**UNIVERSIDADE ESTADUAL PAULISTA -UNESP**

**INSTITUTO DE BIOCIÊNCIAS**

***LUZ ENEIDA OCHOA ORREGO***

# ANÁLISE DAS RELAÇÕES FILOGENÉTICAS E PADRÕES DE DIVERSIFICAÇÃO DE TRICHOMYCTERIDAE (TELEOSTEI, SILURIFORMES) UTILIZANDO SEQUÊNCIAS DE DNA

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**Tese apresentada ao Instituto de Biociências, Câmpus 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 Oliveira**

**Co-Orientador: Dr. Fábio Fernandéz Roxo**

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***A meu Esposo e família!!!***

*"The only way to do great work is to love what do you do. If you haven't found it yet, keep looking. Don't settle. As with all matters of the heart, you'll know when you find it "*

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**RESUMO**

Trichomycteridae é uma das famílias mais diversa da superfamília Loricarioidea com aproximadamente 300 espécies válidas, incluídas em 41 gêneros e oito subfamílias, e amplamente distribuídas pelas drenagens da América do Sul e Central. Trichomycteridae é caracterizada morfologicamente pela presença de um sistema opercular altamente modificado, envolvendo os ossos operculares e pré-operculares, assim como pela variação no tamanho do corpo e padrões de coloração. Também apresentam uma ampla diversidade trófica incluindo espécies onívoras, insetívoras, lepidófagas e hematófagas. A monofilia da família e suas subfamílias são bem suportadas por caracteres morfológicos, exceto Trichomycterinae, a qual inclui *Trichomycterus*, um grupo não-monofilético, taxonomicamente complexo, com elevado número de espécies e desconhecida diversidade. Embora múltiplos estudos tenham focado nas relações supragenéricas com reduzida representatividade de espécies, ainda não existem estudos utilizando caracteres moleculares com ampla amostragem de Trichomycteridae. Neste contexto, o presente trabalho tem como objetivo principal estudar as relações filogenéticas de Trichomycteridae através da análise de sequências de DNA, usando duas aproximações: a análise multilocus, incluindo três genes mitocondriais e dois nucleares, e a implementação de análises filogenômicas usando 851 elementos ultraconservados do genoma (*ultraconserved elements*, UCEs). Com base na filogenia obtida, analisamos padrões de origem e diversificação, assim como sua correlação com a evolução do tamanho do corpo. Além disso, foram realizadas análises de biogeografia paramétrica para a reconstrução das áreas ancestrais. Os resultados obtidos pelas duas metodologias corroboram hipóteses morfológicas em relação à monofilia das subfamílias, exceto Glanapteryginae e Sarcoglanidinae, e revelam novas hipóteses de relacionamento dentro do clado Tridentinae-Stegophiliniae-Vandelliinae-Sarcoglanidinae-Glanapteryginae (TSVSG). As análises de divergência indicaram que a origem de Trichomyctreridae data do Cretaceo inferior com múltiplos eventos cladogenéticos ocorridos

durante o final do Eoceno e inicio do Mioceno. A família apresenta uma alta heterogeneidade nas taxas de diversificação, com um *shift* evidente na origem da subfamília Trichomycterinae, o qual não está correlacionado com a evolução do tamanho do corpo. A reconstrução de áreas ancestrais indicou que o ancestral comum mais recente de Trichomycteridae esteve amplamente distribuído na região amazônica e drenagens costeiras do Atlântico Sul do Brasil. Diferentes processos geomorfológicos de dispersão e vicariância, principalmente associados com eventos de captura de cabeceira modelaram a distribuição atual dos membros de Trichomycteridae.

