Pará	Brazil
<i>Myleus schomburgkii</i>	OS uncat	PE10-044	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Parodon nasus</i>	LBP 1135	5635	Upper Rio Paraná	Botucatu, São Paulo	Brazil
<i>Petulanos</i> cf. <i>spiloclistron</i>	AUM 44717	G5207			
<i>Petulanos</i> cf. <i>spiloclistron</i>	AUM 44717	G5208			
<i>Petulanos intermedius</i>	MZUSP 96778	BO6191	Rio Teles Pires/Tapajós, Amazon basin	Peixoto de Azevedo, Mato Grosso	Brazil
<i>Petulanos intermedius</i>	MZUSP 97330	mzusp7160	Rio Jamanxim/Tapajós, Amazon basin	Novo Progresso, Pará	Brazil
<i>Prochilodus nigricans</i>	OS 18792	PE10-045	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Psectrogaster amazonica</i>	OS 18313	PE10-013	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Pseudanos gracilis</i>	FMNH 113530	T57	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Pseudanos gracilis</i>	ANSP 180320	T73	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Pseudanos irinae</i>	AUM 48210	G07-746			
<i>Pseudanos irinae</i>	AUM 48210	G07-747			

<i>Pseudanos trimaculatus</i>	OS 18779	PE10-020	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Pseudanos trimaculatus</i>	OS 18795	PE10-057	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Pseudanos varii</i>	ANSP 182659	V5301	Río Atabapo, Orinoco basin	San Francisco de Atabapo, Amazonas	Venezuela
<i>Pseudanos winterbottomi</i>	AUM 39855	V120			
<i>Pseudanos winterbottomi</i>	AUM 39292	V170			
<i>Rhytidodus argenteofuscus</i>	ANSP 178129	1721	Rio Napo, Amazon basin	Maynas, Loreto	Peru
<i>Rhytidodus argenteofuscus</i>	ANSP 180242	T44	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Rhytidodus lauzannei</i>	LBP 4239	22737	Rio Juruá, Amazon basin	Cruzeiro do Sul, Acre	Brazil
<i>Rhytidodus microlepis</i>	LBP 9781	53201	Rio Itaya, Amazon basin	Iquitos, Loreto	Peru
<i>Rhytidodus microlepis</i>	ANSP 182603	P6326	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Sartor respectus</i>	LBP 15280	62058	Rio Coronel Vanick/Xingu, Amazon basin	Canarana, Mato Grosso	Brazil
<i>Schizodon borellii</i>	LBP 12626	47096	Rio Cuiabá, Paraguay basin	Corumbá, Mato Grosso do Sul	Brazil
<i>Schizodon borellii</i>	LBP 12626	47097	Rio Cuiabá, Paraguay basin	Corumbá, Mato Grosso do Sul	Brazil
<i>Schizodon cf. vittatus</i>	LBP 12752	41006	Rio Araguaia, Amazon basin	Cocalinho, Mato Grosso	Brazil
<i>Schizodon cf. vittatus</i>	LBP 12752	41007	Rio Araguaia, Amazon basin	Cocalinho, Mato Grosso	Brazil
<i>Schizodon fasciatus</i>	OS 18310	PE10-104	Rio Nanay, Amazon basin	Maynas, Loreto	Peru
<i>Schizodon fasciatus</i>	FMNH 113522	T55	Rio Itaya, Amazon basin	Iquitos, Loreto	Peru
<i>Schizodon intermedius</i>	LBP 3305	19845	Rio Tietê, upper Rio Paraná	Botucatu, São Paulo	Brazil
<i>Schizodon intermedius</i>	LBP 3305	19846	Rio Tietê, upper Rio Paraná	Botucatu, São Paulo	Brazil
<i>Schizodon isognathus</i>	LBP 2162	15161	Rio Tietê, upper Rio Paraná	Botucatu, São Paulo	Brazil
<i>Schizodon isognathus</i>	LBP 2735	17482	Rio Paranapanema, upper Rio Paraná	Paranapanema, São Paulo	Brazil
<i>Schizodon knerii</i>	LBP 11334	45534	Rio São Francisco	Pirapora, Minas Gerais	Brazil
<i>Schizodon knerii</i>	LBP 11334	45535	Rio São Francisco	Pirapora, Minas Gerais	Brazil
<i>Schizodon nasutus</i>	LBP 3708	21859	Rio Paraná	Ilha Solteira, São Paulo	Brazil
<i>Schizodon nasutus</i>	LBP 3708	21860	Rio Paraná	Ilha Solteira, São Paulo	Brazil
<i>Schizodon scotorhabdotus</i>	LBP 3046	19130	Rio Orinoco basin	Caicara del Orinoco, Bolívar	Venezuela
<i>Schizodon scotorhabdotus</i>	AUM 54067	T09044	Rio Orinoco basin		
<i>Schizodon scotorhabdotus</i>	AUM 53654	T09707	Rio Orinoco basin		
<i>Schizodon vittatus</i>	LBP 3994	23098	Rio Araguaia, Amazon basin	São Félix do Araguaia, Mato Grosso	Brazil
<i>Schizodon vittatus</i>	LBP 12849	53463	Rio Tapajós, Amazon basin	Itaituba, Pará	Brazil
<i>Semaprochilodus taeniurus</i>	LBP 1691	12757	Rio Amazonas	Manaus, Amazonas	Brazil
<i>Synaptolaemus latofasciatus</i>	MZUSP 97460	mzusp7230	Rio Jamanxim/Tapajós, Amazon basin	Novo Progresso, Pará	Brazil
<i>Synaptolaemus latofasciatus</i>	AUM 54407	T09316	Rio Orinoco basin		Venezuela

LITERATURA CITADA

- Abe K.T., Mariguela T.C., Avelino G.S., Castro R.M.C., Oliveira C. 2013. Multilocus molecular phylogeny of Gasteropelecidae (Ostariophysi: Characiformes) reveals the existence of an unsuspected diversity. *Mol. Phylogenet. Evol.* 69:1209–14.
- Abe K.T., Mariguela T.C., Avelino G.S., Foresti F., Oliveira C. 2014. Systematic and historical biogeography of the Bryconidae (Ostariophysi: Characiformes) suggesting a new rearrangement of its genera and an old origin of Mesoamerican ichthyofauna. *BMC Evol. Biol.* 14:152.
- Aguilera O., Lundberg J., Birindelli J., Sabaj Pérez M., Jaramillo C., Sánchez-Villagra M.R. 2013. Palaeontological evidence for the last temporal occurrence of the ancient western Amazonian river outflow into the Caribbean. *PLoS One*. 8:e76202.
- Albert J.S., Reis R.E. 2011a. Introduction to Neotropical Freshwaters. In: Albert J.S., Reis R.E., editors. *Historical Biogeography of Neotropical Freshwater Fishes*. Berkeley and Los Angeles: University of California Press. p. 3–19.
- Albert J.S., Reis. 2011b. *Historical Biogeography of Neotropical Freshwater Fishes*. Berkeley and Los Angeles: University of California Press.
- Araújo-Lima C.A.R.M., Ruffino M.L. 2003. Migratory fishes of the Brazilian Amazon. In: Carolsfeld J., Harvey B., Ross C., Baer A., editors. *Migratory Fishes of South America: Biology, Fisheries and Conservation Status*. Victoria: World Fisheries Trust. p. 233–301.
- Arce H M., Reis R.E., Geneva A.J., Sabaj Pérez M.H. 2013. Molecular phylogeny of thorny catfishes (Siluriformes: Doradidae). *Mol. Phylogenet. Evol.* 67:560–77.
- Arroyave J., Denton J.S.S., Stiassny M.L.J. 2013. Are characiform fishes Gondwanan in origin? Insights from a time-scaled molecular phylogeny of the Citharinoidei (Ostariophysi: Characiformes). *PLoS One*. 8:e77269.
- Arroyave J., Stiassny M.L.J. 2011. Phylogenetic relationships and the temporal context for the diversification of African characins of the family Alestidae (Ostariophysi: Characiformes): evidence from DNA sequence data. *Mol. Phylogenet. Evol.* 60:385–97.
- Balassa G.C., Fugli R., Hahn N.S., Galina A.B. 2004. Dieta de espécies de Anostomidae (Teleostei, Characiformes) na área de influência do reservatório de Manso, Mato Grosso, Brasil. *Iheringia Ser. Zool.* 94:77–82.
- Bayley P.B., Petrere Jr. M. 1989. Amazon fisheries: assessment methods, current status and management options. In: Dodge D.P., editor. *Proceedings of the International Large River Symposium*. Can. Spec. Publ. Fish. Aquat. Sci. p. 385–398.
- Bejerano G., Pheasant M., Makunin I., Stephen S., Kent W.J., Mattick J.S., Haussler D. 2004. Ultraconserved elements in the human genome. *Science*. 304:1321–5.

- Benine R.C., Mariguela T.C., Oliveira C. 2009. New species of *Moenkhausia* Eigenmann, 1903 (Characiformes: Characidae) with comments on the *Moenkhausia oligolepis* species complex. *Neotrop. Ichthyol.* 7:161–168.
- Bergmann L.A.. 1988. *Schizodon jacuiensis* sp. n., um novo anostomídeo do sul do Brasil e redescritção de *Schizodon kneri* (Steindachner, 1875) e *S. platae* (Garman, 1890) (Pisces, Characiformes, Anostomidae). *Comun. do Mus. Ciências e Tecnol. PUCRS Ser Zool.* 1:13–28.
- Bermingham E., Avise J.C. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics.* 113:939–965.
- Birindelli J.L.O., Britski H. a. 2013. Two new species of *Leporinus* (Characiformes: Anostomidae) from the Brazilian Amazon, and redescription of *Leporinus striatus* Kner 1858. *J. Fish Biol.* 83:1128–60.
- Birindelli J.L.O., Britski H.A., Garavello J.C. 2013a. Two new species of *Leporinus* Agassiz (Characiformes: Anostomidae) from eastern basins of Brazil, and redescription of *L. melanopleura* Günther. *Neotrop. Ichthyol.* 11:9–23.
- Birindelli J.L.O., Britski H.A., Lima F.C.T. 2013b. New Species of *Leporinus* from the Rio Tapajós Basin, Brazil, and Redescription of *L. moralesi* (Characiformes: Anostomidae). *Copeia.* 2013:238–247.
- Birindelli J.L.O., Britski H.A. 2009. New species of the genus *Leporinus* Agassiz (Characiformes: Anostomidae) from the rio Curuá, rio Xingu basin, Serra do Cachimbo, Brazil, with comments on *Leporinus reticulatus*. *Neotrop. Ichthyol.* 7:1–10.
- Birindelli J.L.O., Lima F.C.T., Britski H.A. 2012. New species of *Pseudanos* Winterbottom, 1980 (Characiformes: Anostomidae), with notes on the taxonomy of *P. gracilis* and *P. trimaculatus*. *Zootaxa.* 3425:55–68.
- Birindelli J.L.O., Peixoto L.A.W., Wosiacki W.B., Britski H.A. 2013c. New Species of *Hypomasticus* Borodin, 1929 (Characiformes: Anostomidae) from Tributaries of the Lower Rio Amazonas, Brazil. *Copeia.* 2013:464–469.
- Bogan S., Sidlauskas B., Vari R.P., Agnolin F. 2012. *Arrhinolemur scalabrinii* Ameghino, 1898, of the late Miocene - a taxonomic journey from the Mammalia to the Anostomidae (Ostariophysi: Characiformes). *Neotrop. Ichthyol.* 10:555–560.
- Bouckaert R., Heled J., Kühnert D., Vaughan T., Wu C.-H., Xie D., Suchard M.A., Rambaut A., Drummond A.J. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* 10:e1003537.
- Boulenger G. 1904. Fishes (Systematic Account of the Teleostei). In: Harmer S., Shipley A., editors. *The Cambridge Natural History*. London: Macmillan and Co. Ltd. p. 475–727.
- Bowen S.H., Bonetto A.A., Ahlgren M.O. 1984. Microorganisms and detritus in the diet of a typical neotropical riverine detritivore, *Prochilodus platensis* (Pisces: Prochilodontidae). *Limnol. Oceanogr.* 29:1120–1122.

- Bowen S.H. 1983. Detritivory in neotropical fish communities. *Environ. Biol. Fishes.* 9:137–144.
- Britski H.A., Birindelli J.L.O., Garavello J.C. 2012. A new species of *Leporinus* Agassiz, 1829 from the upper Rio Paraná basin (Characiformes, Anostomidae) with redescription of *L. elongatus* Valenciennes, 1850 and *L. obtusidens* (Valenciennes, 1837). *Pap. Avulsos Zool.* 52:441–476.
- Britski H.A., Garavello J.C. 1993. Descrição de duas espécies novas de *Leporinus* da bacia do Tapajós (Pisces, Characiformes). *Comun. do Mus. Ciências e Tecnol. PUCRS Ser Zool.* 6:29–40.
- Britski H.A., Silimon K.Z.S., Lopes B.S. 2007. Peixes do Pantanal: manual de identificação. Brasília: Embrapa Informação Tecnológica.
- Buckup P. 1998. Relationships of the Characidiinae and phylogeny of characiform fishes (Teleostei: Ostariophysi). In: Malabarba L.R., Reis R.E., Vari R.P., Lucena Z.M.S., Lucena C.A.S., editors. *Phylogeny and Classification of Neotropical Fishes*. Porto Alegre: Edipucrs.
- Buckup P. 2011. The Eastern Brazilian Shield. In: Albert J.S., Reis R.E., editors. *Historical Biogeography of Neotropical Freshwater Fishes*. Berkeley and Los Angeles: University of California Press. p. 203–210.
- Bussing W.A. 1966. New species and new records of Costa Rican freshwater fishes with a tentative list of species. *Rev. Biol. Trop.* 13:205–249.
- Calcagnotto D., Schaefer S. a, DeSalle R. 2005. Relationships among characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences. *Mol. Phylogenet. Evol.* 36:135–53.
- Camargo S.A.F., Petrere Jr. M. 2001. Social and financial aspects of the artisanal São Francisco River, Minas Gerais, Brazil. *Fish. Manag. Ecol.* 8:163–171.
- Carolsfeld J., Harvey B., Ross C., Baer A. 2003. *Migratory Fishes of South America*. Victoria: World Fisheries Trust.
- Carvalho-Costa L.F., Hatanaka T., Galetti Jr P. 2008. Evidence of lack of population substructuring in the Brazilian freshwater fish *Prochilodus costatus*. *Genet. Mol. Biol.* 31:377–380.
- Castro R.M.C., Vari R.P. 2003. Family Prochilodontidae. In: Reis R.E., Kullander S.O., Ferraris C.J., editors. *Check List of the Freshwater Fishes of South and Central America (CLOFFSCA)*. Porto Alegre: Edipucrs. p. 65–70.
- Castro R.M.C., Vari R.P. 2004. Detritivores of the South American fish family Prochilodontidae (Teleostei: Ostariophysi: Characiformes): a phylogenetic and revisionary study. *Smithson. Contrib. to Zool.* 1–189.
- Chen W.-J., Lavoué S., Mayden R.L. 2013. Evolutionary origin and early biogeography of otophysan fishes (Ostariophysi: Teleostei). *Evolution (N. Y.)*. 67:2218–39.
- Chen W.-J., Mayden R.L. 2010. A Phylogenomic Perspective on the New Era of Ichthyology. *Bioscience*. 60:421–432.

- Chiachio M.C., Oliveira C., Montoya-Burgos J.I. 2008. Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). *Mol. Phylogenet. Evol.* 49:606–617.
- Crawford N.G., Faircloth B.C., McCormack J.E., Brumfield R.T., Winker K., Glenn T.C. 2012. More than 1000 ultraconserved elements provide evidence that turtles are the sister group of archosaurs. *Biol. Lett.* 8:783–6.
- Dahdul W. 2010. Review of the phylogenetic relationships and fossil record of Characiformes. In: Grande T., Poyato-Ariza F., Diogo R., editors. *Goniorhynchiformes and ostariophysan relationships: A comprehensive review*. Enfield: Science Publishers. p. 409–440.
- Darriba D., Taboada G.L., Doallo R., Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods.* 9:772.
- Dimmick W.W., Larson A. 1996. A molecular and morphological perspective on the phylogenetic relationships of the otophysan fishes. *Mol. Phylogenet. Evol.* 6:120–33.
- Drummond A.J., Ho S.Y.W., Phillips M.J., Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4:e88.
- Drummond A.J., Suchard M.A., Xie D., Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29:1969–73.
- Eigenmann C. 1917. The American Characidae [Part 1]. *Mem. Museum Comp. Zool.* 43:1–102.
- Eschmeyer W.N., Fong J.D. 2015. Species by family/subfamily. Available from <http://research.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp>.
- Faircloth B.C., Branstetter M.G., White N.D., Brady S.G. 2015. Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. *Mol. Ecol. Resour.* 15:489–501.
- Faircloth B.C., McCormack J.E., Crawford N.G., Harvey M.G., Brumfield R.T., Glenn T.C. 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Syst. Biol.* 61:717–26.
- Faircloth B.C., Sorenson L., Santini F., Alfaro M.E. 2013. A Phylogenomic Perspective on the Radiation of Ray-Finned Fishes Based upon Targeted Sequencing of Ultraconserved Elements (UCEs). *PLoS One.* 8:e65923.
- Figueiredo F.J., Costa-Carvalho B.C.M. 1999. *Plesiocurimata alvarengai* gen. et sp. nov. (Teleostei: Ostariophysi: Curimatidae) from the Tertiary of Taubaté basin, São Paulo. *An. Acad. Bras. Ciências.* 71:885–894.
- Figueiredo J., Hoorn C., van der Ven P., Soares E. 2009. Late Miocene onset of the Amazon River and the Amazon deep-sea fan: Evidence from the Foz do Amazonas Basin. *Geology.* 37:619–622.

- Fink S., Fink W.L. 1981. Interrelationships of the ostariophysian fishes (Teleostei). Zool. J. Linn. Soc. 72:297–353.
- Flecker A.S. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical stream. Ecology. 77:1845–1854.
- Garavello J.C., Britski H.A. 1990. Duas novas espécies do gênero *Schizodon* Agassiz da bacia do alto Paraná, Brasil, América do Sul (Ostariophysi, Anostomidae). Nat. (São Paulo). 15:153–170.
- Garavello J.C., Britski H.A. 2003. Family Anostomidae. In: Reis R.E., Kullander S.O., Ferraris Jr. C., editors. Check List of the Freshwater Fishes of South and Central America. Porto Alegre: p. 71–84.
- Garavello J.C. 1979. Revisão taxonômica do gênero *Leporinus* Spix, 1829. Universidade de São Paulo.
- Garavello J.C. 2000. Two new species of *Leporinus* Spix with a review of the blotched species of the Rio Orinoco system and redescription of *Leporinus muyscorum* Steindachner (Charciformes: Anostomidae). Proc. Acad. Nat. Sci. Philadelphia. 150:193–201.
- Garcia A., Tello S., Vargas G., Duponchelle F. 2009. Patterns of commercial fish landings in the Loreto region (Peruvian Amazon) between 1984 and 2006. Fish Physiol. Biochem. 35:53–67.
- Géry J., Planquette P., Le Bail P.-Y. 1991. Faune characoïde (poissons ostariophysaires) de l'Oyapock, l'Approuague et la Rivière de Kaw (Guyane Française). Cybium. 15:1–69.
- Géry J. 1961a. Contributions à l'étude des poissons Characoïdes 13, structures et évolution des Anostominae. Bull. Aquat. Biol. 2:93–112.
- Géry J. 1961b. Contributions a l'étude des poissons characoïdes. n14. Révision de la super-espèce *Anostomus Anostomus* (L.) et description de formes nouvelles: *A. brevior* et *A. anostomus longus* (Erythrinidae, Anostomidae). Bull. du Muséum Natl. d'Histoire Nat. 32:498–505.
- Géry J. 1977. Characoids of the World. Neptune City: TFH Publications.
- Godinho A.L., Kynard B. 2006. Migration and Spawning of Radio-Tagged Zulega *Prochilodus argenteus* in a Dammed Brazilian River. Trans. Am. Fish. Soc. 135:811–824.
- Greenwood P., Rosen D., Weitzman S.H., Myers G. 1966. Phyletic studies of teleostean fishes with a provisional classification of living forms. Bulletin Am. Museum Nat. Hist. 131:339–456.
- Gregory W., Conrad G. 1938. The Phylogeny of the Characin Fishes. Zoologica. 23:319–360.
- Guerrero J. 1997. Stratigraphy, sedimentary environments, and the Miocene uplift of the Colombian Andes. In: Kay R., Madden R., Cifelli R., Flynn J., editors. Vertebrate Paleontology in the Neotropics: The Miocene Fauna of La Venta, Colombia. Washington DC: Smithsonian Institution Press. p. 15–43.
- Günther A. 1864. Catalogue of the Fishes in the Collection of the British Museum. London: .
- Harris H. 2007. Improved pairwise alignment of genomic DNA. The Pennsylvania State University.