**ABSTRACT**

Trichomycteridae is one of the most specious families in the superfamily Loricarioidea with approximately 300 valid species including 41 genera and eight subfamilies, widely distributed through the rivers in South and Central America. Trichomycteridae is characterized morphologically by the presence of a highly modified opercular system, involving the opercular and pre-opercular bones, as well as by variation in body size and coloration patterns. Trichomycterids also present a wide trophic diversity including omnivorous, insectivorous, lepidophagous and hematophagous species. The monophyly of the family and its subfamilies are well supported by morphological characters except Trichomycterinae, which includes Trichomycterus, a taxonomically complex non-monophyletic group with a high number of species and unknown diversity. Although, multiple studies have focused on suprageneric relationships with reduced species representativity, there are no studies using molecular characters with a large sample of Trichomycteridae. In this context, the main objective of this research is to study the phylogenetic relationships of Trichomycteridae through the analysis of DNA sequences using two approaches: multilocus analysis, including three mitochondrial and two nuclear genes, and the implementation of phylogenetic analyzes using 851 ultraconserved elements of the genome (ultraconserved elements, UCEs). Based on the phylogeny obtained, we analyzed patterns of origin and diversification, as well as their correlation with the evolution of body size. In addition, analyzes of parametric biogeography were carried out for the reconstruction of the ancestral areas. The results obtained by the two methodologies corroborate morphological hypotheses supporting the monophyly of the subfamilies, except Glanapteryginae and Sarcoglanidinae, and reveal new hypotheses of relationship within the clade Tridentinae-Stegophiliniae-Vandelliinae-Sarcoglanidinae-Glanapteryginae (TSVSG). The analysis of divergence indicated that the origin of Trichomyctreridae dates from the lower Cretaceous with multiple cladogenetic events occurring during the late Eocene and early

Miocene. The family shows a high heterogeneity in the rates of diversification, with an evident shift in the origin of the subfamily Trichomycterinae, which is not correlated with the evolution of body size. The reconstruction of ancestral areas indicated that the most recent common ancestor of Trichomycteridae was widely distributed in the Amazon region and coastal drains of the South Atlantic of Brazil. Different geomorphological processes of dispersal and vicariance mainly associated with river capture events modeled the current distribution of Trichomycteridae species.

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# Apresentação

Dentro da ordem Siluriformes, a família Trichomycteridae corresponde a um dos maiores grupos monofiléticos de peixes Neotropicais, devido a sua excepcional riqueza de espécies, ampla gama de especializações morfológicas, fisiológicas e ecológicas. Estudos filogenéticos, principalmente baseados em caracteres morfológicos têm contribuído no conhecimento da história evolutiva do grupo, entanto inúmeros problemas sistemáticos e taxonômicos persistem. Dessa forma, visando contribuir para o conhecimento das relações filogenéticas dos peixes de água-doce na região Neotropical, o objetivo principal deste trabalho foi analisar as relações filogenéticas da família Trichomycteridae com base em caracteres moleculares.

O manuscrito começa com uma introdução geral, destacando prévios estudos das relações filogenéticas da família, apresentando as principais hipóteses propostas até o momento; em seguida são apresentadas a justificativa e objetivos do trabalho.

Os resultados foram organizados em dois capítulos inéditos. Cada um contendo um artigo em preparação.

No primeiro capítulo são apresentados os resultados da análise genômica usando 851 Elementos Ultraconservados do genoma (UCE´s) para 150 espécies, que representam aproximadamente 41% da diversidade de espécies da família. Novos relacionamentos entre as subfamílias foram identificados assim como o reconhecimento de vários clados dentro do gênero Trichomycterus. Neste artigo é incluída também a analise macroevolutiva avaliando o tamanho do corpo e a evolução dos hábitos alimentares.

No segundo capitulo, o manuscrito está focado a testar quais processos biogeográficos (dispersão, vicariância, evento fundador) tem modelado a distribuição da família na região tropical e sua relação com a extensa história geológica da América do Sul.