- Heled J., Drummond A.J. 2010. Bayesian inference of species trees from multilocus data. *Mol. Biol. Evol.* 27:570–80.
- Hennig W. 1950. *Grundzüge einer Theorie der phylogenetischen Systematik*. Berlin: Deutsche Zentralverlag.
- Hennig W. 1966. *Phylogenetic Systematics*. Urbana: University of Illinois.
- Hoorn C., Wesselingh F.P., ter Steege H., Bermudez M. a, Mora A., Sevink J., Sanmartín I., Sanchez-Meseguer A., Anderson C.L., Figueiredo J.P., Jaramillo C., Riff D., Negri F.R., Hooghiemstra H., Lundberg J., Stadler T., Särkinen T., Antonelli A. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* (80-.). 330:927–31.
- Isbrücker I.J.H., Nijssen H. 1988. Review of the South American characiform fish genus *Chilodus*, with description of a new species, *C. gracilis* (Pisces, Characiformes, Chilodontidae). *Beaufortia*. 38:47–56.
- Javonillo R., Malabarba L.R., Weitzman S.H., Burns J.R. 2010. Relationships among major lineages of characid fishes (Teleostei: Ostariophysi: Characiformes), based on molecular sequence data. *Mol. Phylogenet. Evol.* 54:498–511.
- Jerep F.C., Vari R.P. 2014. New Species of *Nannocharax* (Characiformes: Distichodontidae) from the Northwestern Congo River Basin, with an Appraisal of *Hemigrammocharax*. *Copeia*. 2014:44–49.
- Katoh K., Kuma K., Toh H., Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res.* 33:511–8.
- Kocher T.D. 2004. Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev. Genet.* 5:288–98.
- Kubatko L.S., Degnan J.H. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Syst. Biol.* 56:17–24.
- Lanfear R., Calcott B., Ho S.Y.W., Guindon S. 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.* 29:1695–701.
- Langeani F. 1998. Phylogenetic study of the Hemiodontidae (Ostariophysi: Characiformes). In: Malabarba L.R., Reis R.E., Vari R.P., Lucena Z.M.S., Lucena C., editors. *Phylogeny and Classification of Neotropical Fishes*. Porto Alegre: Edipucrs. p. 145–160.
- Lavoué S., Miya M., Poulsen J.Y., Møller P.R., Nishida M. 2008. Monophyly, phylogenetic position and inter-familial relationships of the Alepocephaliiformes (Teleostei) based on whole mitogenome sequences. *Mol. Phylogenet. Evol.* 47:1111–21.
- Lemmon A.R., Emme S.A., Lemmon E.M. 2012. Anchored hybrid enrichment for massively high-throughput phylogenomics. *Syst. Biol.* 61:727–44.
- Lemmon E.M., Lemmon A.R. 2013. High-Throughput Genomic Data in Systematics and Phylogenetics. *Annu. Rev. Ecol. Evol. Syst.* 44:99–121.

- Li C., Lu G., Ortí G. 2008. Optimal data partitioning and a test case for ray-finned fishes (Actinopterygii) based on ten nuclear loci. *Syst. Biol.* 57:519–39.
- Li C., Ortí G., Zhang G., Lu G. 2007. A practical approach to phylogenomics: the phylogeny of ray-finned fish (Actinopterygii) as a case study. *BMC Evol. Biol.* 7:44.
- Lopera-Barrero N.M., Povh J.A., Ribeiro R.P., Gomes P.C., Jacometo C.B., Lopes T. da S. 2008. Comparison of DNA extraction protocols of fish fin and larvae samples: modified salt (NaCl) extraction. *Cienc. e Investig. Agrar.* 35:65–74.
- López-Fernández H., Winemiller K.O., Honeycutt R.L. 2010. Multilocus phylogeny and rapid radiations in Neotropical cichlid fishes (Perciformes: Cichlidae: Cichlinae). *Mol. Phylogenet. Evol.* 55:1070–1086.
- Lovejoy N.R., Collette B.B. 2001. Phylogenetic relationships of new world needlefishes (Teleostei: Belonidae) and the biogeography of transitions between marine and freshwater habitats. *Copeia*. 1:324–338.
- Lowe-McConnell R.H. 1975. Fish communities in tropical freshwaters. New York: Longman Publishing.
- Lucena C.A.S., Menezes N.A. 1998. A phylogenetic analysis of *Roestes* Günther and *Gilbertolus* Eigenmann, with a hypothesis on the relationships of the Cynodontidae and Acestrorhynchidae (Teleostei: Ostariophysi: Characiformes). In: Malabarba L.R., Reis R.E., Vari R.P., Lucena Z.M.S., Lucena C.A.S., editors. *Phylogeny and Classification of Neotropical Fishes*. Porto Alegre: Edipucrs. p. 261–278.
- Lucinda P.F., Vari R. 2009. New *Steindachnerina* Species (Teleostei: Characiformes: Curimatidae) from the Rio Tocantins Drainage. *Copeia*. 2009:142–147.
- Lujan N., Armbruster J. 2011. The Guiana Shield. In: Albert J.S., Reis R.E., editors. *Historical Biogeography of Neotropical Freshwater Fishes*. Berkeley and Los Angeles: University of California Press. p. 211–224.
- Lundberg J.G., Marshall L., Guerrero J., Horton B., Malabarba M.C.S.L., Wesselingh F. 1998. The stage for Neotropical fish diversification: A history of tropical South American rivers. In: Malabarba L.R., Reis R.E., Vari R.P., Lucena Z., Lucena C., editors. *Phylogeny and Classification of Neotropical Fishes*. Porto Alegre: Edipucrs. p. 13–48.
- Lundberg J.G., Sullivan J.P., Hardman M. 2011. Phylogenetics of the South American Catfish Family Pimelodidae (Teleostei: Siluriformes) using Nuclear and Mitochondrial Gene Sequences. *Proc. Acad. Nat. Sci. Philadelphia*. 161:153–189.
- Lundberg J.G. 1997. Freshwater fishes and their paleobiotic implications. In: Kay R.F., Madden R., Cifelli R., Flynn J., editors. *Vertebrate Paleontology in the Neotropics: The Miocene Fauna of La Venta, Colombia*. Washington: Smithsonian Institution. p. 67–91.
- Malabarba L.R., Weitzman S.H. 2003. Description of a new genus with six new species from southern Brazil, Uruguay and Argentina, with a discussion of a putative characid clade (Teleostei: Characiformes: Characidae). *Comun. do Mus. Ciências e Tecnol. PUCRS Ser Zool.* 16:67–151.

- Malabarba L.R. 1998. Characters and major clades of the Cheirodontinae (Ostariophysi: Characidae). In: Malabarba L.R., Reis R.E., Vari R.P., Lucena C.A.S., Lucena Z.M.S., editors. *Phylogeny and Classification of Neotropical Fishes*. Porto Alegre: Edipucrs. p. 199–233.
- Malabarba M.C., Malabarba L.R. 2010. Biogeography of Characiformes: an evaluation of the available information of fossil and extant taxa. In: Nelson J.S., Schultze H.P., Wilson M.V.H., editors. *Origin and Phylogenetic Interrelationships of Teleosts*. Munich: Verlag Dr. Friedrich Pfeil. p. 317–336.
- Malabarba M.C. 1996. Reassessment and relationships of *Curimata mosesi* Travassos & Santos, a fossil fish (Teleostei: Characiformes: Curimatidae) from the tertiary of São Paulo, Brazil. *Comun. do Mus. Ciências e Tecnol. PUCRS Ser Zool.* 9:55–63.
- Mariguela T.C., Benine R.C., Abe K.T., Avelino G.S., Oliveira C. 2013a. Molecular phylogeny of *Moenkhausia* (Characidae) inferred from mitochondrial and nuclear DNA evidence. *J. Zool. Syst. Evol. Res.* 51:327–332.
- Mariguela T.C., Ortí G., Avelino G.S., Abe K.T., Oliveira C. 2013b. Composition and interrelationships of a large Neotropical freshwater fish group, the subfamily Cheirodontinae (Characiformes: Characidae): a case study based on mitochondrial and nuclear DNA sequences. *Mol. Phylogenet. Evol.* 68:23–34.
- Mateus L.A.F., Penha J.M.F., Petrere M. 2004. Fishing resources in the rio Cuiabá basin, Pantanal do Mato Grosso, Brazil. *Neotrop. Ichthyol.* 2:217–227.
- Mattox G.M.T., Bifi A.G., Oyakawa O.T. 2014. Taxonomic study of *Hoplias microlepis* (Günther, 1864), a trans-Andean species of trahiras (Ostariophysi: Characiformes: Erythrinidae). *Neotrop. Ichthyol.* 12:343–352.
- Mattox G.M.T., Toledo-Piza M. 2012. Phylogenetic study of the Characinae (Teleostei: Characiformes: Characidae). *Zool. J. Linn. Soc.* 165:809–915.
- Mautari K.C., Menezes N.A. 2006. Revision of the South American freshwater fish genus *Laemolyta* Cope, 1872 (Ostariophysi: Characiformes: Anostomidae). *Neotrop. Ichthyol.* 4:27–44.
- McCormack J.E., Faircloth B.C., Crawford N.G., Gowaty P.A., Brumfield R.T., Glenn T.C. 2012. Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species-tree analysis. *Genome Res.* 22:746–54.
- McCormack J.E., Harvey M.G., Faircloth B.C., Crawford N.G., Glenn T.C., Brumfield R.T. 2013. A phylogeny of birds based on over 1,500 loci collected by target enrichment and high-throughput sequencing. *PLoS One.* 8:e54848.
- Melo B.F., Benine R.C., Mariguela T.C., Oliveira C. 2011. A new species of *Tetragonopterus* Cuvier, 1816 (Characiformes: Characidae: Tetragonopterinae) from the rio Jari, Amapá, northern Brazil. *Neotrop. Ichthyol.* 9:49–56.
- Melo B.F., Sato Y., Foresti F., Oliveira C. 2013. The roles of marginal lagoons in the maintenance of genetic diversity in the Brazilian migratory fishes *Prochilodus argenteus* and *P. costatus*. *Neotrop. Ichthyol.* 11:625–636.

- Melo B.F., Sidlauskas B.L., Hoekzema K., Vari R.P., Oliveira C. 2014. The first molecular phylogeny of Chilodontidae (Teleostei: Ostariophysi: Characiformes) reveals cryptic biodiversity and taxonomic uncertainty. *Mol. Phylogenet. Evol.* 70:286–95.
- Melo B.F., Vari R.P. 2014. New species of *Cyphocharax* (Characiformes: Curimatidae) from the upper rio Negro, Amazon basin. *Neotrop. Ichthyol.* 12:327–332.
- Menezes N.A. 1972. Distribuição e origem da fauna de peixes de água doce das grandes bacias fluviais do Brasil. *Poluição e Piscicultura - Notas sobre Poluição, Ictiologia e Piscicultura*. São Paulo: Faculdade de Saúde Pública da USP, Secretaria de Agricultura, Instituto de Pesca.
- Meredith R.W., Janečka J.E., Gatesy J., Ryder O.A., Fisher C.A., Teeling E.C., Goodbla A., Eizirik E., Simão T.L.L., Stadler T., Rabosky D.L., Honeycutt R.L., Flynn J.J., Ingram C.M., Steiner C., Williams T.L., Robinson T.J., Burk-Herrick A., Westerman M., Ayoub N.A., Springer M.S., Murphy W.J. 2011. Impacts of the Cretaceous Terrestrial Revolution and KPg extinction on mammal diversification. *Science*. 334:521–4.
- Miller M., Pfeiffer W., Schwartz T. 2010. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. *Proc. Gatew. Comput. Environ. Work. (GCE)*, New Orleans, LA.:1–8.
- Mirande J.M. 2009. Weighted parsimony phylogeny of the family Characidae (Teleostei: Characiformes). *Cladistics*. 25:1–40.
- Mirande J.M. 2010. Phylogeny of the family Characidae (Teleostei: Characiformes): from characters to taxonomy. *Neotrop. Ichthyol.* 8:385–568.
- Monsch K. a. 1998. Miocene fish faunas from the northwestern Amazonia basin (Colombia, Peru, Brazil) with evidence of marine incursions. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 143:31–50.
- Moreira C. 2007. Relações Filogenéticas na ordem Characiformes (Teleostei: Ostariophysi). Universidade de São Paulo, unpublished thesis.
- Müller J., Troschel F. 1845. *Horae ichthyologicae. Beschreibung und Abbildung neuer Fische; die Familie Characinen*. Berlin: .
- Müller J. 1842. Beobachtungen über die Schwimmblase der Fische, mit Bezug auf einige neue Fischgattungen. *Arch. Ana. Physiol.*:307–329.
- Nakatani M., Miya M., Mabuchi K., Saitoh K., Nishida M. 2011. Evolutionary history of Otophysi (Teleostei), a major clade of the modern freshwater fishes: Pangaeen origin and Mesozoic radiation. *BMC Evol. Biol.* 11:177.
- Near T.J., Eytan R.I., Dornburg A., Kuhn K.L., Moore J. a, Davis M.P., Wainwright P.C., Friedman M., Smith W.L. 2012. Resolution of ray-finned fish phylogeny and timing of diversification. *Proc. Natl. Acad. Sci. U. S. A.* 109:13698–703.
- Nelson J.S. 2006. *Fishes of the World*. Hoboken, NJ: John Wiley & Sons, Inc.

- Netto-Ferreira A.L., Vari R.P. 2011. New Species of *Steindachnerina* (Characiformes: Curimatidae) from the Rio Tapajós, Brazil, and Review of the Genus in the Rio Tapajós and Rio Xingu Basins. *Copeia*. 2011:523–529.
- Netto-Ferreira L., Birindelli J.L.O., Sousa L.M., Mariguela C., Oliveira C. 2013. A New Miniature Characid (Ostariophysi: Characiformes: Characidae), with Phylogenetic Position Inferred from Morphological and Molecular Data. *PLoS One*. 8:e52098.
- Nylander J.A.A. 2004. MrAIC.pl. Program distributed by the author. Evolutionary Biology Center, Uppsala University.
- Oliveira C., Avelino G.S., Abe K.T., Mariguela T.C., Benine R.C., Ortí G., Vari R.P., Corrêa e Castro R.M. 2011. Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. *BMC Evol. Biol.* 11:275.
- Ortega H., Vari R.P. 1986. Annotated checklist of the freshwater fishes of Peru. *Smithson. Contrib. to Zool.* 437:1–25.
- Ortí G., Meyer A. 1996. Molecular evolution of ependymin and the phylogenetic resolution of early divergences among euteleost fishes. *Mol. Biol. Evol.* 13:556–73.
- Ortí G., Meyer A. 1997. The radiation of characiform fishes limits of resolution of mitochondrial ribosomal DNA sequences. *Syst. Biol.* 46:75–100.
- Ortí G., Sivasundar A., Dietz K., Jégu M. 2008. Phylogeny of the Serrasalminae (Characiformes) based on mitochondrial DNA sequences. *Genet. Mol. Biol.* 31:343–351.
- Oyakawa O.T., Mattox G.M.T. 2009. Revision of the Neotropical trahiras of the *Hoplias lacerdae* species-group (Ostariophysi: Characiformes: Erythrinidae) with descriptions of two new species. *Neotrop. Ichthyol.* 7:117–140.
- Paiva M.P., Bastos S.A. 1981. Marcação de peixes nas regiões do alto e médio São Francisco. *Cienc. Cult.* 34:1362–1365.
- Palumbi S. 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis D., Moritz C., Mable B., editors. *Molecular Systematics*. Massachusetts: Sinauer Associates Inc. p. 205–247.
- Paradis E., Claude J., Strimmer K. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics*. 20:289–90.
- Paradis E. 2013. Molecular dating of phylogenies by likelihood methods: a comparison of models and a new information criterion. *Mol. Phylogenet. Evol.* 67:436–44.
- Pavanelli C.S., Britski H.A. 1999. Description of a new species of *Steindachnerina* (Teleostei: Characiformes: Curimatidae) from the upper Rio Paraná basin, Brazil. *Ichthyol. Explor. Freshwaters*.

- Peng Z., He S., Wang J., Wang W., Diogo R. 2006. Mitochondrial molecular clocks and the origin of the major Otocephalan clades (Pisces: Teleostei): A new insight. *Gene*. 370:113–24.
- Pennisi E. 2013. Large-Scale Gene Comparisons Boost Tree of Life Studies. *Science* (80-.). 342:26–27.
- Pereira T.L., Santos U., Schaefer C.E., Souza G.O., Paiva S.R., Malabarba L.R., Schmidt E.E., Dergam J. a. 2013. Dispersal and vicariance of *Hoplias malabaricus* (Bloch, 1794) (Teleostei, Erythrinidae) populations of the Brazilian continental margin. *J. Biogeogr.* 40:905–914.
- Poulsen J.Y., Møller P.R., Lavoué S., Knudsen S.W., Nishida M., Miya M. 2009. Higher and lower-level relationships of the deep-sea fish order Alepocephaliformes (Teleostei: Otocephala) inferred from whole mitogenome sequences. *Biol. J. Linn. Soc.* 98:923–936.
- R Development Core Team. 2013. R: A language and environment for statistical computing. .
- Rambaut A., Suchard M.A., Xie D., Drummond A.J. 2014. Tracer v1.6. <http://beast.bio.ed.ac.uk/Tracer>.
- Regan C. 1911. The Classification of the Teleostean Fishes of the Order Ostariophysi, I: Cyprinoidea. *Ann. Mag. Nat. Hist.* 8:13–32.
- Reis R.E. 1989. Systematic Revision of the Neotropical Characid Subfamily Stethaprioninae (Pisces, Characiformes). *Comun. do Mus. Ciências e Tecnol. PUCRS Ser Zool.* 2:3–86.
- Reis R.E. 1998. Systematics, biogeography, and the fossil record of the Callichthyidae: a review of the available data. In: Malabarba L.R., Reis R.E., Vari R., Lucena Z., Lucena C., editors. *Phylogeny and Classification of Neotropical Fishes*. Porto Alegre: Edipucrs. p. 351–362.
- Ribeiro A.C. 2006. Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil : an example of faunal evolution associated with a divergent continental margin. *Neotrop. Ichthyol.* 4:225–246.
- Ribeiro M.C.L.B., Petrere Jr. M. 1990. Fisheries ecology and management of the jaraqui (*Semaprochilodus taeniurus*, *S. insignis*) in central Amazonia. *Regul. Rivers Res. Manag.* 5:195–215.
- Riccomini C., Appi C., Freitas E., Arai M. 1987. Tectônica e sedimentação no sistema de rifts continentais da Serra do Mar (Bacia de Volta Redonda, Resende, Taubaté e São Paulo). *Simpósio Geol. RJ-ES.*:253–298.
- Riccomini C., Suguio K., Alvarenga H., Fittipaldi F. 1991. Estratigrafia e ambientes de sedimentação da porção central da Bacia de Taubaté. *Congr. Bras. Paleontol.*:12: 1–29.
- Roberts T. 1969. Osteology and relationships of characoid fishes, particularly the genera *Hepsetus*, *Salminus*, *Hoplias*, *Ctenolucius*, and *Acestrorhynchus*. *Proc. Calif. Acad. Sci.* 36:391–500.
- Roberts T. 1973. Osteology and Relationships of the Prochilodontidae, a South American Family of Characoid Fishes. *Bull. Museum Comp. Zool.* 145:213–235.
- Roberts T. 1975. Characoid fish teeth from Miocene deposits in the Cuenca basin. *J. Zool.* 175:259–271.