Finalmente em anexo são apresentados dois manuscritos publicados durante o desenvolvimento do projeto. O primeiro, contem à primeira hipótese molecular para a família baseada numa análise multilocus, e o segundo corresponde a descrição de uma nova espécie do gênero *Trichomycterus.*

# Introdução

A ictiofauna Neotropical de água-doce é bastante rica, incluindo mais de 7000 espécies válidas, representando aproximadamente 10% de todas as espécies conhecidas de vertebrados (Lundberg et al., 2000; Berra, 2001; Reis et al., 2003; Lévêque et al., 2005; Lévêque et al., 2008; Petry, 2008; Albert e Reis, 2011; Eschmeyer e Fong 2017). Os peixes das ordens Siluriformes e Characiformes juntas compreendem cerca de 74% das espécies de peixes Neotropicais e formam as duas ordens mais especiosas, com 3.700 espécies para os Siluriformes e 2.100 para Characiformes (Reis et al., 2016; Eschmeyer e Fong, 2017). Tal diversidade é também refletida na alta variação morfológica e ecológica destes grupos, constituindo um excelente modelo para investigar processos macroevolutivos.

No contexto da biologia evolutiva, compreender os processos que modelaram a diversidade de espécies é desafiador (Ricklefs, 2007). A base para estes estudos está em conhecer os relacionamentos ancestrais-descendentes entre as espécies. Neste sentido, os estudos filogenéticos têm sido foco de importantes avanços na ultima década, principalmente relacionado ao progressivo avanço das técnicas de sequenciamento de DNA, o qual tem aumentado consideravelmente o número de caracteres usados nas inferências filogenéticas, e potenciam seu poder de resolução (Zou et al., 2012; McCormarck et al., 2012; Faircloth et al., 2013).

Dentro da ordem Siluriformes, a superfamília Loricarioidea (sensu de Pinna, 1998), ou também chamada de subordem Loricarioidei (sensu Sullivan et al., 2006), representa o mais diverso e amplamente distribuído grupo de peixes neotropicais, encontrado em praticamente todos os hábitats de água-doce na região tropical (Reis et al., 2003; Nelson, 2006). Além de sua excepcional riqueza de espécies, esse grupo exibe uma ampla gama de especializações morfológicas, fisiológicas e ecológicas, ocupando muitos hábitats e níveis tróficos (Reis, 1998; de Pinna, 1998; Nelson, 1999; Brito, 2002).

Com aproximadamente 1.420 espécies válidas (Eschmeyer e Fong, 2017), Loricarioidea é composto por seis famílias sendo as mais diversas Loricariidae e Trichomycteridae. A primeira tem sido foco de diversos estudos filogeneticos (e.g. Howes, 1983; Schaefer, 1987, 1991, 1998,2003; Amrbruster, 1998, 2004) e moleculares (e.g. Montoya Burgos et al., 1997, 1998, 2002; Zawadzki et al., 2005; Chiachio et al., 2008; Roxo et al., 2012a, b, 2014, 2017; Lujan et al., 2014; Covain et al., 2015). Estes estudos, com uma ampla

amostragem de táxons em grupos específicos dentro de Loricariidae, tem levantado questões biogeográficas e evolutivas importantes e têm auxiliado no esclarecimento dos mecanismos e processos geradores da ampla diversidade de espécies. As relações entre famílias de Loricarioidea encontram-se relativamente bem estabelecidas (Arratia, 1987; 1990; 1998; de Pinna, 1992), com base em dados morfológicos e moleculares (de Pinna, 1998; Britto, 2002; Sullivan et al., 2006; Lundberg et al., 2007). Por outro lado, as relações entre espécie, dentro de cada família ainda necessitam de estudos adicionais (e.g., Reis et al., 2003; Alexandrou et al., 2011; Roxo et al., 2012a, b).