- Rodríguez-Olarte D., Mojica-Corzo J., Taphorn D.C. 2011. Northern South America, Magdalena and Maracaibo Basins. In: Albert J.S., Reis R.E., editors. Historical Biogeography of Neotropical Freshwater Fishes. Berkeley and Los Angeles: University of California Press. p. 243–257.
- Ronquist F., Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*. 19:1572–1574.
- Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M. a, Huelsenbeck J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61:539–42.
- Roxo F.F., Albert J.S., Silva G.S.C., Zawadzki C.H., Foresti F., Oliveira C. 2014. Molecular Phylogeny and Biogeographic History of the Armored Neotropical Catfish Subfamilies Hypoptopomatinae, Neoplecostominae and Otothyridae (Siluriformes: Loricariidae). *PLoS One*. 9:e105564.
- Saadi A., Machette M., Haller K., Dart R., Bradley L., Souza A. 2002. Map and database of Quaternary faults and lineaments in Brazil. Reston: U.S. Geological Survey.
- Saadi A. 1995. A geomorfologia da Serra do Espinhaço em Minas Gerais e suas margens. *Geonomos*. 3:41–63.
- Sabaj Pérez M.H. 2013. Standard Symbolic Codes for Institutional Resource Collections in Herpetology and Ichthyology. Available from <http://www.asih.org>.
- Saitoh K., Miya M., Inoue J.G., Ishiguro N.B., Nishida M. 2003. Mitochondrial genomics of ostariophysan fishes: perspectives on phylogeny and biogeography. *J. Mol. Evol.* 56:464–72.
- Sanderson M.J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol. Biol. Evol.* 19:101–9.
- Sanderson M.J. 2012. r8s version 1.71. Analysis of rates (“r8s”) of evolution. Section of Evolution and Ecology. :Accessed 15 January 2012.
- Santos G.M., Jégu M., Mérona B. 1985. Catálogo de peixes comerciais do baixo rio Tocantins. Manaus: Eletronorte.
- Santos G.M., Rosa P.S. 1988. Alimentação de *Anostomus ternetzi* e *Synaptolaemus cingulatus*, duas espécies de peixes amazônicos com boca superior. *Rev. Bras. Biol.* 58:255–262.
- Scharcansky A., Lucena C.A.S. 2007. *Caenotropus schizodon*, a new chilodontid fish from the Rio Tapajós drainage, Brazil (Ostariophysi: Characiformes: Chilodontidae). *Zootaxa*. 1557:59–66.
- Seehausen O. 2006. African cichlid fish: a model system in adaptive radiation research. *Proc. Biol. Sci.* 273:1987–98.
- Sidlauskas B. 2007. Testing for unequal rates of morphological diversification in the absence of a detailed phylogeny: a case study from characiform fishes. *Evolution (N. Y.)*. 61:299–316.

- Sidlauskas B.L., Mol J.H., Vari R.P. 2011. Dealing with allometry in linear and geometric morphometrics: a taxonomic case study in the *Leporinus cylindriformis* group (Characiformes: Anostomidae) with description of a new species from Suriname. *Zool. J. Linn. Soc.* 162:103–130.
- Sidlauskas B.L., Santos G.M. 2010. *Pseudanos winterbottomi*: A New Anostomine Species (Teleostei: Characiformes: Anostomidae) from Venezuela and Brazil, and Comments on Its Phylogenetic Relationships. 2005. 2005:109–123.
- Sidlauskas B.L., Vari R.P. 2008. Phylogenetic relationships within the South American fish family Anostomidae (Teleostei, Ostariophysi, Characiformes). *Zool. J. Linn. Soc.* 154:70–210.
- Sidlauskas B.L., Vari R.P. 2012. Diversity and distribution of anostomoid fishes (Teleostei: Characiformes) throughout the Guianas. *Cybium*. 36:71–103.
- Sidlauskas B.L. 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylomorphospace approach. *Evolution* (N. Y). 62:3135–56.
- Silva G.S.C., Melo B.F., Oliveira C., Benine R.C. 2013. Morphological and molecular evidence for two new species of *Tetragonopterus* (Characiformes: Characidae) from central Brazil. *J. Fish Biol.* 82:1613–31.
- Sivasundar A., Bermingham E., Ortí G. 2001. Population structure and biogeography of migratory freshwater fishes (*Prochilodus*: Characiformes) in major South American rivers. *Mol. Ecol.* 10:407–17.
- Smith B.T., McCormack J.E., Cuervo A.M., Hickerson M.J., Aleixo A., Cadena C.D., Pérez-Emán J., Burney C.W., Xie X., Harvey M.G., Faircloth B.C., Glenn T.C., Derryberry E.P., Prejean J., Fields S., Brumfield R.T. 2014. The drivers of tropical speciation. *Nature*. 515:406–9.
- Stamatakis A., Hoover P., Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* 57:758–771.
- Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*. 22:2688–2690.
- Steindachner F. 1879. Zur fisch-fauna des Magdalenen Stromes. *Denkschriften der Akad. der Wissenschaften*. 39:19–78.
- Stephen S., Pheasant M., Makunin I. V, Mattick J.S. 2008. Large-scale appearance of ultraconserved elements in tetrapod genomes and slowdown of the molecular clock. *Mol. Biol. Evol.* 25:402–8.
- Tagliacollo V.A., Souza-Lima R., Benine R.C., Oliveira C. 2012. Molecular phylogeny of Aphyocharacinae (Characiformes, Characidae) with morphological diagnoses for the subfamily and recognized genera. *Mol. Phylogenet. Evol.* 64:297–307.
- Taylor B.W., Flecker A.S., Hall R.O. 2006. Loss of a harvested fish species disrupts carbon flow in a diverse tropical river. *Science* (80-.). 313:833–836.

- Thompson A.W., Betancur-R. R., López-Fernández H., Ortí G. 2014. A time-calibrated, multi-locus phylogeny of piranhas and pacus (Characiformes: Serrasalminidae) and a comparison of species tree methods. *Mol. Phylogenet. Evol.*
- Timme R.E., Bachvaroff T.R., Delwiche C.F. 2012. Broad phylogenomic sampling and the sister lineage of land plants. *PLoS One*. 7:e29696.
- Toledo-Piza M. 2000. The Neotropical Fish Subfamily Cynodontinae (Teleostei: Ostariophysi: Characiformes): A Phylogenetic Study and a Revision of *Cynodon* and *Rhaphiodon*. *Am. Museum Novit.* 3286:1–88.
- Travassos H., Santos R.S. 1955. Caracídeos fósseis da bacia do Paraíba. *An. Acad. Bras. Ciências.* 27:313–321.
- Turner G.F. 2007. Adaptive radiation of cichlid fish. *Curr. Biol.* 17:827–831.
- Turner T., McPhee M. V, Campbell P., Winemiller K.O. 2004. Phylogeography and intraspecific genetic variation of prochilodontid fishes endemic to rivers of northern South America. *J. Fish Biol.* 64:186–201.
- Vari R.P., Blackledge T.A. 1996. New Curimatid, *Cyphocharax laticlavus* (Ostariophysi, Characiformes), from Amazonian Ecuador, with a Major Range Extension for *C. gouldingi*. *Copeia*. 1996:109–113.
- Vari R.P., Castro R.M.C., Raredon S.J. 1995. The neotropical fish family Chilodontidae (Teleostei: Characiformes): a phylogenetic study and a revision of *Caenotropus* Günther. *Smithson. Contrib. to Zool.*:1–32.
- Vari R.P., Chang F. 2006. *Cyphocharax derhami*, a new species (Ostariophysi: Characiformes: Curimatidae) from northeastern Peru. *Ichthyol. Explor. Freshwaters.* 17:93–96.
- Vari R.P., Harold A.S. 2001. Phylogenetic Study of the Neotropical Fish Genera *Creagrutus* Gunther and *Piabina* Reinhardt (Teleostei: Ostariophysi: Characiformes), with a Revision of the Cis-Andean Species. *Smithson. Contrib. to Zool.* 613:1–239.
- Vari R.P., Malabarba L.R. 1998. Neotropical ichthyology: an overview. In: Malabarba L.R., Reis R.E., Vari R.P., Lucena Z.M.S., Lucena C.A.S., editors. *Phylogeny and Classification of Neotropical Fishes*. Porto Alegre: Edipucrs. p. 1–11.
- Vari R.P., Ortega H. 1997. A new *Chilodus* species from southeastern Peru (Ostariophysi: Characiformes: Chilodontidae): description, phylogenetic discussion, and comments on the distribution of other chilodontids. *Ichthyol. Explor. Freshwaters.* 8:71–80.
- Vari R.P., Reis R.E. 1995. *Curimata acutirostris*, a new fish (Teleostei: Characiformes: Curimatidae) from the rio Araguaia, Brazil: description and phylogenetic relationships. *Ichthyol. Explor. Freshwaters.* 6:297–304.

- Vari R.P., Sidlauskas B.L., Le Bail P.-Y. 2012. New species of *Cyphocharax* (Ostariophysi: Characiformes: Curimatidae) from Suriname and French Guiana and a discussion of curimatid diversity on the Guiana Shield. *Cybium*. 36:63–69.
- Vari R.P., Williams A.M. 1987. Headstanders of the Neotropical anostomid genus *Abramites* (Pisces: Characiformes: Anostomidae). *Proc. Biol. Soc. Washingt.* 100:89–103.
- Vari R.P., Zanata A.M., Camelier P. 2010. New Species of *Cyphocharax* (Ostariophysi: Characiformes: Curimatidae) from the Rio de Contas Drainage, Bahia, Brazil. *Copeia*. 2010:382–387.
- Vari R.P. 1979. Anatomy, relationships and classification of the families Citharinidae and Distichodontidae (Pisces, Characoidea). *Bull. Br. Museum Nat. Hist.* 36:261–344.
- Vari R.P. 1982a. Systematics of the Neotropical characoid genus *Curimatopsis* (Pisces: Characoidei). *Smithson. Contrib. to Zool.*:1–28.
- Vari R.P. 1982b. *Curimatopsis myersi*, a new curimatid characiform fish (Pisces: Characiformes) from Paraguay. *Proc. Biol. Soc. Washingt.* 95:788–792.
- Vari R.P. 1983. Phylogenetic relationships of the families Curimatidae, Prochilodontidae, Anostomidae, and Chilodontidae (Pisces, Characiformes). *Smithson. Contrib. to Zool.* 378:1–60.
- Vari R.P. 1984. Systematics of the Neotropical characiform genus *Potamorhina* (Pisces, Characiformes). *Smithson. Contrib. to Zool.*:1–36.
- Vari R.P. 1988. The Curimatidae: A lowland Neotropical fish family (Pisces: Characiformes); distribution, endemism, and phylogenetic biogeography. In: Heyer W.R., Vanzolini P.E., editors. *Proceedings of a workshop on Neotropical distribution patterns*. Rio de Janeiro: Academia Brasileira de Ciências. p. 343–377.
- Vari R.P. 1989a. A Phylogenetic Study of the Neotropical Characiform Family Curimatidae (Pisces: Ostariophysi). *Smithson. Contrib. to Zool.*:1–71.
- Vari R.P. 1989b. Systematics of the Neotropical characiform genus *Curimata* Bosc (Pisces: Characiformes). *Smithson. Contrib. to Zool.*:1–63.
- Vari R.P. 1989c. Systematics of the Neotropical Characiform Genus *Psectrogaster* Eigenmann and Eigenmann (Pisces: Characiformes). *Smithson. Contrib. to Zool.*:43.
- Vari R.P. 1989d. Systematics of the Neotropical Characiform Genus *Pseudocurimata* Fernández-Yépez (Pisces: Ostariophysi). *Smithson. Contrib. to Zool.*:28.
- Vari R.P. 1991. Systematics of the Neotropical Characiform Genus *Steindachnerina* Fowler (Pisces: Ostariophysi). *Smithson. Contrib. to Zool.*:118.
- Vari R.P. 1992a. Systematics of the Neotropical Characiform Genus *Cyphocharax* Fowler (Pisces: Ostariophysi). *Smithson. Contrib. to Zool.*:137.

- Vari R.P. 1992b. Systematics of the neotropical characiform genus *Curimatella* Eigenmann and Eigenmann (Pisces: Ostariophysi), with summary comments on the Curimatidae. *Smithson. Contrib. to Zool.*:1–48.
- Vari R.P. 1993. On the Status of the Nominal Curimatid Species *Steindachnerina varii* Géry et al., 1991, and *S. runa* Vari, 1991 (Ostariophysi, Characiformes). *Copeia*. 1993:894–896.
- Wagner C.E., Harmon L.J., Seehausen O. 2012. Ecological opportunity and sexual selection together predict adaptive radiation. *Nature*. 487:366–9.
- Weitzman S.H., Vari R.P. 1988. Miniaturization in South American freshwater fishes; an overview and discussion. *Proc. Biol. Soc. Washingt.* 101:444–465.
- Winemiller K.O., Willis S. 2011. The Vaupes Arch and Casiquiare Canal. In: Albert J.S., Reis R.E., editors. *Historical Biogeography of Neotropical Freshwater Fishes*. Berkeley and Los Angeles: University of California Press. p. 225–242.
- Winterbottom R. 1980. Systematics, osteology and phylogenetic relationships of fishes of the ostariophysan subfamily Anostominae (Characoidei, Anostomidae). *Life Sci. Contrib. R. Ontario Museum*. 123:1–112.
- Wosiacki W.B., Miranda D.P.S.M. 2013. Description of a New Small Species of the Genus *Cyphocharax* (Characiformes: Curimatidae) from the Lower Amazon Basin. *Copeia*. 2013:627–633.
- Xia X. 2013a. DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. *Mol. Biol. Evol.* 30:1720–8.
- Xia X. 2013b. DAMBE5: A Comprehensive Software Package for Data Analysis in Molecular Biology and Evolution. *Mol. Biol. Evol.* 30:1720–1728.
- Zanata A.M., Vari R.P. 2005. The family Alestidae (Ostariophysi, Characiformes): a phylogenetic analysis of a trans-Atlantic clade. *Zool. J. Linn. Soc.* 145:1–144.

ANEXO 1

**The first molecular phylogeny of Chilodontidae (Teleostei: Ostariophysi: Characiformes)
reveals cryptic biodiversity and taxonomic uncertainty**



The first molecular phylogeny of Chilodontidae (Teleostei: Ostariophysi: Characiformes) reveals cryptic biodiversity and taxonomic uncertainty



Bruno F. Melo^{a,*}, Brian L. Sidlauskas^{b,c}, Kendra Hoekzema^b, Richard P. Vari^c, Claudio Oliveira^a

^a Departamento de Morfologia, Instituto de Biociências, UNESP – Universidade Estadual Paulista, Distr. Rubião Jr., 18618-970 Botucatu SP, Brazil

^b Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, 97331-3803, Corvallis OR, USA

^c Department of Vertebrate Zoology, MCR-159, National Museum of Natural History, Smithsonian Institution, PO Box 37012, 20013-7012, Washington DC, USA

ARTICLE INFO

Article history:

Received 13 June 2013

Revised 29 August 2013

Accepted 27 September 2013

Available online 10 October 2013

Keywords:

Amazon

Freshwater fishes

Headstanders

Multilocus analysis

Systematics

ABSTRACT

Chilodontidae is a small family of eight described characiform species popularly known as headstanders. These small to moderately sized fishes are well known to aquarists, who prize their striking spotted pigmentation and unusual behaviors, and to systematists, who have revised both chilodontid genera in recent memory and studied their phylogenetic relationships using a comprehensive morphological dataset. However, no molecular phylogeny for the family has ever been proposed. Here, we reconstruct phylogenetic relationships for all eight known chilodontid species using three mitochondrial and two nuclear loci. Results largely agree with the previous morphological hypothesis, and confirm the monophyly of the family as well as its included genera, *Caenotropus* and *Chilodus*. The molecular topology differs slightly from the morphological hypothesis by placing *Caenotropus maculosus* rather than *C. mestomorgmatus* as the sister to the remaining three congeners, and by reconstructing the Curimatidae as the closest out-group family, rather than the Anostomidae. However, the topologies supported by the morphological data were only slightly less likely and could not be rejected via Shimodaira-Hasegawa tests. Within *Chilodus*, two described species with distinctive pigmentation (*C. fritillus* and *C. zunevei*) appear embedded within the broad distributed *C. punctatus* clade, suggesting the presence of cryptic taxa with polymorphic pigmentation within the present concept of *C. punctatus*. Future work should combine morphological and molecular data to revisit the taxonomy and systematics of *Chilodus* and determine species limits within the *C. punctatus*-group *sensu lato*.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

The 275 species in the fish superfamily Anostomoidea of the order Characiformes (Eschmeyer and Fong, 2013), are widely distributed in Central and South American freshwater ecosystems from Costa Rica to Argentina (Vari, 1983) and in northern South America occur in river systems to both sides of the Andean Cordilleras. Anostomoidea includes the families Anostomidae, Chilodontidae, Curimatidae and Prochilodontidae, that together form a major recognized monophyletic assemblage (Vari, 1983; Buckup, 1998; Sidlauskas, 2008) supported by synapomorphies related to modifications to the gill arches, musculature and dentition. The major clades within the superorder are defined by multiple derived features of those body systems, plus the neurocranium, jaws, connective tissues, pectoral girdle and axial skeleton (Vari, 1983, 1989; Vari et al., 1995; Castro and Vari, 2004; Sidlauskas and Vari, 2008).

Species of Chilodontidae (Fig. 1), one of the four families in the Anostomoidea, adopt a typical head-down orientation while swimming and resting (Vari et al., 1995), an orientation unusual within Characiformes, and are consequently popularly known as headstanders. Given this unusual orientation, small to moderate body sizes and striking pigmentation patterns, chilodontids are well known among aquarists (Isbrücker and Nijssen, 1988). Members of Chilodontidae are broadly distributed in the Amazon and Orinoco river basins, the coastal rivers draining the Guianas, and the Rio Parnaíba basin in northeastern Brazil (Vari and Raredon, 2003; Vari et al., 2009) where they feed on a combination of small invertebrates, sponges and detritus (Goulding et al., 1988; Vari and Raredon, 2003). The family is composed by eight recognized species, six of which were previously studied in taxonomic reviews of *Chilodus* (Isbrücker and Nijssen, 1988) and *Caenotropus* (Vari et al., 1995). Two additional species were described in subsequent decades: *Chilodus fritillus* by Vari and Ortega (1997) and *Caenotropus schizodon* by Scharcansky and Lucena (2007).

Vari (1983) and Vari et al. (1995) proposed a series of synapomorphies for Chilodontidae, as well as for its two included genera, *Caenotropus* and *Chilodus*. In the latter publication, Vari et al.

* Corresponding author.

E-mail address: melo@ibb.unesp.br (B.F. Melo).

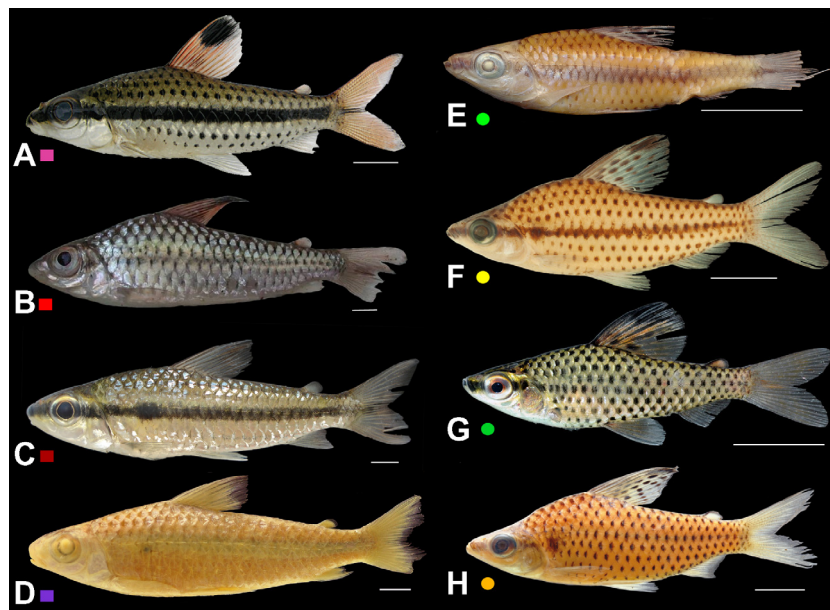


Fig. 1. Representative specimens of the eight known species in Chilodontidae. (A) *Caenotropus maculosus* (live), ANSP 189147, Suriname, Litanie River, Marowijne River basin; (B) *Caenotropus mestomorgmatos* (live), MZUSP 92545, Brazil, Rio Tiquié, tributary of Rio Negro, Amazon basin; (C) *Caenotropus labyrinthicus* (live), MZUSP 95908, Brazil, Rio Teles Pires, tributary of Rio Tapajós, Amazon basin; (D) *Caenotropus schizodon* (preserved), LBP 13847, Brazil, Rio Tapajós, Amazon basin; (E) *Chilodus gracilis* (preserved), LBP 6962, Brazil, Rio Negro, Amazon basin; (F) *Chilodus punctatus* (preserved), AUM 36902, Guyana, Rupununi River, Essequibo River basin; (G) *Chilodus fritillus* (live), AUM 51355, Rio Madre de Diós, a tributary of Mamoré-Madeira system, Amazon basin; (H) *Chilodus zunevei* (preserved), MHNG 2608.040, French Guiana, Kaw River basin. Scale bars indicate one centimeter. Photos by M. Sabaj Pérez (A), F.C.T. Lima (B), J.L.O. Birindelli (C), B.F. Melo (D, E), J. Armbruster (F), N.K. Lujan (G) and B.L. Sidlauskas (H).

(1995) proposed intrageneric phylogenetic relationships for *Caenotropus* on the basis of 10 morphological characters. Scharcansky and Lucena (2007) more recently expanded that analysis to address the phylogenetic placement for *C. schizodon* described in the same publication. No set of phylogenetic relationships within *Chilodus* has ever been proposed. Using a multilocus molecular dataset, Oliveira et al. (2011) corroborated the monophyly of Anostomoidea and of Chilodontidae, though their analysis included only two chilodontid species (*Caenotropus labyrinthicus* and *Chilodus punctatus*). No more detailed molecular hypothesis of phylogenetic relationships within Chilodontidae is available.

Herein, we present the first phylogenetic analysis including all eight species in the Chilodontidae. Our aims were to test the monophyly of Chilodontidae (sensu Vari, 1983) and both genera in the family and to infer their interspecific relationships using a model-based phylogenetic analysis of molecular data. We also discuss the biogeographic distribution of the family, apparent morphological convergences, and the possibility of unrecognized cryptic species within the context of the molecular results.

2. Material and methods

2.1. Taxon sampling

At least one specimen of all eight species of Chilodontidae (Table 1) was included in the analysis. We sampled broadly across the distributional range of *Caenotropus labyrinthicus* and *Chilodus punctatus*, which are the most common species of chilodontids and among the most widespread of the species that have been the subject of recent analysis among all New World characiforms. The map in Fig. 2 illustrates the sampling localities for the ingroup taxa and was prepared using the Quantum GIS 1.7.1 and Cartographer module of Mesquite (Maddison and Maddison, 2013). We included several species of each of the other three anostomoid families (Anostomidae, Curimatidae and Prochilodontidae) as outgroups, as well as one species in Hemiodontidae that was used to

root the tree (Table 1). Tissues were preserved in 95% ethanol or a saturated DMSO/NaCl solution. Voucher specimens were formalin-fixed, alcohol-preserved and deposited in collections (Table 1; abbreviations follow <http://www.asih.org/codons.pdf>).

2.2. DNA extraction and sequencing

Total DNA was extracted from ethanol-fixed muscle tissue with a DNeasy Tissue kit (Qiagen Inc.; <http://www.qiagen.com>) following the instructions of the manufacturer, or following a modified NaCl extraction protocol adapted from Lopera-Barrero et al. (2008). Partial sequences of the genes 16S rRNA (16S, 608 bp), cytochrome oxidase C subunit 1 (COI, 633 bp) and cytochrome B (Cytb, 985 bp) were amplified using one round of polymerase chain reaction (PCR). Additionally, we obtained sequences of the myosin heavy chain 6 gene (Myh6, 704 bp), and recombination activating gene 1 (Rag1, 1210 bp) through nested-PCR following the procedures detailed in Oliveira et al. (2011). PCR amplifications were performed in 12.5 µl reactions containing 9.075 µl of double-distilled water, 1.25 µl 5 × reaction buffer, 0.375 µl MgCl₂, 0.25 µl dNTP mix at 8 mM, 0.25 µl of each primer at 10 µM (list of primers in Table 2), 0.05 µl Platinum Taq DNA polymerase enzyme (Invitrogen; www.invitrogen.com) and 1.0 µl genomic DNA (10–50 ng). The amplification cycles consisted of an initial denaturation (4 min at 95 °C) followed by 28 cycles of chain denaturation (30 s at 95 °C), primer hybridization (30–60 s at 52–54 °C), and nucleotide extension (30–60 s at 72 °C). All PCR products were visually identified in a 1% agarose gel. Samples were cleaned using ExoSAP (Hanke and Wink, 1994) and subsequently sequenced using dye terminators (Big Dye™ Terminator v 3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems; <http://www.appliedbiosystems.com>) purified again through ethanol precipitation and loaded onto an automatic sequencer ABI 3130-Genetic Analyzer (Applied Biosystems) at either the Universidade Estadual Paulista, Botucatu, São Paulo, Brazil, or Oregon State University, Corvallis, Oregon, USA. Consensus sequences were assembled and edited in BioEdit 7.0.9.0 (Hall,

Table 1
Genus and species, voucher specimens, locality information and GenBank accession number of chironomid taxa used in this study. Asterisks show sequences obtained by Oliveira et al. (2011).

Species	Voucher	Specimen Locality	Coordinates	City, State	Country	16s	COI	Cytb	Myh6	Rag1
<i>Caenotropus labyrinthicus</i>	LBP 1828	Rio Araguaia, Amazon basin	15°53'35.6"S/52°15'01"W	Aragarças, Goiás	Brazil	–	EU185613	HQ289538	–	HQ289154*
<i>Caenotropus labyrinthicus</i>	LBP 9216	Rio Apure, Orinoco basin	07°37'24.4"N/66°24'48"W	Cabruta, Guárico	Venezuela	HQ171428*	–	–	HQ289136*	HQ289327*
<i>Caenotropus labyrinthicus</i>	LBP 3050	Rio Orinoco, Orinoco basin	07°38'11.6"N/66°19'4.2"W	Caicara del Orinoco, Bolívar	Venezuela	KF562379	–	KF562437	KF562462	KF562485
<i>Caenotropus labyrinthicus</i>	OS 18770	Rio Nanay, Amazon basin	3.751667S/73.287222 W	Iquitos, Loreto	Peru	KF562380	KF562408	KF562438	KF562463	KF562486
<i>Caenotropus maculosus</i>	MHNG 2705.038	Sipaliwini, Corantijn basin	04°38'48.3"N/57°12'53"W	Sipaliwini	Suriname	KF562381	KF562409	KF562439	KF562464	KF562487
<i>Caenotropus maculosus</i>	MHNG 2717.052	Tapanahony river, Marowijne basin	04°15'0"N/54°31'33.2"W	Sipaliwini	Suriname	KF562382	KF562410	KF562440	KF562465	KF562488
<i>Caenotropus maculosus</i>	ANSP 189156	Marowijne river basin	3°17'24"N/54°4'38"W	Sipaliwini	Suriname	KF562383	KF562411	KF562441	–	KF562489
<i>Caenotropus mestomorgmatos</i>	ANSP 180516	Rio Nanay, Amazon basin	3°46'45"S/73°22'6"W	Iquitos, Loreto	Peru	KF562384	KF562412	KF562442	KF562466	KF562490
<i>Caenotropus mestomorgmatos</i>	OS 18346	Rio Nanay, Amazon basin	3.751667S/73.31625W	Iquitos, Loreto	Peru	KF562385	KF562413	KF562443	KF562467	KF562491
<i>Caenotropus mestomorgmatos</i>	OS 18772	Rio Nanay, Amazon basin	3.751667S/73.287222W	Iquitos, Loreto	Peru	KF562386	KF562414	KF562444	KF562468	KF562492
<i>Caenotropus mestomorgmatos</i>	OS 18323	Rio Nanay, Amazon basin	3.780972S/73.363889W	Iquitos, Loreto	Peru	KF562387	KF562415	KF562445	–	–
<i>Caenotropus mestomorgmatos</i>	OS 18323	Rio Nanay, Amazon basin	3.780972S/73.363889W	Iquitos, Loreto	Peru	KF562388	KF562416	KF562446	–	–
<i>Caenotropus schizodon</i>	LBP 13847	Rio Tapajós, Amazon basin	04°16'49"S/59°59'26.1"W	Itaituba, Pará	Brazil	KF562389	–	KF562447	–	–
<i>Caenotropus schizodon</i>	LBP 13847	Rio Tapajós, Amazon basin	04°16'49"S/59°59'26.1"W	Itaituba, Pará	Brazil	KF562390	KF562417	KF562448	–	KF562494
<i>Chilodus fritillus</i>	AUM 51355	Rio Madre de Dios, Mamoré-Madeira system, Amazon basin	12.27713S/69.15237W	Madre de Dios	Peru	KF562391	KF562418	–	–	KF562495
<i>Chilodus gracilis</i>	LBP 6962	Rio Negro, Amazon basin	00°00'32.1"N/66°55'35.7"W	São Gabriel da Cachoeira, Amazonas	Brazil	KF562392	KF562419	KF562449	KF562470	KF562496
<i>Chilodus gracilis</i>	LBP 6962	Rio Negro, Amazon basin	00°00'32.1"N/66°55'35.7"W	São Gabriel da Cachoeira, Amazonas	Brazil	KF562393	KF562420	KF562450	KF562471	KF562497
<i>Chilodus gracilis</i>	LBP 7026	Rio Negro, Amazon basin	00°16'25.9"N/66°38'36.5"W	São Gabriel da Cachoeira, Amazonas	Brazil	KF562394	KF562421	KF562451	KF562472	KF562498
<i>Chilodus gracilis</i>	LBP 7026	Rio Negro, Amazon basin	00°16'25.9"N/66°38'36.5"W	São Gabriel da Cachoeira, Amazonas	Brazil	KF562395	KF562422	KF562452	KF562473	KF562499
<i>Chilodus punctatus</i>	LBP 11921	Rio Purus, Amazon basin	10°04'44.3"S/67°32'33.9"W	Rio Branco, Acre	Brazil	KF562396	KF562423	KF562453	KF562474	KF562500
<i>Chilodus punctatus</i>	LBP 11921	Rio Purus, Amazon basin	10°04'44.3"S/67°32'33.9"W	Rio Branco, Acre	Brazil	KF585008	KF562424	KF585014	KF585017	–
<i>Chilodus punctatus</i>	LBP 4090	Rio Juruá, Amazon basin	07°34'28.8"S/72°55'24.9"W	Mâncio Lima, Acre	Brazil	HQ171309*	–	HQ289598*	–	HQ289211*
<i>Chilodus punctatus</i>	LBP 12041	Rio Purus, Amazon basin	07°56'11.0"S/63°27'35.3"W	Lábrea, Amazonas	Brazil	KF562398	KF562425	KF562455	KF562475	KF562502
<i>Chilodus punctatus</i>	LBP 9391	Rio Guamá, Amazon basin	01°34'00"S/47°09'51.4"W	Ourém, Pará	Brazil	KF562399	KF585011	KF562456	KF562476	KF562503
<i>Chilodus punctatus</i>	LBP 7202	Rio Araguaia, Amazon basin	15°32'25.8"S/52°26'18.7"W	Barra do Garças, Mato Grosso	Brazil	KF562400	KF562426	KF562457	KF562477	KF562504
<i>Chilodus punctatus</i>	ANSP 180521	Rio Nanay, Amazon basin	3°52'21"S/73°32'43"W	Iquitos, Loreto	Peru	KF562401	KF562427	–	–	KF562505
<i>Chilodus punctatus</i>	OS 18781	Rio Nanay, Amazon basin	3.751667S/73.287222 W	Iquitos, Loreto	Peru	KF562402	KF562428	–	KF562479	KF562506
<i>Chilodus punctatus</i>	OS 18318	Rio Nanay, Amazon basin	3.780972S/73.363889 W	Iquitos, Loreto	Peru	KF562403	KF562429	KF562458	–	KF562507
<i>Chilodus punctatus</i>	LBP 15541	Rio Taktutu, Rio Branco, Amazon basin	03°22'55.9"N/59°51'28.3"W	Bonfim, Roraima	Brazil	KF562404	KF562430	–	–	–
<i>Chilodus punctatus</i>	LBP 15541	Rio Taktutu, Rio Branco, Amazon basin	03°22'55.9"N/59°51'28.3"W	Bonfim, Roraima	Brazil	KF562405	–	KF562459	KF562482	KF562508
<i>Chilodus zunevei</i>	MHNG 2705.043	Commewijne river basin	05°23'47.50"N/54°44'9.17"W	Para	Suriname	KF585009	KF562431	KF585015	KF562483	KF562509
<i>Anostomus ternetzi</i>	LBP 4375	Rio Branco, Amazon basin	02°18'02.0"N/60°55'20.7"W	Mucajá, Roraima	Brazil	HQ171317*	–	HQ289606*	HQ289026*	HQ289219*
<i>Anostomus ternetzi</i>	MZUSP 97271	Rio Tapajós, Amazon basin	08°11'40"S/55°10'47.0"W	Novo Progresso, Pará	Brazil	KF585016	KF585013	KF585016	KF585019	KF585021
<i>Leporinus altipinnis</i>	LBP 4459	Rio Negro, Amazon basin	00°40'03.1"S/62°58'23.5"W	Barcelos, Amazonas	Brazil	HQ171321*	–	HQ289610*	HQ289030*	HQ289223*
<i>Schizodon scotorhabdatus</i>	LBP 3046	Rio Orinoco, Orinoco basin	07°38'11.6"N/66°19'04.2"W	Caicara del Orinoco, Bolívar	Venezuela	HQ171270*	KF562432	HQ289559*	HQ288980*	HQ289177*

Table 1 (continued)

Species	Voucher	Specimen	Locality	Coordinates	City, State	Country	16s	COI	Cytb	Myh6	Rag1
<i>Schizodon vittatus</i>	LBP 3994	23098	Rio Araguaia, Amazon basin	11°40'9"S/50°51'0.30"W	São Félix do Araguaia, Mato Grosso	Brazil	HQ171308*	–	HQ289597*	HQ289018*	HQ289210*
<i>Curimatella dorsalis</i>	LBP 3759	22034	Rio Paraguay, La Plata basin	19°34'33.7"S/56°14'49.5"W	Aquidauana, Mato Grosso do Sul	Brazil	HQ171290*	KF562433	HQ289579*	HQ289000*	HQ289194*
<i>Cyphocharax aspiros</i>	LBP 6109	29560	Lago Maracaibo	09°38'53.8"N/72°34'56.4"W	Machiques de Perijá, Zulia	Venezuela	HQ171363*	–	HQ289650*	HQ289071*	HQ289264*
<i>Cyphocharax gouldingi</i>	LBP 1537	11889	Rio Araguaia, Amazon basin	15°53'53.4"S/52°13'00.6"W	Aragarcas, Goiás	Brazil	HQ171243*	KF562434	HQ289534*	HQ288953*	HQ289150*
<i>Potamorhina altamazonica</i>	LBP 2571	17020	Rio Purus, Amazon basin	08°51'21.5"S/68°42'22.6"W	Boca do Acre, Amazonas	Brazil	HQ171261*	–	HQ289552*	HQ288971*	HQ289168*
<i>Steindachnerina brevipinna</i>	LBP 5185	26336	Rio Paraná, La Plata basin	22°47'29"S/53°20'58"W	Porto Rico, Paraná	Brazil	HQ171339*	–	HQ289628*	HQ289048*	HQ289241*
<i>Hemiodus immaculatus</i>	LBP 1725	12849	Rio Negro, Amazon basin	02°03'10.0"S/60°06'31.7"W	Manaus, Amazonas	Brazil	HQ171246*	–	HQ289537*	HQ288956*	HQ289153*
<i>Prochilodus reticulatus</i>	LBP 6127	29514	Rio Catacumbo, Lago Maracaibo	09°05'08.3"N/72°13'50.5"W	Catacumbo, Zulia	Venezuela	HQ171358*	KF562435	HQ289647*	HQ289067*	HQ289260*
<i>Semaprochilodus laticeps</i>	LBP 1383	12728	Rio Orinoco, Orinoco basin	02°03'10.0"S/60°06'31.7"W	Caicara del Orinoco, Bolívar	Venezuela	HQ171245*	KF562436	HQ289536*	HQ288955*	HQ289152*

1999) and Geneious 6.1 (Biomatters, 2013). Where uncertainty of nucleotide identity was detected, IUPAC ambiguity codes were applied.

2.3. Alignment and phylogenetic analyses

Consensus sequences of each gene for each individual were independently aligned using the Muscle program (Edgar, 2004) under default parameters. The resulting alignments were inspected by eye for obvious misalignments that were then corrected manually. GenBank accession numbers appear in Table 1 and the matrix was deposited in TreeBase (<http://treebase.org>) under number 14605. To evaluate the occurrence of substitution saturation, the index of substitution saturation (Iss) as described by Xia et al. (2003) and Xia and Lemey (2009) was estimated using Dambé 5.3.38 (Xia, 2013). The nucleotide frequencies were computed in MEGA 5.0 (Tamura et al., 2011).

Maximum likelihood (ML) analyses were generated in a partitioned (13 partitions, Table 3) RAXML (Stamatakis, 2006) analysis using the CIPRES web server (Miller et al., 2010). Random starting trees were used for ML tree search and all other parameters were set to default values. All ML analyses were performed under GTR + G since RAXML only applies this model (Stamatakis et al., 2008). The robustness of the topology was investigated using 1000 bootstrap pseudoreplicates.

Maximum parsimony (MP) analysis was performed using PAUP* 4.0b10 (Swofford, 2003). Heuristic searches were performed with minimally 1000 random addition replicates and TBR branch swapping. All characters were unordered and all transformation series were equally weighted. Branches with maximum length of zero were collapsed. Gaps were treated as missing data. The resulting topologies were statistically tested with the bootstrap method (Felsenstein, 1985) using 1000 pseudoreplicates.

We inferred a Bayesian topology (BI) with a partitioned matrix using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) via the CIPRES web portal (Miller et al., 2010). Schema ranging from 1 to 13 partitions was tested following Li et al. (2007) under the Akaike information criterion (AIC) as detailed in Table 3 and in Supplementary data 1. Parameters were estimated using ModelTest 3.6 (see Posada and Crandall, 1998 for model symbols) implemented in PAUP* 4.0b10 (Swofford, 2003) on each partition (Table 3). We performed two runs of four independent MCMC chains with 10 million replicates each, sampling one tree every one thousand generations. The distributions of log likelihood scores were examined using Tracer 1.5 (Rambaut and Drummond, 2007) in order to determine stationarity and decide if extra runs were required to achieve convergence. The first one million generations (10%) were discarded as burn-in, and the remaining trees were used to construct a 50% majority rule consensus tree in PAUP* (Swofford, 2003).