Trichomycteridae é a segunda família mais diversa em número de espécies dentro de Loricarioidea, foco de diversos estudos filogenéticos usando caracteres morfológicos (Baskin, 1973; de Pinna, 1988, 1989, 1992, 1998; de Pinna e Starnes, 1990; Costa e Bockman, 1993; Wosiacki, 2002; Datovo e Bockmann, 2010). Porém, poucos estudos têm sido realizados usando caracteres moleculares, além de se focar nas relações ao nível infrafamiliar (Fernandez e Schaefer, 2009) e na identificação genética de espécies (da Silva et al., 2010). Sete das oito subfamílias reconhecidas em Trichomycteridae (Copionodontinae, Trichogeninae, Sarcoglanidinae, Glanapteryginae, Tridentinae, Stegophilinae e Vandelliinae) são diagnosticadas por caracteres exclusivos; entretanto a subfamília Trichomycterinae e seu gênero mais diverso, *Trichomycterus* são considerados polifiléticos, basicamente diagnosticados pela ausência de especializações das outras subfamílias (Baskin, 1973; Costa e Bockmann, 1993; de Pinna, 1998; Datovo e Bockmann, 2010). Adicionalmente, o incompleto conhecimento da diversidade de espécies, hipóteses conflitantes ou incompletamente resolvidas para a família representa um dos maiores desafios na ictiologia neotropical. O acelerado descobrimento de novas espécies e a dificuldade em identificar caracteres sinapomorficos, requerem da aplicação de novas metodologias assim como o incremento na representatividade de espécies para tentar estabelecer as relações filogenéticas em Trichomycteridae.

*Família Trichomycteridae*

A família Trichomycteridae é um dos mais diversos grupos monofiléticos de peixes de água-doce (de Pinna, 1998), distribuído nas Américas Central e do Sul, desde a Costa Rica até a Patagônia (sul da Argentina e Chile), de ambos lados dos Andes, em águas turvas até os rios costeiros (Wosiacki, 2004). A família atualmente é representada por aproximadamente 300 espécies válidas (Eschmeyer e Fong, 2017), 41 gêneros e oito subfamílias (i.e.

Copionodontinae, Glanapteryginae, Sarcoglanidinae, Stegophylinae, Trichogeninae, Trichomycterinae, Tridentinae, Vandelliinae). Trichomycteridae é considerada a segunda família mais diversa de Loricarioidea e sua diversidade é ainda subestimada (de Pinna e Wosiack, 2003), com várias espécies identificadas, porém ainda não foram descritas formalmente (Reis et al., 2003). A família é também de especial interesse devido a sua posição chave na filogenia dos Siluriformes, que junto com Nematogenyidae, Callichthyidae, Scoloplacidae, Astroblepidae e Loricariidae constituem a primeira linhagen em diversificar (Datovo e Bockmann, 2010) da subordem Loricarioidei (*sensu* Sullivan et al., 2006).

A monofilia de Trichomycteridae é suportada por um grande número de sinapomorfias, sendo a mais conspícua, o aparato opercular altamente modificado (Baskin, 1973; de Pinna, 1992a, 1998; Datovo e Bockmann, 2010), que inclui a presença de vários odontódeos sobre o opérculo e inter-opérculo; estrutura que em muitos membros, forma um complexo sistema músculo-esquelético que permite aos peixes ancorar-se ao substrato ou ao corpo de seus hospedeiros, no caso dos semiparásitos "candirus" (Datovo e Bockmann, 2010), nome popular relacionado com as espécies da subfamília Vandelliinae, mas algumas vezes usado para todas as espécies da família.

Inicialmente a família Trichomycteridae foi alocada como subfamília "Trichomycteriformes" na família Siluroidei segundo Bleeker (1863). Posteriormente foi reconhecida como família por Gill (1872). Embora, Eigenmann e Eigenmann (1888, 1890) e Eigenmann (1918) usaram o nome Pygidiidae, não existe duvida que o nome adequado para a família é Trichomycteridae (Miranda-Ribeiro, 1922) como foi estabelecido por Tchernavin (1944) que revisou a questão nomenclatural dos membros da família e tratou Trichomycteridae como sinônimo junior de Pygiididae.

Um dos primeiros e mais importantes estudos da família foi realizado por Eigenmann (1918) que revisou todas as espécies descritas até esse momento e subdividiu Pygiididae (=Trichomycteridae) em seis subfamílias: Nematogeninae, Pygiidinae, Pareiodontinae, Stegophilinae, Vandelliinae e Tridentinae.