To test the degree of support for the resulting molecular phylogenies versus the previously published morphological hypothesis, we compared the maximum likelihood unconstrained tree to the maximum likelihood trees generated under two different constraint trees in RAXML. In the first, we constrained the four anostomoid families to conform to the arrangement proposed by Vari (1983) in which Anostomidae and Chilodontidae are sister taxa, as are Curimatidae and Prochilodontidae. In the second, the ingroup Chilodontidae was constrained to conform to the morphological hypothesis of relationships within *Caenotropus* of Vari et al. (1995), modified by the addition of *C. schizodon* as sister to *C. labryrinthicus* as proposed by Scharcansky and Lucena (2007). Constraint trees were constructed in Mesquite (Maddison and Maddison, 2013). We compared the maximum likelihood topologies inferred under these three scenarios using the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) as implemented

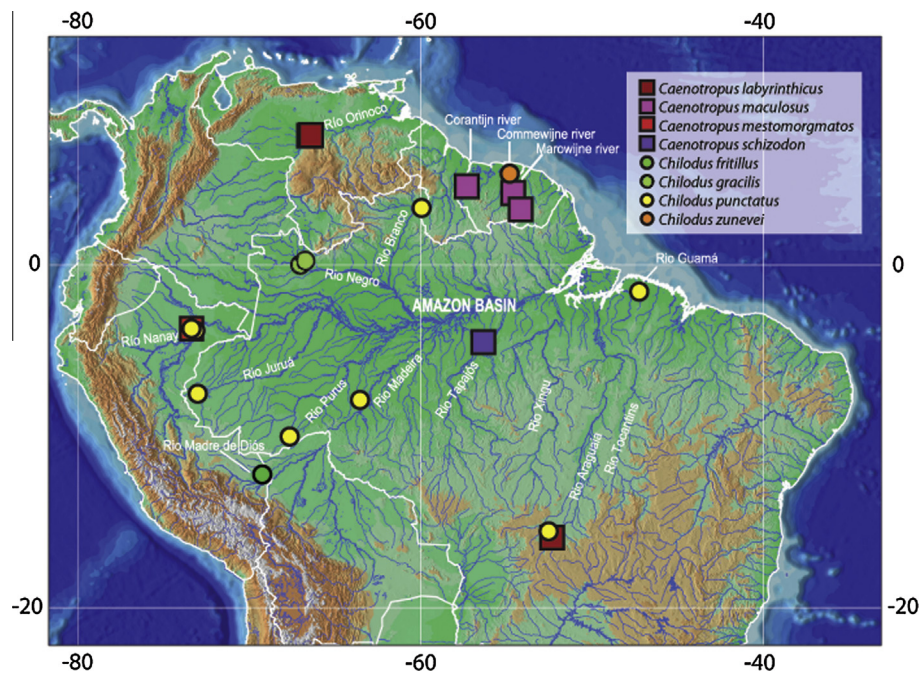


Fig. 2. Distribution map of sampled specimens of Chilodontidae. Outgroups are not shown.

Table 2
Information content and characteristics of each gene.

Gene	Bp after alignment	PCR	Primer sequence (5'–3')	Reference	Π _A	Π _C	Π _G	Π _T
16S	608	1 PCR	16Sa-L – ACGCCTGTTTATCAAAAACAT 16Sb-H – CCGGTCTGAACCTCAGATCACGT	Palumbi (1996)	0.308	0.242	0.221	0.228
COI	633	1 PCR	L6252-Asn – AAGCCGGGAAAGCCCGGCAG H7271-COXI – TCCTATGTAGCCGAATGGTTCTTTT	Melo et al. (2011)	0.245	0.258	0.181	0.314
Cytb	985	1 PCR	LNF – GACTTGAAAACCAACGTTGT H08R2 – GCTTTGGGAGTTAGDGGTGGGACTTAGAATC	Oliveira et al. (2011)	0.276	0.297	0.140	0.285
Myh6	704	1st PCR	F329 – CCGCMTGGATGATCTACAC	Li et al. (2007)	0.306	0.221	0.249	0.241
		2nd PCR	A3R1 – ATTCTCACCACCATCCAGTTGAA A3F2 – GGAGAATCARTCKGTGCTCATCA A3R2 – CTCACCACCATCCAGTTGAACAT					
Rag1	1210	1st PCR	Rag1CF1 – ACCCTCCGTACTGCTGAGAA Rag1CR1 – CGTCGGAAGAGCTTGTGCC	Oliveira et al. (2011)	0.257	0.238	0.281	0.222
		2nd PCR	Rag1CF2 – TACCGCTGAGAAGGAGCTTC Rag1CR2 – TGTTGCCAGACTATTGCCCTC					

Table 3
Gene partitions and their models as selected by ModelTest.

Gene and position	Partitions	Best-fit model
16S	1–608	GTR + G + I
COI 1st position	609–1241/3	TrN + G
COI 2nd position	610–1241/3	F81
COI 3rd position	611–1241/3	GTR + G
Cytb 1st position	1242–2226/3	TrN + I
Cytb 2nd position	1243–2226/3	SYM + I + G
Cytb 3rd position	1244–2226/3	TrN + I
Myh6 1st position	2227–2930/3	GTR + G + I
Myh6 2nd position	2228–2930/3	GTR + I
Myh6 3rd position	2229–2930/3	HKY + G
Rag1 1st position	2931–4140/3	TVM + I + G
Rag1 2nd position	2932–4140/3	TVM + G
Rag1 3rd position	2933–4140/3	TVM + I + G

in the phangorn (Schliep, 2011) package in R (R Development Core Team, 2013). Within phangorn, we compared the likelihood fits assuming a GTR substitution model, four discrete intervals of the gamma distribution ($k = 4$), and 10,000 bootstrap replicates. We computed likelihoods and p -values with and without optimizing

the rate matrix and base frequencies in phangorn (Table 4, Supplementary figures 1 and 2). We also filtered the combined results from both Bayesian Markov chains to determine the percentage of trees in the posterior distribution that were consistent with bipartitions present in the morphological reconstruction, but not present in the best-supported molecular reconstruction.

3. Results

Most sequences from the outgroup species were previously published in the phylogeny of the Characidae by Oliveira et al. (2011); however, the sequences from the cytochrome oxidase C subunit I (COI) for these species were newly generated for this study. The concatenated matrix from 13 outgroups and 32 specimens of the Chilodontidae include 4140 bp and 1506 variable sites of which 1179 were parsimony informative. The I_{ss} index indicated no saturation in transitions or transversions in both asymmetrical (I_{ss}-cAsym) and symmetrical (I_{ss}-cSym) topologies. Table 2 contains numbers of base pairs (bp) after alignment, primer sequences, and nucleotide composition for each analyzed gene. Comparisons of log likelihoods, AIC and BIC values among different partitioning

schemes (from 1 to 13 partitions) were tested and are presented in [Supplementary data 1](#).

Throughout the text and in [Fig. 3](#), measures of support are indicated as a series of three numbers on selected internal branches of the trees subtending labeled clades, starting with posterior probabilities in Bayesian Inference (BI) analysis and followed by non-parametric bootstrap percentages from maximum likelihood (ML) and parsimony (MP) analyses, respectively (e.g., 1/100/100, see [Fig. 3](#)); dashes represent values lower than 0.9 (BI) or 50% (ML and MP) and asterisks represent nodes that have different topologies in different analytical methods. Nodes without support values greater than 0.9 (BI) and 50% (ML and MP) were collapsed.

The Bayesian results represent a majority rule consensus of 18002 post-burn-in trees, the likelihood analysis yielded a single tree with a sum of branch lengths (SBL) of 1.931, and the parsimony analysis returned a single tree (TL: 4726; CI: 0.439; RI: 0.714). [Fig. 3](#) shows the maximum likelihood topology, along with bootstrap and posterior probabilities values from the three analyses, all of which returned very similar results. Anostomidae, Chilodontidae, Curimatidae and Prochilodontidae were all corroborated as monophyletic by all three analyses with 100% bootstrap support or posterior probability equal to one. We obtained a well-supported clade (1/93/70) composed of the Chilodontidae, Curimatidae and Prochilodontidae. The Bayesian consensus differs from the maximum likelihood topology only in a single relationship among the species of the outgroup Curimatidae. The clade composed by Chilodontidae and Curimatidae was well-supported in BI (0.9) and ML (82) but with low support in the MP reconstruction (41). Otherwise, optimal topologies from the three analyses agree completely.

Within Chilodontidae (clade 1), the monophyly of *Caenotropus* (clade 2) was also well-supported with *C. maculosus* (clade 3) as the sister of a clade composed by its three congeners. In clade 4, the analysis placed *C. mestomorgmatus* as sister to clade 5 composed of *C. labyrinthicus* and *C. schizodon*. Clade 6 is composed by two specimens of *C. schizodon* from the Rio Tapajós in the eastern portions of the Amazon basin and one specimen of *C. labyrinthicus* from the Río Nanay in Peru ([Fig. 2](#)), in the western portion of that river system, thereby rendering *C. labyrinthicus* paraphyletic (albeit with only moderate statistical support: -/71/63).

Our results corroborated *Chilodus* as a monophyletic genus (clade 7). Within *Chilodus*, *C. gracilis* (from the Rio Negro in Brazil) appeared as monophyletic (clade 8) and as sister to clade 9 containing the other three currently recognized species of the genus (*C. fritillus*, *C. punctatus* and *C. zunevei*). *Chilodus punctatus*, however, was not recovered as a monophyletic group as a consequence of *C. fritillus* and *C. zunevei* nesting among the twelve sampled individuals of *C. punctatus*.

Within clade 9, two lineages were recovered. The first is composed solely by *Chilodus punctatus* from the Rio Araguaia, a large river south of the mainstream Amazon that drains a portion of the Brazilian Shield and flows into the Rio Tocantins and through that river to the lower Rio Amazonas ([Fig. 2](#)). The second lineage (clade 9, 0.8/92/68) is composed by specimens of *Chilodus punctatus* from the remainder of the Amazon, including the Rios Juruá, Purus and Nanay in the west, the Rio Takutu at the border of Brazil

and Guyana (a tributary of the upper Rio Branco) in the northeast, and the Rio Guamá (a southern tributary of the lower Amazon) in the east ([Fig. 2](#)). Clade nine also includes *C. fritillus* from south-eastern Peru in the Río Madre de Diós, an upper tributary to the Rio Madeira in the western Amazon and a specimen of *C. zunevei* from the Atlantic slope of the Guianas (Commewijne River, Suriname) in north-eastern South America.

While the tree topology discussed above is that best supported by the available molecular data, the maximum likelihood trees under the topological constraints based on previous morphological results ([Supplementary figures 1 and 2](#)) are only slightly less likely ([Table 4](#)). Shimodaira-Hasegawa tests failed to reject these two alternative topologies ([Table 4](#)). Nevertheless, no sampled trees in the Bayesian posterior distributions are fully congruent with the morphological topologies.

4. Discussion

4.1. Interfamilial relationships within Anostomoidea

Our molecular study returns the same hypothesis of close relationship among Chilodontidae, Curimatidae and Prochilodontidae obtained by [Oliveira et al. \(2011\)](#). That congruence is perhaps unsurprising given the similarity of loci and taxa examined in the two studies. This arrangement differs from the interfamilial hypothesis ((Anostomidae + Chilodontidae) + (Curimatidae + Prochilodontidae)) of [Vari \(1983, 1989\)](#), which was based on synapomorphies of multiple morphological systems. The latter result was subsequently obtained by [Buckup \(1998\)](#) using much of the same data. The arrangement of families suggested by the molecular data would imply a large number of morphological convergences or reversals and indicate a much more complex evolutionary history of these fishes than previously suspected. Of particular note would be the very distinctive shared modifications of the gill arches in the Chilodontidae and Anostomidae, which include pronounced enlargement of the upper and lower pharyngeal dentition, the presence of two or more pointed cusps on those teeth, a shift in alignment of the fourth upper pharyngeal tooth plate, vertical expansion of the fifth upper pharyngeal tooth plate, a highly developed *obliqueus dorsalis* muscle on the fourth infra-pharyngobranchial and cord-like ligaments joining the ectopterygoid and ventral wing of the lateral ethmoid; none of which occur elsewhere in Characiformes ([Vari, 1983](#)). Under the scenario implied by the molecular data, either all of these characters are convergent, or they were present in the common ancestor of Anostomoidea and subsequently lost during the evolution of Curimatidae and Prochilodontidae.

The strength of the morphological data makes it noteworthy that the currently available genetic data do not strongly reject the morphological hypothesis of interfamilial relationships. Support for the novel arrangement based on molecular evidence was inconsistent among the three methods that we employed, and support for the maximum likelihood tree that conforms to the morphological hypothesis ([Supplementary figure 1](#)) was similar enough to the support for the unconstrained tree that a Shimoda-

Table 4

Results of Shimodaira-Hasegawa tests of alternative topologies with and without optimization of base frequencies and rate matrices in Phangorn. *P*-values lower than 0.05 would indicate statistical rejection of equivalence of the topologies.

Constraint	No optimization			Optimized		
	lnL	ΔL	<i>p</i> -Value	lnL	ΔL	<i>p</i> -Value
None	-13134.40	0.00	0.7224	-12047.94	0.00	0.7317
<i>Caenotropus</i> (Vari et al., 1995)	-13136.61	2.21	0.4502	-12048.90	0.97	0.5345
Outgroups (Vari, 1983)	-13141.15	6.76	0.1506	-12050.72	2.78	0.3413

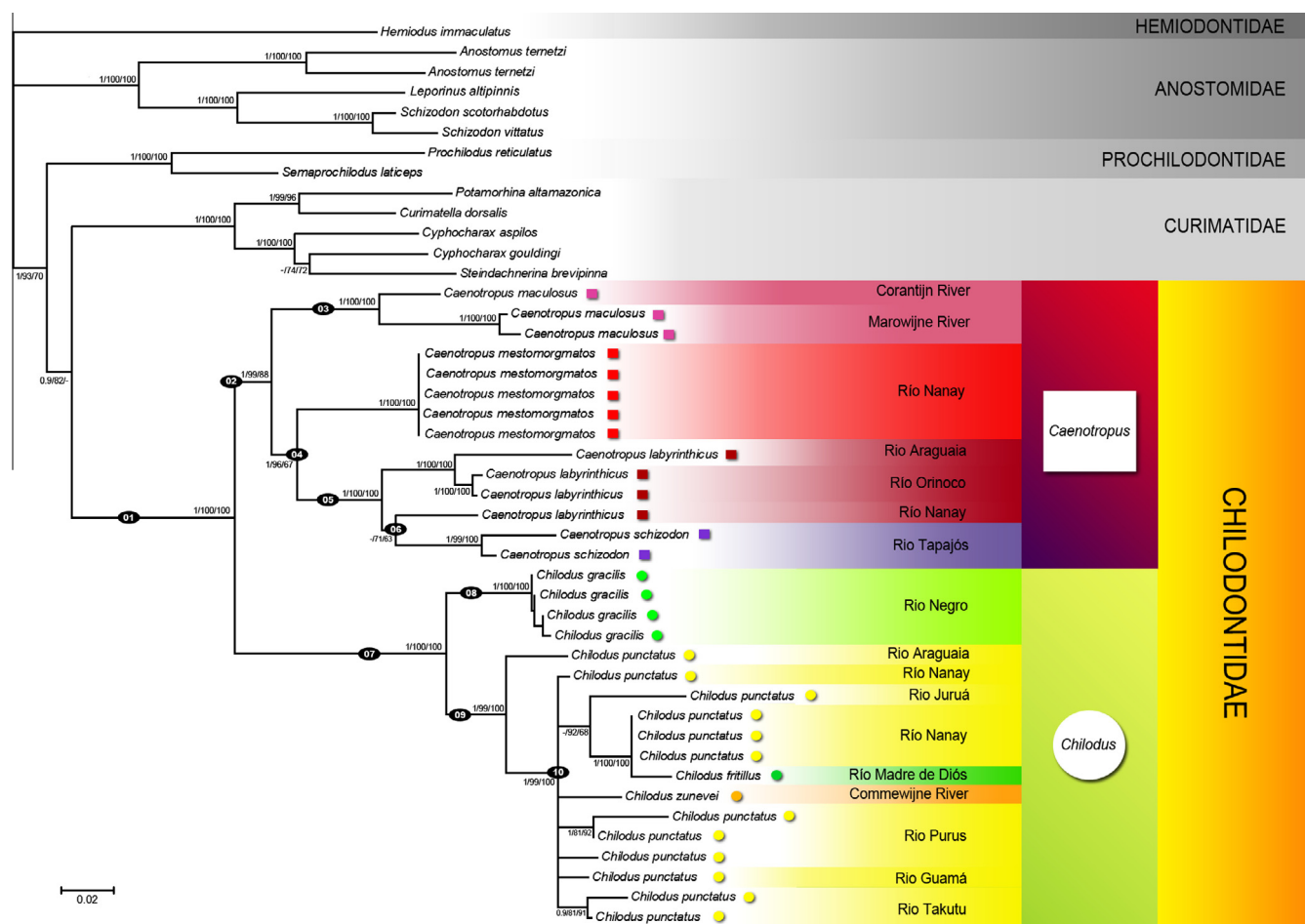


Fig. 3. Relationships among species of Chilodontidae and others taxa of the Anostomoidea obtained by a ML partitioned analyses of the concatenated dataset. A series of three numbers (e.g. 1/100/100) at each of the main nodes shows the posterior probability for that split obtained in BI, percentage of bootstrap support obtained by ML, and percentage of bootstrap obtained by MP analysis, respectively (1000 bootstrap pseudoreplicates). Dashes show values lower than 0.9 (BI) or 50% (ML, MP) and nodes not supported by values higher than 0.9 (BI) or 50% (ML, MP) were collapsed. Asterisks represent nodes that were not obtained by BI or MP analyses. Clades with dark numbered ovals are discussed in the text.

ira-Hasegawa test was unable to distinguish between these hypotheses (Table 4). None of the sampled trees in the Bayesian posterior conform exactly to the morphological hypothesis of relationships among these families, but substantial variation in results exist. The posterior distribution includes a moderate percentage (4.44%) of topologies in which Chilodontidae appears as sister to Prochilodontidae as well as more limited occurrence (<1%) of sister relationships between Anostomidae and Curimatidae, Anostomidae and Prochilodontidae, and Curimatidae and Prochilodontidae. We plan to examine these alternatives more thoroughly in an upcoming analysis with more complete taxon and gene sampling. Such further tests are outside the scope of the present paper, which focuses on Chilodontidae.

4.2. Monophyly of Chilodontidae and included genera

Our results support the monophyly of the family Chilodontidae, corroborating the previous morphological hypothesis of Vari (1983) and Vari et al. (1995) and agreeing with Greenwood et al. (1966), who in a pre-cladistic analysis were the first authors to elevate chilodontids to the familial level. Subsequently, Vari (1983) proposed the monophyly of the family Chilodontidae based on 26 synapomorphies (his characters 74–99) mostly related to the gill-arches, suspensorium, pectoral girdle, and anterior portions of the vertebral column. In a taxonomic revision of *Caenotropus*, Vari et al. (1995) discovered 10 additional synapomorphies (their

characters 27–36) for the family involving a variety of osteological and scale-based characters. Oliveira et al. (2011) later obtained monophyly of the two species of Chilodontidae included in their molecular study.

Clade 2, containing the four species of *Caenotropus*, corroborates the monophyly of that genus as hypothesized by Vari et al. (1995) on the basis of a series of eight synapomorphies involving details of the bones of the infraorbital series, jaws, neurocranium and scales among others. Similarly, clade 7, comprising all species and multiple specimens of *Chilodus* from different localities, appears as a strongly supported monophyletic lineage (1/100/100). Vari et al. (1995) has previously proposed monophyly of *Chilodus* based on eight synapomorphies related to modifications of the bones of the infraorbital series, the lower jaw, the hyoid series, the pattern of the laterosensory canals in the dentary, infraorbitals and neurocranium and the complete loss of the third postcleithrum.

4.3. Interspecific relationships in *Caenotropus*

Vari et al. (1995) distinguished *Caenotropus mestomorgmatos* from its congeners as a new species on the basis of the combination of various features. Two of these, the presence of three scales (versus four) above the lateral line to the dorsal fin and a broad region of dusky pigmentation across most of the dorsal fin (versus no pigmentation or just a distal spot of pigmentation) were unique to the species in the genus and with the latter proposed as an autapomorphy for

the species. Those authors proposed that *C. mestomorgmatos* was, in turn, the sister species to a clade composed of *C. maculosus* and *C. labyrinthicus* and supported their proposal with three synapomorphies involving different portions of the body. Scharcansky and Lucena (2007) subsequently described *C. schizodon* and hypothesized a sister relationship between that new species and *C. labyrinthicus*.

Our results are incongruent with those hypotheses, and reverse the position of *C. maculosus* and *C. mestomorgmatos* proposed by Vari et al. (1995). The results of this study consequently place *C. maculosus* as sister to the group containing the remaining three species in the genus (*C. labyrinthicus*, *C. mestomorgmatos* and *C. schizodon*) with *C. mestomorgmatos* as sister to a clade formed by *C. labyrinthicus* and *C. schizodon* (Fig. 3). The arrangement supported by the morphological analysis is only slightly less likely than that based on the molecular data, and the two topologies could not be distinguished by the Shimodaira-Hasegawa test (Table 4). However, no trees in the Bayesian posterior place *C. mestomorgmatos* as sister to its remaining congeners, and the constrained maximum likelihood tree (Supplementary figure 2) includes a very short internode that effectively creates a polytomy among *C. mestomorgmatos*, *C. maculosus* and a clade containing *C. labyrinthicus* and *C. schizodon*. Thus, the molecular support for the novel hypothesis of relationships within *Caenotropus* is relatively robust, and it is worth exploring whether morphological support for the basal position of *C. maculosus* exists.

As pointed out by Vari et al. (1995), all species of *Chilodus* have darkly pigmented distal portions of the dorsal fin (Fig. 1, see also Isbrücker and Nijssen, 1988). Under our molecular reconstruction, *Caenotropus maculosus* shares this plesiomorphic condition, as does *C. mestomorgmatos* to a fainter extent. Their remaining congeners would then possess a synapomorphic reduction in dorsal-fin pigmentation (Fig. 1).

Our results also suggest the possibility of cryptic diversity within the present concept of *Caenotropus maculosus*. A deep split occurs within Clade 3, separating a specimen from the Corantijn River basin of western Suriname from two others that originated in the Marowijne River system of eastern Suriname and western French Guiana. Within the intervening drainages, *C. maculosus* is only known from the Suriname River which lies close to the Marowijne basin (Sidlauskas and Vari, 2012; Mol et al., 2012). The evidence suggests the presence of two genetically isolated populations. Additional studies should investigate whether these merit species status.

Our results corroborate the close relationship between *Caenotropus labyrinthicus* and *C. schizodon* (clade 5) as proposed by Scharcansky and Lucena (2007). These species share the three character states originally discovered by Vari et al. (1995) as autapomorphies for *C. labyrinthicus* and later proposed as synapomorphies for that species and *C. schizodon* by Scharcansky and Lucena (2007). *Caenotropus labyrinthicus* resolved as paraphyletic in our analyses (clade 5) due to a single individual of that species from the western Amazon clustering with two individuals of *C. schizodon* of the eastern Amazon (clade 6). This relationship received only moderate statistical support (−71/63), and a substantial fraction (22.4%) of the trees in the posterior distribution include a monophyletic *C. labyrinthicus*. The apparent paraphyly of that species in the best-supported tree may represent an artifact of locus selection or model choice, or gene-tree conflict resulting from incomplete lineage sorting. Alternatively, it could reflect the presence of multiple species within the present concept of *C. labyrinthicus*, or indicate that *C. labyrinthicus* and *C. schizodon* are morphotypes of the same species. The samples of *C. schizodon* used herein originated in the Rio Tapajós, a drainage which includes the type locality of that species. However, several individuals of *C. labyrinthicus* with bifid premaxillary teeth, the purported distinguishing feature of *C. schizodon*, have been found in other portions of the Amazon basin including

the Rio Madeira of the western Amazon (J. Zuanon, pers. com.) and Rio Xingu of the Brazilian Shield (observed in this study). Studies of other populations of *C. labyrinthicus* and *C. schizodon* across the range of those species within the Amazon and Tocantins basins are required to evaluate the distribution and intrapopulational consistency of the dentition characters that putatively separate these species, and to further evaluate their reciprocal monophyly.

As a final unanswered question within *Caenotropus*, we note that the known distribution of *Caenotropus mestomorgmatos* is pronouncedly disjunct. One population is distributed in blackwater portions of the southern Río Orinoco of Venezuela and the adjoining upper reaches of the Rio Negro of Brazil (Vari et al., 1995), and another in the blackwater Río Nanay of northeastern Peru (Vari and Ortega, 1997). All of our sequenced samples are from the Peruvian population. In a future study, it would be of great interest to determine whether these two populations have diverged enough genetically to merit possible recognition as separate species.

4.4. Phylogenetic relationships and comments on the taxonomy of *Chilodus*

Chilodus is a well known genus among aquarists and includes four currently valid species (Vari and Ortega, 1997) and we herein present the first phylogenetic reconstruction for the genus. Within the monophyletic *Chilodus*, *C. gracilis* appears as the sister group of a major clade (9) including the remaining three species. Relationships within clade 9 indicate that the present concept of *C. punctatus* is paraphyletic unless *C. fritillus* and *C. zunevei* are included within *C. punctatus*. Within this broadly defined *C. punctatus* species-complex (clade 9), we found two distinct genetic lineages. The first is restricted to the Rio Araguaia, a lowland river draining into the Rio Tocantins system to the southeast of the Amazon basin and which represents an ecoregion with one of the highest degrees of ichthyological endemism in the Neotropics (Albert et al., 2011). Our phylogeny shows *C. punctatus* from the Rio Araguaia as the sister lineage of the remaining specimens which are distributed throughout the Amazon basin (Fig. 3). This arrangement is congruent to the area cladogram for Neotropical fishes constructed by Albert and Carvalho (2011) under which a clade composed by the Tocantins, Araguaia and Xingu basins is sister to a clade composed by the remaining drainages within the Amazon.

Although Isbrücker and Nijssen (1988) did not report specimens of *Chilodus* from the Rio Araguaia basin, Lowe-McConnell (1991) cited *Chilodus* sp. from a tributary of the Rio das Mortes in the Araguaia drainage. Later, Vari and Ortega (1997) described the occurrence of *C. punctatus* from many Amazonian tributaries including several in the Rio Araguaia basin and Lucinda et al. (2007) reported *C. punctatus* in the middle Rio Tocantins. Additional and more detailed taxonomic studies are required to evaluate whether this lineage of *C. punctatus* from the Rio Araguaia basin merits recognition as a distinct species.

The second clade within the *Chilodus punctatus* species-complex (clade 10) is distributed throughout the Amazon and Guianas. For the most part, our analysis failed to resolve relationships within this clade, but it did group *Chilodus punctatus* from the Rio Juruá and Río Nanay with *C. fritillus* from the Río Madre de Dios in Peru, a tributary of the Rio Mamoré-Madeira (Figs. 2 and 3). These river basins are included in the major ecoregion of the western Amazon and Mamoré-Madre de Dios as detailed by Albert et al. (2011). The close genetic similarity between *C. fritillus* and these western *C. punctatus* (particularly those from the Río Nanay) is striking given the pronounced separation of these regions in linear distance, but much more so in terms of distances along rivers. It further suggests that the intense spotting and the absence of a midlateral stripe that was used to diagnose *C. fritillus* (Vari and Ortega, 1997) may be a function of pronounced regional variation within a chromatically

plastic species. Resolution of this question necessitates in-depth analysis of multiple population samples of the genus from the western Amazon.

The remaining individuals of *C. punctatus* within clade 10 are distributed throughout the Amazon drainage, including the Takutu-Branco system in the northeast of the basin, the Rio Guamá in the east of the system and the Rio Purus in the western Amazon. Notably, the clade also includes a specimen of *C. zunevei* from the independent Atlantic-draining Commewijne River in Suriname northeast of the Amazon basin (Figs. 2 and 3).

If *Chilodus punctatus* is eventually split into multiple species, the lineage marked herein as Clade 10 will be the most likely to bear the original name. The holotype of *C. punctatus* was described from Lake Amuku within the Rupununi Savannas of the upper Essequibo River system (Müller and Troschel, 1844). This locality lies about 50 linear km from the Takutu River in the upper Rio Branco drainage, where two samples of *C. punctatus* used in this analysis originated. During high water periods, the Rupununi Savannas (the so called Rupununi portal) connect the Essequibo River basin (via the Rupununi River and the Takutu River) with the Rio Branco, the major tributary of the Rio Negro which is, in turn, the largest northern tributary of the mainstream Amazon. This interconnection allows ichthyofaunal exchange between these otherwise separate Essequibo and Amazon biogeographic provinces (Hubert and Renno, 2006; Souza et al., 2012). Considering that *C. punctatus* has been reported from both the Essequibo and Takutu rivers (Sidlauskas and Vari, 2012; Souza et al., 2012), it is likely that our analyzed specimens from the Takutu River are genetically similar to those living in the type-locality within the upper Essequibo basin.

The taxonomic history of *Chilodus zunevei* is complex and the results of this study further contribute to the uncertainty. *Chilodus zunevei* was originally described as a distinct species by Puyo (1945). That nominal form was soon thereafter reduced to the subspecies level by Géry (1964) who soon thereafter synonymized it into *C. punctatus* (Géry, 1977) only to have the species later resurrected by Isbrücker and Nijssen (1988) (see discussion in Vari and Ortega, 1997). The single available specimen of *C. zunevei* appears as nested within *C. punctatus* and potentially related to specimens from near the type locality of the latter species. Although, it seems possible that *C. zunevei* will again become a junior synonym of *C. punctatus* in an eventual taxonomic revision of *Chilodus*, we prefer to not make a formal taxonomic change on the basis of a single sequenced individual.

Overall, our results suggest that substantial unrecognized diversity exists within the Chilodontidae and that the alpha-taxonomy of both *Caenotropus* and *Chilodus* merits revision. Such efforts should include broader geographic sampling than was possible in the present contribution, as well as renewed attention to the patterns of color variation, squamation and tooth structure that served to diagnose the present species limits within the family. Given the increased ease of integration of comprehensive morphological and molecular datasets afforded by recent and ongoing ichthyological collecting programs and new collaborations, it seems likely that such continent-wide studies will soon reveal greater biodiversity than suspected previously not only among the Chilodontidae but also among other groups of Neotropical freshwater fishes.

Acknowledgments

The authors thank Osvaldo T. Oyakawa (MZUSP), Mark Sabaj Perez (ANSP), Nathan K. Lujan and Jonathan W. Armbruster (AUM), Raphael Covain (MHNG), Hernán Ortega and Vanessa C. Roldán (MUSM) for loans of tissues and vouchers and/or facilitating sample collections. We similarly thank Fábio F. Roxo and Guilherme J.C. Silva for their helpful advice on phylogenetic analyses,

and Joseph Brown for assistance troubleshooting RAXML. Authors are individually supported by CNPq (C.O.), FAPESP proc. 2011/08374-1 (B.F.M. and C.O.) and NSF DEB-1257898 (B.L.S., K.H., and R.P.V.).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jmpev.2013.09.025>.

References

- Albert, J.S., Carvalho, T.P., 2011. Neogene assembly of modern faunas. In: Albert, J.S., Reis, R.E. (Eds.), *Historical Biogeography of Neotropical Freshwater Fishes*. University of California Press, Berkeley and Los Angeles, pp. 119–136.
- Albert, J.S., Petry, P., Reis, R.E., 2011. Major Biogeographic and Phylogenetic Patterns. In: Albert, J.S., Reis, R.E. (Eds.), *Historical Biogeography of Neotropical Freshwater Fishes*. University of California Press, Berkeley and Los Angeles, pp. 21–57.
- Biomatters, 2013. Geneious. Version 6.1. <<http://www.geneious.com>> (accessed February 2013).
- Buckup, P.A., 1998. Relationships of the Characidiinae and phylogeny of characiform fishes (Teleostei: Ostariophysi). In: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M.S., Lucena, C.A.S. (Eds.), *Phylogeny and Classification of the Neotropical Fishes*. Edipucrs, Porto Alegre, pp. 123–144.
- Castro, R.M.C., Vari, R.P., 2004. Detritivores of the South American fish family Prochilodontidae (Teleostei: Ostariophysi: Characiformes): a phylogenetic and revisionary study. *Smith. Contrib. Zool.* Washington 622, 1–189.
- Edgar, R.C., 2004. Muscle: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5, 1–19.
- Eschmeyer, W.N., Fong, J.D., 2013. Species of Fishes by Family/Subfamily. <<http://research.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp>> (accessed February 2013).
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Géry, J., 1964. A review of the chilodinae, with a key to the species. *Trop. Fish Hobbyist* 12 (9), pp. 5–10, 63–67.
- Géry, J., 1977. *Characoids of the World*. T.F.H. Publications, Neptune City, New Jersey, 672 p.
- Goulding, M., Leal Carvalho, M., Ferreira, E., 1988. Rio Negro, rich life in poor water. Amazonian diversity and foodchain ecology as seen through fish communities. SPB Academic Publishing, The Hague.
- Greenwood, P.H., Rosen, D.E., Weitzman, S.H., Myers, G.S., 1966. Phyletic studies of teleostean fishes with a provisional classification of living forms. *Bull. Am. Mus. Nat. Hist.* 131 (4), 339–456.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41, 95–98.
- Hanke, M., Wink, M., 1994. Direct DNA sequencing of PCR-amplified vector inserts following enzymatic degradation of primer and dNTPs. *Biotechniques* 17, 858–860.
- Hubert, N., Renno, J.F., 2006. Historical biogeography of South American freshwater fishes. *J. Biogeogr.* 33, 1414–1436.
- Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- Isbrücker, I.J.H., Nijssen, H., 1988. Review of the South American characiform fish genus *Chilodus*, with description of a new species, *C. gracilis* (Pisces, Characiformes, Chilodontidae). *Beaufortia* 38 (3), 47–56.
- Li, C., Ortí, G., Zhang, G., Lu, G., 2007. A practical approach to phylogenomics: the phylogeny of ray-finned fish (Actinopterygii) as a case study. *BMC Evolut. Biol.* 7, 44.
- Lopera-Barrero, M., Povh, J.A., Ribeiro, R.P., Gomes, P.C., Jacometo, C.B., Silva Lopes, T., 2008. Comparison of DNA extraction protocols of fish fin and larvae samples: modified salt (NaCl) extraction. *Cienc. Investig. Agrar.* 35 (1), 65–74.
- Lowe-McConnell, R.H., 1991. Natural history of fishes in Araguaia and Xingu Amazonian tributaries, Serra do Roncador, Mato Grosso, Brazil. *Ichthyol. Explor. Freshw.* 2, 63–82.
- Lucinda, P.H.F., Freitas, I.S., Soares, A.B., Marques, E.E., Agostinho, C.S., Oliveira, R.J., 2007. Fish, Lajeado Reservoir, rio Tocantins drainage, State of Tocantins, Brazil. *Check List* 3 (2), 70–83.
- Maddison, W.P., Maddison, D.R., 2013. Mesquite: A Modular System for Evolutionary Analysis. Version 2.75, University of Arizona, Tucson.
- Melo, B.F., Benine, R.B., Mariguela, T.C., Oliveira, C., 2011. A new species of *Tetragonopterus* Cuvier, 1816 (Characiformes: Characidae: Tetragonopterinae) from the rio Jari, Amapá, northern Brazil. *Neotrop. Ichthyol.* 9 (1), 49–56.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. In *Gateway Computing Environments Workshop (GCE)*.
- Mol, J.H., Vari, R.P., Covain, R., Willink, P., Fisch-Muller, S., 2012. Annotated checklist of the freshwater fishes of Suriname. *Cybio* 36 (1), 293–319.

- Müller, J., Troschel, F.H., 1844. Synopsis generum et specierum familiae Characinarum (Prodonus descriptionis novorum generum et specierum). Archiv für Naturgeschichte 10, 81–98.
- Oliveira, C., Avelino, G.S., Abe, K.T., Mariguela, T.C., Benine, R.C., Ortí, G., Vari, R.P., Castro, R.M.C., 2011. Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. BMC Evolut. Biol. 11, 275.
- Palumbi, S.R., 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis, D., Moritz, C., Mable, B. (Eds.), Molecular Systematics. Sinauer Associates Inc., Massachusetts, pp. 205–247.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.
- Puyo, J., 1945. *Chilodus zunevei* poisson d'eau douce de la Guyane française. Bull. Soc. Hist. Nat. Toulouse 80, 183–185.
- R Development Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. <<http://beast.bio.ed.ac.uk/Tracer>> (accessed February 2013).
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
- Scharcansky, A., Lucena, C.A.S., 2007. *Caenotropus schizodon*, a new chilodontid fish from the Rio Tapajós drainage, Brazil (Ostariophysi: Characiformes: Chilodontidae). Zootaxa 1557, 59–66.
- Schliep, K.P., 2011. Phangorn: phylogenetic analysis in R. Bioinformatics 27, 592–593.
- Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114.
- Sidlauskas, B.L., 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylomorphospace approach. Evolution 62 (12), 3135–3156.
- Sidlauskas, B.L., Vari, R.P., 2008. Phylogenetic relationships within the South American fish family Anostomidae (Teleostei, Ostariophysi, Characiformes). Zool. J. Linnean Soc. 154, 70–210.
- Sidlauskas, B.L., Vari, R.P., 2012. Diversity and distribution of anostomoid fishes (Teleostei: Characiformes) throughout the Guianas. Cybium 36, 71–103.
- Souza, L.S., Armbruster, J.W., Werneke, D.C., 2012. The influence of the Rupununi portal on distribution of freshwater fish in the Rupununi district, Guyana. Cybium 36, 31–43.
- Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
- Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol. 57, 758–771.
- Swofford, D.L., 2003. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates Inc., Sunderland, MA.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
- Vari, R.P., 1983. Phylogenetic relationships of the families Curimatidae, Prochilodontidae, Anostomidae and Chilodontidae (Pisces, Characiformes). Smith. Contrib. Zool. Washington 378, 1–60.
- Vari, R.P., 1989. A phylogenetic study of the Neotropical characiform family Curimatidae. Smith. Contrib. Zool. Washington 471, 1–71.
- Vari, R.P., Castro, R.M.C., Raredon, S.J., 1995. The Neotropical fish family Chilodontidae (Teleostei: Characiformes): a phylogenetic study and a revision of *Caenotropus* Günther. Smith. Contrib. Zool. Washington 577, 1–32.
- Vari, R.P., Ferraris Jr., C.J., Radosavljevic, A., Funk, V.A., 2009. Checklist of the freshwater fishes of the Guiana Shield. Bull. Biol. Soc. Washington 17, 1–95.
- Vari, R.P., Ortega, H., 1997. A new *Chilodus* species from southeastern Peru (Ostariophysi: Characiformes: Chilodontidae): description, phylogenetic discussion, and comments on the distribution of other chilodontids. Ichthyol. Explor. Freshw. 8 (1), 71–80.
- Vari, R.P., Raredon, S.J., 2003. Family Chilodontidae. In: Reis, R.E., Kullander, S.O., Ferraris, C.J., Jr. (Eds.), Check List of the Freshwater Fishes of South and Central America. Edipucrs, Porto Alegre, pp. 85–86.
- Xia, X., Lemey, P., 2009. Assessing substitution saturation with DAMBE. In: Lemey, P., Salemi, M., Vandamme, A.M. (Eds.), The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny, second ed. University Press, Cambridge, pp. 615–630.
- Xia, X., 2013. DAMBE5: A Comprehensive Software Package for Data Analysis in Molecular Biology and Evolution. Mol. Biol. Evol. 30 (7), 1720–1728.
- Xia, X., Xie, Z., Salemi, M., Chen, L., Wang, Y., 2003. An index of substitution saturation and its application. Mol. Phylogenet. Evol. 26, 1–7.