Entre 1944 e 1966 foram propostas duas subfamílias, a primeira para alocar dois novos gêneros e duas novas espécies, *Pygidianops eigenmanni* e *Typhlobelus ternetzi*, além de alocar a espécie *Glanaptaeryx anguila* previamente descrita por Myers (Myers, 1944, 1927). Em 1966 Myers e Weitzman descreveram a subfamília, Sarcoglanidinae, para alocar dois novos gêneros e duas novas espécies, *Sarcoglanis simplex* e *Malacoglanis gelatinosus,* baseados em três

espécimes, um único espécime de *S. simplex* do Alto rio Negro e dois espécimes de *M. gelatinosus* do rio Orteguaza, bacia do rio Caquetá na Colômbia.

A análise mais detalhada das relações filogenéticas da subfamília Trichomycterinae e a primeira em proporcionar suporte cladístico para a monofilia de Trichomycteridae (de Pinna, 2016), foi realizada por Baskin em 1973, com seu trabalho "*Structure and relationships of the Trichomycteridae*", recentemente publicada (de Pinna, 2016). O autor dividiu todos os trichomycteridos conhecidos em dois grupos monofileticos, o primeiro denominado "Trichomycterinae group" que incluiu as subfamílias Glanapteryginae, Sarcoglanidinae e Trichomycterinae; e o segundo "Vandelliinae group" composto pelas subfamílias Stegophilinae, Tridentinae e Vandelliinae. Baskin (1973) não achou evidências suficientes para suportar o monofiletismo da subfamília Trichomycterinae, hipóteses que posteriormente foi testada por de Pinna (1989), apontando para uma possível polifilia do grupo.

Posteriormente de Pinna (1992) descreveu a subfamília Copionodontinae, diagnosticada pela posição anterior da nadadeira dorsal, a presença de uma nadadeira adiposa desenvolvida e a forma espatulada evidente dos dentes mandibulares. Essa subfamília, composta por dois gêneros e três espécies, foi suportada como monofilética e proposta como uma das linhagens irmãs dos Trichomycteridos devido a que apresenta as seguintes condições plesiomórficas: presença de *ductus pneumaticus*; separação do "Pterosfenotico", "esfenotico" e "prootico", a presença de "intercalarium", canal infraorbital incompleto, presença de "interhyal" e a ampla abertura da capsula da bexiga natatoria; unicamente compartilhadas com *Trichogenes* (de Pinna, 1992).

Novas espécies e gêneros foram descobertas nos anos seguintes –e.g. *Ituglanis* Costa e Bockmann (1993), que não foi assinalado a nenhuma subfamília definida, porém os autores sugeriram ser o grupo irmão do clado composto por Vandelliinae, Stegophilinae, Tridentinae, Glanapteryginae e Sarcoglanidinae (clado-VSTGS). *Ituglanis* é considerado essencial, para compreender o surgimento das adaptações morfológicas e ecológicas dos membros de Trichomycteridae, devido a sua posição filogenética intermediária entre as formas mais generalistas (Trichogeninae, Copionodontinae e Trichomycterinae), assim como das formas mais especializadas que compreendem o clado-TSVSG (Lima et al., 2013).

Após o estudo de Baskin (1973), uma nova hipótese para a família Trichomycteridae foi apresentada e discutida por de Pinna (1998). Nessa hipótese os gêneros *Scleronema* e *Ituglanis* não foram assinalados para nenhuma subfamília conhecida, e as subfamílias

Copionodontinae e Trichogeninae formaram uma tricotomia com os demais membros da subfamília Trichomycterinae. Posteriormente, a maior parte dos estudos relacionados com a família concentrou-se na subfamília Trichomycterinae. O primeiro deles foi a descrição de *Silvinichthys* (Arratia 1998) e a proposta de quatro sinapomorfias para Trichomycterinae (Arratia 1990), sendo a subfamília composta pelos gêneros *Eremophilus*, *Rhizosomichthys*, *Scleronema* e *Trichomycterus*. Entretanto, a autora deixou claro que estabelecer os limites de Trichomycterinae e de *Trichomycterus* requereria uma análise completa de todas as espécies da subfamília.

Wosiacki (2002) estudando 205 caracteres morfológicos de 73 espécies de Trichomycteridae incluindo 49 espécies válidas, assim como espécies não descritas, encontrou quatro árvores igualmente parcimoniosas. O cladograma de consenso estrito resultou na identificação de 70 clados. Para uma adequada organização das espécies, frente aos resultados obtidos, Wosiacki propôs 14 novos gêneros e 14 novas subfamílias. Além disso, propôs *Scleronema* como grupo irmão do clado-VSTGS e *Ituglanis* como grupo irmão de *Scleronema*+VSTGS. Wosiacki (2002) estudando as relações filogenéticas de Trichomycterinae também confirma a polifilia desta subfamilia, já apontado anteriormente por de Pinna (1998). Mais recentemente, análises moleculares empregando genes mitocondriais foram realizadas com o objetivo de testar as relações entre algumas espécies de Trichomycteridae (Fernandez e Schaefer, 2009). Os resultados obtidos com seis subfamílias, 17 gêneros e 21 espécies foram congruentes com hipóteses propostas previamente usando dados morfológicos (Eigenmann, 1918; Myers, 1944; Baskin, 1973; de Pinna, 1998) as quais estabeleceram que as subfamílias Stegophilinae e Vandelliinae conformam clados irmãos.

O estudo mais recente incluindo 35 espécies representantes de todas as subfamílias de Trichomycteridae foi realizado por Datovo e Bockmann (2010). Estes autores propuseram uma hipótese de relacionamento baseada exclusivamente em caracteres morfológicos da musculatura da região dorsolateral da cabeça. Os resultados obtidos corroboram hipóteses previas de relacionamento entre os membros da família, além de invalidar muitos dos caracteres morfológicos inicialmente propostos para definir grupos dentro de Trichomycteridae. Os resultados indicaram que as subfamílias Copionodontinae e Trichogeninae formaram uma linhagem monofilética, correspondente ao grupo-irmão de todos os demais trichomycteridos, além de corroborar o monofiletismo do clado C ou clado -TSVSG (Tridentinae, Stegophilinae, Vandelliinae, Glanapteryginae). Entretanto, neste trabalho duas hipóteses são discordantes com relação às hipóteses anteriores para a família: a relação de

grupo irmão entre Tridentinae e Stegophilinae e o monofiletismo de Trichomycterinae *lato sensu* (Datovo e Bockmann, 2010), *i.e.*, incluindo os gêneros *Trichomycterus*, *Scleronema*, *Ituglanis*, *Bullockia* e *Hatcheria*.

Nesse contexto, considerando as hipóteses conflitantes para as relações de parentesco entre os membros de Trichomycteridae, o incremento acelerado na descrição de novas espécies, a dificuldade em identificar caracteres morfológicos únicos para os membros de *Trichomycterus*, a ausência de estudos baseados em caracteres moleculares, e a importância da família para o entendimento das relações evolutivas em Silurifomres, faz-se necessário a realização de uma filogenia molecular incluindo uma grande amostragem de espécies, como base fundamental para a realização de análises macroevolutivas e biogeográficas, que permitam identificar os mecanismos e processos evolutivos responsáveis pela origem e diversificação dos membros de Trichomycteridae.

# Justificativa

Compreender as relações filogenéticas entre organismos é um pré-requisito de todos estudos evolutivos. Até os anos 1970, a reconstrução filogenética foi baseada em análises de caracteres morfológicos ou ultraestruturais, contudo, esta abordagem é dificultada pelo número limitado de caracteres homólogos. No final de 1980, o acesso a sequências de DNA aumentou o número de caracteres que podem ser comparados de menos de 100 para mais de 1.000, melhorando consideravelmente o poder de resolução das inferências filogenéticas (Delsuc et al. 2005).

As análises empregando dados moleculares são considerados uma ótima ferramenta na resolução de problemas taxonômicos e evolutivos (ancestrais-descendente). A utilização de caracteres moleculares nas análises filogenéticas em peixes tem crescido consideravelmente nas últimas décadas contribuindo significativamente na compreensão das relações entre grupos complexos (Bermingham e Avise 1986; Alves-Gomes et al. 1995; Ortí e Meyer 1997; Sivasundar et al. 2001; Shimabukuro-Dias et al. 2004; Calcagnotto et al. 2005; López-Fernandez et al. 2005; Chiachio et al. 2008; Javonillo et al. 2010; Lovejoy et al. 2010; Alexandrou et al., 2011; Oliveira et al., 2011; Carvalho-Costa et al. 2011; entre outros).