ANEXO 2

**New species of *Cyphocharax* (Characiformes: Curimatidae) from the upper rio Negro,
Amazon basin**

New species of *Cyphocharax* (Characiformes: Curimatidae) from the upper rio Negro, Amazon basin

Bruno F. Melo¹ and Richard P. Vari²

A new species of *Cyphocharax*, Curimatidae, apparently endemic to the blackwater upper rio Negro of the Amazon basin in northern Brazil, is described. The new species is readily distinguished from its congeners by the presence of a distinctly longitudinally elongate, posteriorly vertically expanding patch of dark pigmentation along the midlateral surface of the caudal peduncle, with the patch extending from the base of the middle caudal-fin rays anteriorly past the vertical through the posterior terminus of the adipose fin. The new species additionally differs from all congeners in details of body and fin pigmentation and meristic and morphometric ratios. Evidence for the assignment of the species to *Cyphocharax* and the occurrence of other species of the Curimatidae apparently endemic to the upper rio Negro catchment is discussed.

Uma espécie nova de *Cyphocharax*, Curimatidae, aparentemente endêmica das águas escuras do rio Negro, bacia amazônica no norte do Brasil, é descrita. A espécie nova é prontamente distinguida de suas congêneres pela presença de uma mancha escura distintamente alongada longitudinalmente e posteriormente expandida na vertical ao longo da superfície lateral do pedúnculo caudal, sendo estendida da base dos raios medianos da nadadeira caudal até a linha vertical do término da nadadeira adiposa. Adicionalmente, a espécie nova difere de todas as congêneres nos detalhes do corpo, pigmentação das nadadeiras e nas proporções merísticas e morfométricas. As evidências para alocação da espécie no gênero *Cyphocharax* e a ocorrência de outras espécies de Curimatidae aparentemente endêmicas do alto rio Negro são discutidas.

Key words: Biodiversity, Blackwater, Neotropical fish, Pigmentation, Taxonomy.

Introduction

The genus *Cyphocharax* Fowler, 1906, is widely distributed throughout South American freshwaters and has the greatest geographic range of any genus in the family Curimatidae. At the northern limit of the range of the genus, the trans-Andean species *C. magdalenae* extends from the Pacific versant rivers of Costa Rica (Vari, 1992a) south into western Colombia (Maldonado-Ocampo *et al.*, 2010). At the other extreme of the range of the genus, *C. voga* and *C. saladensis* inhabit estuarine rivers of the lower portions of río de La Plata basin in Argentina (Vari, 1992a). Intervening regions of South America are home to numerous species of *Cyphocharax* whose known distributions range from a single locality to broad regions of the continent. With 37 valid species, *Cyphocharax* is by far the most speciose among the eight genera in the Curimatidae (Vari, 2003; Vari *et al.*, 2012). Of the species in *Cyphocharax*, over half (20 spp.) occur in the Amazon basin (Vari, 1992a) making that catchment the epicenter for the genus in terms of species richness.

Notwithstanding the description of numerous species of the Curimatidae as new in the last quarter century, ichthyological explorations across the range of the family continue to regularly reveal previously unknown species of the family (Vari & Blackledge, 1996; Pavanelli & Britski, 1999; Vari & Chang, 2006; Lucinda & Vari, 2009; Netto-Ferreira & Vari, 2011; Vari *et al.*, 2010, 2012; Wosiacki & Miranda, 2013). A recent expedition to the region of São Gabriel da Cachoeira in the blackwater upper rio Negro of northern Amazonian Brazil yielded a previously unknown species of *Cyphocharax* with a distinctive pigmentation pattern on the caudal peduncle. We herein describe that species as new.

Material and Methods

Counts and measurements follow Vari (1992a). Measurements were point-to-point linear distances taken using digital caliper with a precision of 0.1 mm. In the description, the number of examined specimens with a particular count

¹Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, District Rubião Júnior s/n, 18618-970 Botucatu, SP, Brazil. melo@ibb.unesp.br

²Division of Fishes, Department of Vertebrate Zoology, MCR-159, National Museum of Natural History, PO Box 37012, Smithsonian Institution, 20013-7012 Washington DC, USA. varir@si.edu

is indicated in parentheses and the value of the holotype is designated by an asterisk. Vertebrae were counted from radiographs. Comparisons were made to the subset of the species of *Cyphocharax* most similar to the new form and redescribed or described in Vari (1992a), Vari & Blackledge (1996), Vari & Chang (2006) and Vari *et al.* (2010, 2012). Abbreviations in the text are head length (HL) and standard length (SL). Collection abbreviations are: Laboratório de Biologia e Genética de Peixes da Universidade Estadual Paulista (LBP), Botucatu, SP; Laboratório de Ictiologia de Ribeirão Preto da Universidade de São Paulo (LIRP), Ribeirão Preto, SP, and Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, SP.

***Cyphocharax sanctigabrielis*, new species**

Fig. 1

Holotype. MZUSP 115004, 60.7 mm SL, Brazil, Amazonas, São Gabriel da Cachoeira, Igarapé Nouba Uba, near BR-307 road, upper rio Negro, Amazon basin, 00°00.321'N 66°55.357'W, 9 Aug 2008, C. Oliveira, M. I. Taylor, M. A. Alexandrou & J. I. R. Porto.

Paratypes. LBP 6963, 6, 44.3–67.0 mm SL (tissues 33399 and 33400); collected with holotype.

Diagnosis. *Cyphocharax sanctigabrielis* is distinguished from all other species of *Cyphocharax* by the presence on the lateral surface of the caudal peduncle of a distinct, longitudinally elongate, posteriorly often vertically expanded, darkly pigmented mark extending anteriorly from the base of the median caudal-fin rays to the vertical through the posterior limit of the base of the adipose fin. Dark pigmentation on the caudal peduncle is absent in *C. abramoides*, *C. aspilos*, *C. derhami*, *C. festivus*, *C. leucostictus*, *C. magdalenae*, *C. microcephalus*, *C. multilineatus*, *C. nigripinnis*, *C. notatus*, *C. pinnilepis*, *C. plumbeus*, *C. stilbolepis*, and *C. vexillapinnus*. When present, the dark pigmentation in that region in other congeners can alternatively be rotund (*C. gangamon*, *C. gillii*, *C. gouldingi*, *C. helleri*, *C. mestomylon*, *C. oenas*, *C. punctatus*, *C. spiluroopsis*, and *C. vanderi*), somewhat triangular with its posterior border darker (*C. meniscaprorus*), a vertically oriented ellipsoid (*C. aninha*), in the form of an elongate stripe (*C. laticlavus*, *C. modestus*, *C. nagelii*, and *C. pantostictus*) or longitudinally ovoid but terminating anteriorly distinctly posterior to the vertical through the posterior limit of the base of the adipose fin (*C. biocellatus*, *C. gilbert*, *C. saladensis*, *C. santacatarinae*, *C. signatus*, *C. spilotus*, *C. spilurus*, and *C. voga*). *Cyphocharax sanctigabrielis* can be further diagnosed from various congeners by the absence of



Fig. 1. *Cyphocharax sanctigabrielis*, holotype, MZUSP 115004, 60.7 mm SL, Igarapé Nouba Uba, rio Negro, Amazon basin, São Gabriel da Cachoeira, Amazonas State, Brazil. Views of preserved specimen (a) and radiograph (b).

a series of dark stripes or spots running between the scale rows (vs. the presence of such dark pigmentation in *C. helleri*, *C. multilineatus*, and *C. pantostictus*), the absence of two to eight dark spots distributed along the midlateral surface of the body (vs. the presence of such pigmentation in *C. biocellatus*, *C. punctatus*, and *C. vanderi*), the absence of a patch of dark pigmentation on the dorsal or adipose fins (vs. the presence of such pigmentation in *C. nigripinnis*, *C. notatus* and *C. vexillapinnus*) and a non-fleshy upper lip (vs. lip very fleshy in *C. mestomyllo*). *Cyphocharax sanctigabrielis* can be meristically further distinguished from various other congeners by the possession of 31 pored scales along the lateral line from the supracleithrum to the hypural joint (vs. four to nine pored scales in *C. aninha*, *C. saladensis* and *C. signatus*, 27 in *C. vanderi*, 27 or 28 in *C. gangamon* and in sum 32 to 97 in *C. abramoides*, *C. aspilos*, *C. gilbert*, *C. leucostictus*, *C. magdalenae*, *C. nagelii*, *C. nigripinnis*, *C. pinnilepis*, *C. platanus*, *C. santacatarinae*, *C. stilbolepis*, and *C. voga*), the presence of 9 branched dorsal-fin rays (vs. 10 to 12 in *C. spilotos*), and the possession of 30 or 31 vertebrae (vs. 28 or 29 in *C. vanderi* and 32 to 37 in *C. abramoides*, *C. aspilos*, *C. gilbert*, *C. modestus*, *C. nagelii*, *C. notatus*, *C. platanus*, *C. santacatarinae*, *C. stilbolepis*, and *C. voga*). Morphometric ratios and counts serve to further discriminate *C. sanctigabrielis* from various congeners (for comparative data see Vari, 1992a; Vari & Blackledge, 1996; Vari & Chang, 2006; Vari *et al.*, 2010, 2012).

Description. Morphometric data presented in Table 1. Body moderately elongate; elongation more pronounced in larger specimens. Dorsal profile of head convex from margin of upper lip to vertical through anterior nares, nearly straight from that point to posterior terminus of head. Dorsal profile of body slightly convex from tip of supraoccipital spine to dorsal-fin origin; straight to slightly convex and posteroventrally-slanted from base of last dorsal-fin ray to adipose-fin origin and then slightly concave to origin of anteriormost dorsal procurent ray. Dorsal surface of body with barely apparent median ridge anterior to dorsal-fin base and transversely rounded posterior to fin base. Ventral profile of head very slightly convex to nearly straight from margin of lower lip to isthmus. Ventral profile of body smoothly convex from isthmus to pelvic-fin origin, convex from that point to rear of anal-fin base and then slightly concave to origin of anteriormost ventral procurent ray. Prepelvic region smoothly flattened transversely, with midventral series of scales comparable in size to those on adjoining portions of body. Postpelvic region of body transversely rounded.

Dorsal fin pointed, with distal margin straight and first and second branched rays longest. Pectoral-fin profile pointed. Tip of adpressed pectoral fin falls four or five scales short of vertical through pelvic-fin origin. Pelvic fin profile pointed.

Table 1. Morphometric data for *Cyphocharax sanctigabrielis* (N = 7). Range includes holotype and paratypes. SD = standard deviation.

	Holotype	Range	Mean $\pm$ SD
Standard length (mm)	60.7	44.3-67.0	53.1
Percentage of standard length			
Greatest body depth	33.2	30.4-33.2	32.0 $\pm$ 0.9
Snout to dorsal-fin origin	47.9	46.6-48.6	47.7 $\pm$ 0.7
Snout to pectoral-fin origin	24.6	24.6-27.7	26.4 $\pm$ 1.3
Snout to pelvic-fin origin	54.3	52.2-54.7	53.7 $\pm$ 1.0
Snout to anal-fin origin	80.0	77.6-80.0	79.2 $\pm$ 0.8
Snout to anus	75.7	74.1-75.7	74.6 $\pm$ 0.6
Dorsal-fin origin to hypural joint	57.8	56.4-58.9	57.3 $\pm$ 0.8
Dorsal-fin origin to anal-fin origin	45.6	43.6-45.6	44.7 $\pm$ 0.7
Dorsal-fin origin to pelvic-fin origin	33.0	30.9-33.0	32.0 $\pm$ 0.6
Dorsal-fin origin to pectoral-fin origin	34.0	32.2-34.0	33.0 $\pm$ 0.8
Caudal-peduncle depth	12.7	11.5-12.8	12.4 $\pm$ 0.4
Pectoral-fin length	17.8	16.8-19.1	18.0 $\pm$ 0.8
Pelvic-fin length	19.5	19.3-20.8	19.9 $\pm$ 0.6
Dorsal-fin length	23.5	23.5-27.2	25.3 $\pm$ 1.4
Head length	25.4	23.9-26.6	25.7 $\pm$ 0.9
Percentage of head length			
Snout length	32.3	29.3-32.9	31.7 $\pm$ 1.3
Orbital diameter	38.1	34.1-39.3	36.6 $\pm$ 1.9
Postorbital length	34.6	33.1-39.0	36.3 $\pm$ 1.9
Interorbital width	39.6	33.0-42.8	41.0 $\pm$ 1.2

Tip of adpressed pelvic fin falls one or two scales short of anus. Caudal fin forked with tips of lobes somewhat pointed. Adipose fin well developed. Anal fin emarginate with first branched ray longest and about three times length of ultimate ray. Tip of adpressed anal fin falls five or six scales short of point of origin of ventral most caudal-fin ray.

Head profile anteriorly pointed overall from lateral view, but rounded in region of mouth and snout. Upper jaw very slightly longer than lower jaw with mouth slightly subterminal or jaws equal. Nostrils very close; anterior circular to ovoid, posterior crescent-shaped with aperture closed by thin flap of skin separating nostrils. Adipose eyelid well developed and extending posteriorly onto anterodorsal portion of opercle. Smaller specimens with central aperture in adipose eyelid round and approximately corresponding to limits of pupil. Opening in larger individuals vertically-ovoid with eyelid overlapping anterior and posterior portions of pupil.

All scales of lateral line pored with primary laterosensory canal straight. Pored lateral-line scales from supracleithrum to hypural joint 31* (7). Pored scales on basal portions of caudal fin posterior to hypural joint 2* (5) or 3 (2). Scales in transverse series from dorsal-fin origin to lateral line 5* (6) or 5½ (1). Scales in transverse series from anal-fin origin to lateral line 4*(3), 4½ (3) or 5 (1). Scales between anus and anal-fin origin 2* (6) or 3 (1). Middorsal series of scales from rear of supraoccipital spine to dorsal-fin origin 9* (6) or 10 (1). Smaller individuals lacking scales over caudal-fin lobes. Midsized and larger specimens with field of adherent scales continuing posteriorly onto basal portion of each caudal-fin lobe. Anterior scales similar in size to those on posterior portion of caudal peduncle. Adherent scales present over

basal portions of pelvic fin; scales primarily covering last unbranched fin-ray.

Dorsal-fin rays iii,9* (7), with first unbranched ray very short. Anal-fin rays ii,7* (3) or iii,7 (4), with first ray very short when three unbranched rays present. Pelvic-fin rays ii, 9* (7). Pectoral-fin rays 14 (1) or 15* (6). Total vertebrae 30 (1) or 31* (6).

Coloration in alcohol. Ground coloration of specimens fixed in alcohol brownish; those fixed in formalin yellowish. Overall coloration of larger specimens retaining guanine on scales silvery or silvery golden. Dusky surface coloration darker on dorsal portion of head; head dusky dorsolaterally and light colored ventrally. Chromatophores on postorbital region of head slightly larger than those on snout other than in area posterior of orbit overlapped by adipose eyelid. Overall pigmentation of that portion of postorbital region consequently somewhat lighter than that of adjoining areas.

Dusky surface coloration darker on dorsal and dorsolateral regions of body. Ground coloration of body more yellowish ventrally. Dusky surface coloration darker on dorsal and dorsolateral regions of body. Ground coloration of body more yellowish ventrally. Deep-lying, dark chromatophores forming faint, dusky midlateral stripe on body. Stripe most evident posterior of vertical through base of ultimate dorsal-fin ray and with posterior section of stripe slightly expanded vertically. Stripe continues to anterior margin of patch of dark pigmentation

on midlateral surface of caudal peduncle. Middorsal region of body with series of small dark chromatophores running from tip of supraoccipital spine to anterior border of adipose fin, darker than adjoining areas. Scales on dorsal and dorsolateral regions of body with dark central regions. Light *versus* dark regions cumulatively forming overall reticulate pattern on those portions of body. Dark chromatophores sparsely distributed over central portion of exposed region of scales but more concentrated anteriorly. Dark pigmentation absent on scales on lateral surface of body ventral to horizontal through base of pectoral fin and also on abdomen.

Dorsal, anal, and caudal fins somewhat dusky, with ray margins outlined by small, dark chromatophores. Dark pigmentation most developed distally on caudal-fin lobes, dorsal fin, and anterior rays of anal fin. Pectoral and pelvic fins hyaline overall, but with rays outlined by small, dark chromatophores. Adipose fin speckled with small dark chromatophores.

Distribution. *Cyphocharax sanctigabrielis* is presently known from the upper rio Negro, Amazon basin (Fig. 2). The type locality, Igarapé Nouba Uba (Fig. 3) empties into the rio Negro upriver of the city of São Gabriel da Cachoeira.

Habitat notes. The Igarapé Nouba Uba (Fig. 3) is a shallow (30-50 cm), slow-flowing stream over fine and sand substrate within a well-preserved forested setting with the stream borders lined with grasses and rushes.

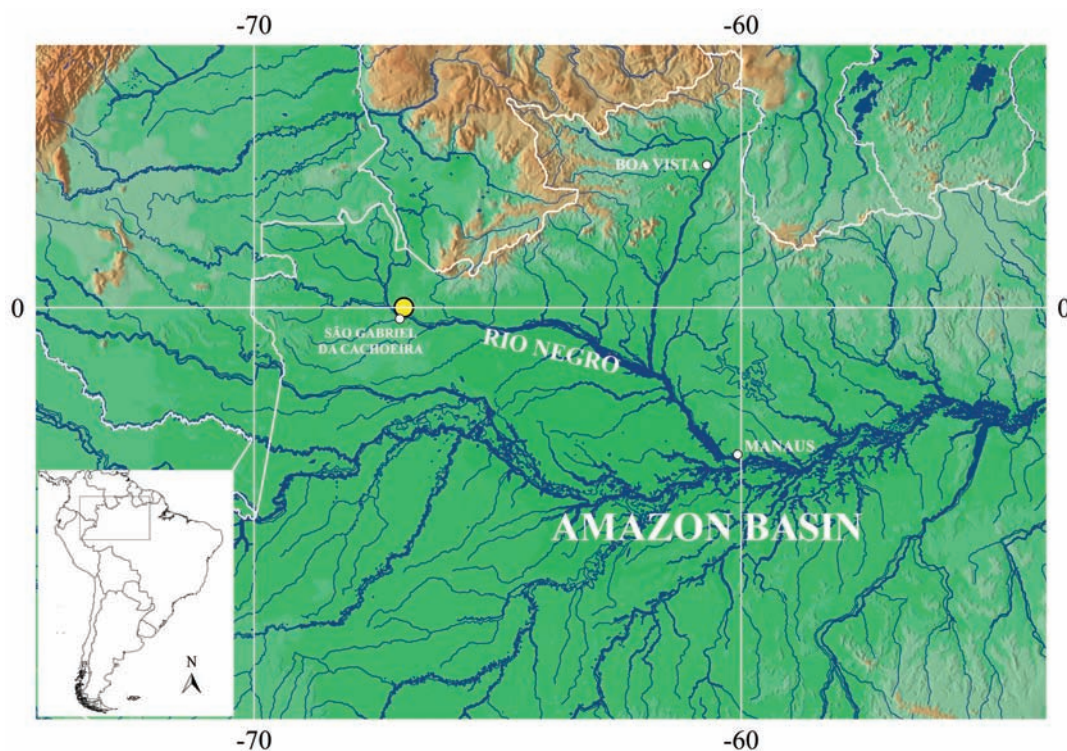


Fig. 2. Map of Western Amazon basin showing the collection locality of *Cyphocharax sanctigabrielis* (yellow dot) in the upper rio Negro near São Gabriel da Cachoeira, Amazonas, Brazil.



Fig. 3. Igarapé Nouba Uba, type locality of *Cyphocharax sanctigabrielis*, upper rio Negro, near São Gabriel da Cachoeira, Amazon basin, northern Brazil.

Etymology. The species name, *sanctigabrielis*, is in reference to the município de São Gabriel da Cachoeira, Amazonas State, within which the new species was discovered.

Generic placement. As presently defined, *Cyphocharax* is delimited by the combination of the possession of the synapomorphies for a quadritomy formed by that genus, *Curimatella*, *Pseudocurimata* and *Steindachnerina* in conjunction with the absence of the synapomorphies diagnostic for each of those three other genera (Vari, 1989a, 1989c, 1991, 1992a, 1992b). The absence of identified derived features common to the species of *Cyphocharax* leaves open the possibility that the closest relatives of *Cyphocharax sanctigabrielis* (and likely some other species in *Cyphocharax*) lie with one of *Curimatella*, *Pseudocurimata*, and *Steindachnerina* rather than with their nominal congeners. That possibility notwithstanding, *Cyphocharax sanctigabrielis* lacks the externally obvious synapomorphies present in *Curimatella* (Vari, 1989a: 58, 1992b: 4), *Pseudocurimata* (Vari, 1989c: 3) and *Steindachnerina* (Vari, 1989: 58; 1991: 23). In the absence of such derived features, the new species is assigned to *Cyphocharax* under the present definition of that genus.

Discussion

The rio Negro is the second largest tributary of the Amazon basin and is characterized by extreme black water due to high concentrations of dissolved humic and fulvic acids, the low levels of dissolved minerals, low pH (between 3.6 and 5.8) and low conductivity (Furch, 1984; Goulding *et al.*, 1988). Goulding *et al.* (1988) noted that these extreme conditions possibly exclude many species and genera from that basin

whereas yet others are endemic to that river system. In the case of the Curimatidae, only a subset of the species of the family known from the Amazon extend into the upper rio Negro between the rapids at São Gabriel da Cachoeira north to the rio Casiquiare. These are *Curimata incompta*, *C. ocellata*, *C. vittata*, *Curimatopsis macrolepis*, *Cyphocharax abramoides*, *C. multilineatus* (collected in sympatry with *C. sanctigabrielis*), *C. plumbeus*, and *C. spilurus* (Vari, 1982, 1989b, 1992a). Of these species, only two, *C. multilineatus* and *C. sanctigabrielis* are known solely from within the rio Negro system with the latter species only known from its type locality above the rapids of São Gabriel da Cachoeira. Species from the upper portions of the rio Orinoco and rio Negro which are conjoined via the rio Casiquiare are presumably isolated from lower portions of their respective drainages by the Atures and Maipures rapids in the southern portions of the rio Orinoco basin and the rapids at São Gabriel along the rio Negro in the northern Amazon (Lima & Ribeiro, 2011; Lujan & Armbruster, 2011). Albert *et al.* (2006) similarly hypothesized that the rapids at São Gabriel might be physical barriers blocking the dispersal of gymnotiforms from the Orinoco to Amazon lowlands. *Cyphocharax sanctigabrielis* is possibly yet another species endemic to the upper portions of the rio Negro above the rapids of São Gabriel da Cachoeira.

Acknowledgments

We thank Hertz F. Santos and Thiago N. Pereira (LIRP) for assistance with the radiographs. We are also grateful to Flávio A. Bockmann and Ricardo M. C. Castro (LIRP) for assistance with the radiography of the type series and to Claudio Oliveira (LBP) and Michel D. Gianeti (MZUSP) for curatorial assistance. Martin I. Taylor provided the photograph in Fig. 3 and habitat information. The authors were financially supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (Proc. 2011/08374-1 and Proc. 2013/16436-2) to BFM and the National Science Foundation (DEB-1257898) to RPV.

Literature Cited

- Albert, J. S., N. R. Lovejoy & W. G. R. Crampton. 2006. Miocene tectonism and the separation of cis- and trans-Andean river basins: Evidence from Neotropical fishes. *Journal of South American Earth Sciences*, 21: 14-27.
- Furch, K. 1984. Water chemistry of the Amazon basin: the distribution of chemical elements among freshwaters. Pp. 167-200. Sioli, H. (Ed.). *The Amazon: Limnology and Landscape Ecology*. Dordrecht, Dr. H. Junk Publishers.
- Goulding, M., M. L. Carvalho & E. J. G. Ferreira. 1988. *Rio Negro: rich life in the poor water*. The Hague, SPB Academic Publishing.
- Lima, F. C. T. & A. C. Ribeiro. 2011. Continental-scale tectonic controls of biogeography and ecology. Pp. 145-164. Albert, J. S. & R. E. Reis (Eds.). *Historical Biogeography of Neotropical Freshwater Fishes*. Berkeley and Los Angeles, University of California Press.

- Lucinda, P. H. F. & R. P. Vari. 2009. New *Steindachnerina* species (Teleostei: Characiformes: Curimatidae) from the Rio Tocantins basin. *Copeia*, 2009: 142-147.
- Lujan, N. K. & J. W. Armbruster. 2011. The Guiana Shield. Pp. 211-224. Albert, J. S. & R. E. Reis (Eds.). *Historical Biogeography of Neotropical Freshwater Fishes*. Berkeley and Los Angeles, University of California Press.
- Maldonado-Ocampo, J. A., R. P. Vari & J. S. Usma. 2008. Checklist of the freshwater fishes of Colombia. *Biota Colombiana*, 9: 143-237.
- Netto-Ferreira, A. L. & R. P. Vari. 2011. New species of *Steindachnerina* (Characiformes: Curimatidae) from the Rio Tapajós, Brazil, and review of the genus in the Rio Tapajós and Rio Xingu basins. *Copeia*, 2011: 523-529.
- Pavanelli, C. S. & H. A. Britski. 1999. Description of a new species of *Steindachnerina* (Teleostei: Characiformes: Curimatidae) from the upper Rio Paraná basin, Brazil. *Ichthyological Exploration of Freshwaters*, 10: 211-216.
- Vari, R. P. 1982. Systematics of the Neotropical characoid genus *Curimatopsis* (Pisces: Characoidei). *Smithsonian Contributions to Zoology*, 373: 1-28.
- Vari, R. P. 1989a. A phylogenetic study of the Neotropical characiform family Curimatidae (Pisces: Ostariophysi). *Smithsonian Contributions to Zoology*, 471: 1-71.
- Vari, R. P. 1989b. Systematics of the Neotropical characiform genus *Curimata* Bosc (Pisces: Characiformes). *Smithsonian Contributions to Zoology*, 474: 1-63.
- Vari, R. P. 1989c. Systematics of the Neotropical characiform genus *Pseudocurimata* Fernández-Yépez (Pisces, Ostariophysi). *Smithsonian Contributions to Zoology*, 490: 1-28.
- Vari, R. P. 1991. Systematics of the Neotropical characiform genus *Steindachnerina* Fowler (Pisces, Ostariophysi). *Smithsonian Contributions to Zoology*, 507: 1-118.
- Vari, R. P. 1992a. Systematics of the Neotropical characiform genus *Cyphocharax* Fowler (Pisces, Ostariophysi). *Smithsonian Contributions to Zoology*, 529: 1-137.
- Vari, R. P. 1992b. Systematics of the Neotropical characiform genus *Curimatella* Eigenmann and Eigenmann (Pisces, Ostariophysi), with summary comments on the Curimatidae. *Smithsonian Contributions to Zoology*, 533: 1-48.
- Vari, R. P. 2003. Family Curimatidae. Pp. 51-64. In: Reis, R. E., S. O. Kullander & C. J. Ferraris Jr. (Eds.). *Check list of the freshwater fishes of South and Central America*. Porto Alegre, Edipucrs.
- Vari, R. P. & T. A. Blackledge. 1996. New curimatid, *Cyphocharax laticlavus* (Ostariophysi: Characiformes), from Amazonian Ecuador, with a major range extension for *C. gouldingi*. *Copeia*, 1996: 109-113.
- Vari, R. P. & F. Chang. 2006. *Cyphocharax derhami*, a new species (Ostariophysi: Characiformes: Curimatidae) from northeastern Peru. *Ichthyological Exploration of Freshwaters*, 17: 93-96.
- Vari, R. P., B. L. Sidlauskas & P. Y. Le Bail. 2012. New species of *Cyphocharax* (Ostariophysi: Characiformes: Curimatidae) from Suriname and French Guiana and a discussion of curimatid diversity on the Guiana Shield. *Cybio*, 36: 63-69.
- Vari, R. P., A. M. Zanata & P. Camelier. 2010. New species of *Cyphocharax* (Ostariophysi: Characiformes: Curimatidae) from the Rio de Contas Drainage, Bahia, Brazil. *Copeia*, 2010: 382-387.
- Wosiacki, W. B. & D. P. S. Miranda. 2013. Description of a new small species of the genus *Cyphocharax* (Characiformes: Curimatidae) from the lower Amazon basin. *Copeia*, 2013: 627-633.

Submitted September 12, 2013

Accepted December 3, 2013 by Francisco Langeani

Published June 30, 2014

ANEXO 3: Produção Acadêmica e Científica no Doutorado: 2011–2015

Artigos completos publicados em periódicos

1. **Melo BF**, Benine RC, Mariguela TC, Oliveira C. 2011. A new species of *Tetragonopterus* Cuvier, 1816 (Characiformes: Characidae: Tetragonopterinae) from the rio Jari, Amapá, northern Brazil. *Neotropical Ichthyology*, 9(1): 49–56. DOI:
2. **Melo BF**, Sato Y, Foresti F, Oliveira C. 2013. The roles of marginal lagoons in the maintenance of genetic diversity in the Brazilian migratory fishes *Prochilodus argenteus* and *P. costatus*. *Neotropical Ichthyology*, 11(3): 625–636. DOI: 10.1590/S1679-62252013000300016.
3. Silva GSC, **Melo BF**, Oliveira C, Benine RC. 2011. Morphological and molecular evidence for two new species of *Tetragonopterus* (Characiformes: Characidae) from central Brazil. *Journal of Fish Biology*, 82: 1613–1631. DOI: 10.1111/jfb.12094.
4. **Melo BF**, Sidlauskas BL, Hoekzema K, Vari RP, Oliveira C. 2014. The first molecular phylogeny of Chilodontidae (Teleostei: Ostariophysi: Characiformes) reveals cryptic biodiversity and taxonomic uncertainty. *Molecular Phylogenetics and Evolution*, 70: 286–295. DOI: 10.1016/j.ympev.2013.09.025.
5. Polaz CNM, **Melo BF**, Britzke R, Resende EK, Machado FA, Lima JAF, Petrere Jr M. 2014. Fishes from the Parque Nacional do Pantanal Matogrossense, upper Paraguai River basin, Brazil. *Check List (Online)*, 10: 122–130. DOI: 10.15560/10.1.122.
6. **Melo BF**, Vari RP. 2014. New species of *Cyphocharax* (Characiformes: Curimatidae) from the upper rio Negro, Amazon basin. *Neotropical Ichthyology*, 12 (2): 327–332. DOI: 10.1590/1982-0224-20130153.
7. Benine RC, **Melo BF**, Castro RMC, Oliveira C. 2015. Taxonomic revision and molecular phylogeny of *Gymnocorymbus* Eigenmann, 1908 (Teleostei, Characiformes, Characidae). *Zootaxa*, 3956: 1–28. DOI: 10.11646/zootaxa.3956.1.1.

Artigos submetidos para publicação

1. Silva GSC, **Melo BF**, Oliveira C, Benine RC. Integrative taxonomy of the Neotropical fish genus *Tetragonopterus* (Teleostei: Characiformes: Characidae). *PLoS ONE*. 2015.
2. **Melo BF**, Vari RP, Oliveira C. A new species of *Curimatopsis* (Characiformes: Curimatidae) from the rio Tapajós, Amazon basin, northern Brazil. *Ichthyological Exploration of Freshwaters*, 2015.

Artigos em preparação

1. Vari RP, **Melo BF**, Oliveira C. A new genus of the Characidae (Teleostei: Characiformes). *Ichthyological Exploration of Freshwaters*. 2015.
2. **Melo BF**, Hoekzema K, Frable BW, Vari RP, Sidlauskas BL, Oliveira C. Miocene radiations in the family Prochilodontidae (Ostariophysi: Characiformes) revealed by a time calibrated molecular phylogeny. *BMC Evolutionary Biology*. 2015/2016.
3. Hoekzema K, **Melo BF**, Alfaro ME, Burns M, Faircloth BC, Frable BW, Oliveira C, Vari RP, Sidlauskas BL. Phylogenomic analysis reveals multiple episodes of diversification and convergence within the radiation of South American anostomoid fishes (Ostariophysi: Characiformes). *Current Biology*. 2016.
4. **Melo BF**, Hoekzema K, Frable BW, Vari RP, Oliveira C, Sidlauskas BL. Molecular systematics of the Neotropical fish family Curimatidae (Teleostei: Ostariophysi: Characiformes). *Molecular Phylogenetics and Evolution*. 2016/2017.
5. Sidlauskas BL, **Melo BF**, Hoekzema K, Burns M, Frable BW, Oliveira C, Vari RP. Molecular phylogeny and evolution of the headstanders of the family Anostomidae (Teleostei: Ostariophysi: Characiformes). 2016.
6. **Melo BF**, Lima FP, Birindelli JLO. New species of *Leporinus* (Characiformes: Anostomidae) from the upper rio Tapajós, Amazon basin. *Neotropical Ichthyology*, 2015/2016.
7. **Melo BF**, Ochoa LE, Sidlauskas BL, Vari RP, Oliveira C. Amazonian rivers reveal multiple genetic lineages of the curimatid fish (*Curimatopsis*) and disclose its cryptic biodiversity. *Zoologica Scripta*, 2016.
8. **Melo BF**, Benine RC, Silva GSC, Oliveira C. Molecular phylogeny of the Neotropical fish genus *Tetragonopterus* (Teleostei, Characiformes, Characidae). *Molecular Phylogenetics and Evolution*. 2016.

Livros publicados/organizados ou edições

1. Furtado EL, Firmino AC, Tozze-Junior HJ, Costa-Silva GJ, Henriques JM, Pereira LHG, **Melo BF**, Zimback L. 2011. Princípios Básicos de Biologia Molecular. 2 ed. FEPAF, Botucatu, Pp. 1–120.

Capítulos de livros publicados

1. Henriques JM, **Melo BF**. Engenharia Genética: DNA Recombinante. In: Furtado EL, Firmino AC, Tozze-Junior HJ, Costa-Silva GJ, Henriques JM, Pereira LHG, Melo BF, Zimback, L (Eds.) Princípios Básicos de Biologia Molecular. 3 ed. FEPAF, Botucatu, 2013, p. 50–57.
2. Henriques JM, **Melo BF**. Engenharia Genética. In: Furtado EL, Firmino AC (Eds.) Princípios Básicos de Biologia Molecular. 3 ed. FEPAF, Botucatu, 2013, p. 32–39.

Capítulos de livros preparados para publicação

1. **Melo BF**, Sidlauskas BL. Family Chilodontidae – Headstanders. In: Van der Sleen, P. & Albert JS (Eds.) Field Guide for Fishes from the Amazon, Orinoco and Guianas. 2016.
2. **Melo BF**, Sidlauskas BL. Family Prochilodontidae – Flannel mouth characiforms. In: Van der Sleen, P. & Albert JS (Eds.) Field Guide for Fishes from the Amazon, Orinoco and Guianas. 2016.

Estágios no exterior

1. **University of California, Los Angeles (UCLA)**, Department of Ecology and Evolutionary Biology, Los Angeles, California, EUA. Preparação de bibliotecas genômicas, enriquecimento e sequenciamento de elementos ultraconservados (UCEs) em conjunto com Brant Faircloth e Kendra Hoekzema. Supervisão: Dr. Michael E. Alfaro.
2. **Oregon State University (OSU)**, Department of Fisheries and Wildlife, Corvallis, Oregon, EUA. Sequenciamento de UCEs, análises e discussão dos resultados na Oregon State Ichthyology Collection (OSIC). Supervisão: Dr. Brian L. Sidlauskas.

Visitas a coleções científicas

1. Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, Brasil.
2. Laboratório de Ictiologia de Ribeirão Preto (LIRP-USP), Ribeirão Preto, Brasil.
3. Oregon State Ichthyology Collection (OS), Corvallis OR, EUA.
4. National Museum of Natural History – SI (USNM), Washington DC, EUA.
5. Academy of Natural Sciences of Philadelphia (ANSP), Philadelphia PY, EUA.

Expedições de campo realizadas

1. Rio São Francisco – Minas Gerais, 2011.
2. Rio Paraguai, Pantanal – Mato Grosso, 2010/2011.
3. Rios Costeiros do Sudeste – São Paulo e Rio de Janeiro, 2011.
4. Rios Takutu e Branco – Roraima, 2012.
5. Rios Araguaia e Tocantins – Goiás, Distrito Federal e Tocantins, 2012 e 2014.
6. Rio Grande, alto Paraná – São Paulo, 2014.
7. Rios Costeiros da Península de Yucatán – Belize e México, 2015.

Participações em congressos nacionais e internacionais

1. XIV Simpósio de Citogenética e Genética de Peixes. Ponta Grossa - PR, 2011.
2. XIV European Congress of Ichthyology, Liège, Bélgica, 2012.
3. I Simpósio Brasileiro de Identificação Molecular de Espécies, Foz do Iguaçu/PR, 2012.
4. II Oficina de Avaliação do Estado de Conservação de Actinopterygii Continentais, Iperó/SP, 2012.
5. XX Encontro Brasileiro de Ictiologia, Maringá/PR, 2013.
6. XIII Workshop de Genética, Botucatu/SP, 2013.
7. 21st Annual Conference of the Society for Molecular Biology and Evolution, Chicago/IL, EUA, 2013.
8. IV Oficina de Avaliação do Estado de Conservação de Actinopterygii Continentais, Iperó/SP, 2013.
9. Research Advances in Fisheries, Wildlife and Ecology, Corvallis/OR, EUA, 2014.
10. Evo-WIBO 2014 Evolutionary Biology in the Pacific Northwest, Port Townsend/WA, EUA, 2014.

11. V Simpósio Programa Pós-Graduação Ciências Biológicas – Genética, Botucatu/SP, 2014.
12. Evolution 2015, Guarujá/SP, 2015.

Cursos de Extensão Ministrados ou Organizados

1. I Curso Teórico-Prático em Sistemática Molecular, IBB-UNESP, 2011 – 40h.
2. II Curso Extra-Curricular de Introdução à Biologia Molecular, FCA-UNESP, 2011 – 40h.
3. II Curso Teórico-Prático em Sistemática Molecular, IBB-UNESP, 2012 – 80h.

Palestras oferecidas

1. Engenharia genética: DNA recombinante. II Curso Extra-Curricular de Introdução à Biologia Molecular, FCA-UNESP, 2011.
2. Diversidade e Morfologia de Peixes de Água Doce Neotropicais. V Curso de Ecologia de Peixes de Rios e Represas, IBB-UNESP, 2012.
3. Evolução molecular e a origem das espécies. XII Workshop de Genética, IBB-UNESP, 2012.
4. Sequenciamento de DNA. I Curso Teórico Prático de Introdução à Biologia Molecular, FCA-UNESP, 2013.
5. Evolução Molecular e a Origem das Espécies. XIII Workshop de Genética, IBB-UNESP, 2013.

Parecer *ad hoc* para periódicos

1. Check List, 2013.
2. River Research and Applications, 2014.
3. Evolutionary Bioinformatics Online, 2014.
4. Journal of Fish Biology, 2015.