No caso dos siluriformes a maioria dos estudos filogenéticos de nível superior têm-se centrado em grupos de poucas famílias, mas com amostragem de táxons mais completa internamente, por exemplo, loricarídeos (Baskin, 1973; Pinna, 1992; Schaefer, 1990); cetopsídeos (Vari e Pinna, 1995), pimelodídeos (Bockmann, 1998, Lundberg e McDade, 1986; Lundberg et al., 1991a, b; de Pinna, 1998); doradídeos e ariídeos (Ferraris, 1988, Lundberg, 1993; Royero, 1987). Contudo, não existem até o momento estudos em sistemática molecular abordando as relações da família Trichomycteridae com o uso de um grande número de táxons e representantes de todas subfamílias.

A pesar dos múltiplos estudos desenvolvidos usando caracteres morfológicos para estabelecer o relacionamento evolutivo da família Trichomycteridae (Eigenmann, 1918; Peyer 1922; Berg 1940; Baskin 1973; de Pinna 1998; Datovo e Bockmann, 2010), existem conflitos ainda não resolvidos. Os únicos estudos que abordaram essas relações foram realizados com grupos abrangentes, suprafamíliares (de Pinna, 1998; Britto, 2002; Sullivan et al., 2006) ou intrafamíliares (Arratia, 1990; Costa e Bockmann, 1993, Wosiacki, 2002, Datovo 2010), porém, utilizando poucos táxons no grupo interno de Trichomycteridae. Após Datovo e

Bockmann (2010) nenhum estudo com o propósito de testar as relações filogenéticas de Trichomycteridae foi realizado. A pequena representatividade da diversidade de espécies da família em todos os estudos já realizados, o incremento no numero de espécies descritas e o limitado numero de caracteres morfológicos únicos para suportar relacionamentos dentro de Trichomycteridae, evidenciam a importância de um estudo aprofundado das relações filogenéticas utilizando uma amostragem mais ampla assim como o uso de caracteres moleculares e novas metodologias genéticas. A disponibilidade de uma hipótese filogenética robusta em Trichomycteridae pode fornecer suporte para a análise dos fatores que determinam a alta diversidade e padrões biogeográficos do grupo na região Neotropical.

# Objetivo Geral

Inferir hipóteses de relacionamento entre os táxons constituintes de Trichomycteridae usando caracteres moleculares.

*Objetivos específicos*

- Testar a hipótese de monofiletismo para as subfamílias de Trichomycteridae.
- Estimar taxas de diversificação de acordo com a hipóteses filogenética e analizar sua correlação com a evolução morfológica do tamanho do corpo.
- Identificar padrões biogeográficos relacionados com a diversificação das espécies através de análises de biogeografia paramétrica.

*"The tree of life was always there. Evolution just fills in the gaps"*

*Simon Conway Morris*

# Chapter 1

## Phylogenomic analysis of trichomycterid catfishes (Teleostei:Siluriformes) inferred from Ultraconserved Elements.

# Phylogenomic analysis of trichomycterid catfishes (Teleostei:Siluriformes) inferred from Ultraconserved Elements.

**By**

**Luz Eneida Ochoa Orrego**

## Abstract

The Trichomycteridae family is one of the most diverse groups of freshwater catfishes in South America; with approximately 290 valid species, eight subfamilies and 41 genera. Its members are widely distributed through out South America and the family is recognized by a high trophic diversity including generalized predators, algivores, carrion-feeders, scale and mucus eaters and the specialized parasites. Likewise, this group shows a high morphological variation and unevenly diversity distribution. Different studies using morphological characters and molecules have been addressed to understand the phylogenetic relationships within each subfamily corroborating their monophyletic status. Nevertheless, the increased knowledge of the taxonomic diversity of Glanapteryginae and Sarcoglanidinae has revealed a series of slightly differentiated taxa that have been difficult to confidently assign to one of these subfamilies. In order to assess the phylogenetic relationships of Trichomycteridae, we collected sequence data from ultraconserved elements (UCE) of the genome from 132 members of Trichomycteridae and 11 species of the outgroups. We used a concatenated matrix to infer the relationships by Bayesian (B) and Maximum Likelihood (ML) inferences. The results show a highly-resolved phylogeny with broad agreement between B and ML trees. The results provide overwhelming support for the monophyletic status of Trichomycterinae including *Ituglanis* and *Scleronema*. Previous hypotheses of relationships among subfamilies, as the sister relationship between Copionodontinae and Trichogeninae forming a sister clade to the remaining trichomycterids and the intrafamilial clade TSVSG are corroborated, while the monophyly of Glanapteryginae and Sarcoglanidinae is not recovered and unexpected novel relationships between members of both subfamilies are found with biogeographic correspondence. The macroevolutionary analysis revels heterogeneity in diversification rates and decouple body size evolution from speciation, suggesting that diversification processes in Trichomycteridae may be more related with ecological specialization.

### *1.1 Introduction*

Unraveling the relationships of major sections of the Tree of Life is one of the most daunting challenges of the evolutionary biology. Next-generation DNA sequencing (so-called massively parallel sequencing) (Crawford *et al.* 2012; Faircloth *et al.* 2012; Mccormack *et al.* 2012; Smith *et al.* 2014) is a promising tool that is helping to resolve the interrelationships of longstanding problematic taxa (Faircloth *et al.* 2013). One of the most common class of phylogenomic methods involves the sequence capture of nuclear regions flanking and including ultraconserved elements (UCEs) (Faircloth *et al.* 2012). Recent studies on ray-finned fishes (Faircloth *et al.* 2013) and flatfishes (Harrington *et al.* 2016), among other vertebrates groups (Mccormack *et al.* 2012; Crawford *et al.* 2015), have shown that UCE's are ideal markers for phylogenetic studies because of their ubiquity among taxonomic groups (Siepel *et al.* 2005), low degrees of paralogy (Derti *et al.* 2006) and low saturation (Mccormack *et al.* 2012). Target enrichment of UCE loci has been used to investigate questions at deep timescales across diverse groups of taxa as consequence of nearly invariant core regions. Simultaneously, the more variable flanking UCE regions allow a better resolution of nodes across a range of evolutionary timescales in a given phylogeny (Faircloth *et al.* 2012). As variation in the flanks increases with distance from the core UCE, this combined approach display a balance between having a high enough substitution rate while minimizing saturation, thus providing information for estimating phylogenies at multiple evolutionary timescales (Faircloth *et al.* 2012; Mccormack *et al.* 2012). UCEs are rarely found in duplicated genomic regions (Derti *et al.* 2006), making the determination of orthology more straightforward than other markers (Mccormack *et al.* 2013). According to Gilbert et al. (2010), the phylogenetic informativeness of the combined flank and core regions of UCEs is superior to the ones derived from sets of protein-coding genes. Additionally, phylogenomic approaches are characterized by their potential to collect data from at least one order of magnitude more loci than the traditional protein-coding sequencing techniques.

The present survey is the first to employ these recent advances in phylogenomics and high-throughput sequencing to address evolutionary relationships in the catfish family Trichomycteridae, which includes the so-called pencil and parasitic catfishes. The family contains approximately 290 species (Eschmeyer & Fong 2017) characterized by a highly modified opercular system, with opercular and interopercular bones usually armed with patches of sharp odontodes. Additionally, the family is also characterized by a high variation in body size with some species miniature due to a small body and paedomorphic features associated

with the degree of development of the laterosensory canal system and reduction of fin rays, as well the bones of the head (Weitzman and Vari 1988). Trichomycterids have one of the broadest ranges of trophic adaptations known within any single catfish family, including insectivores, omnivores, carnivores, necrophagous, mucophagous, lepidophagous, and hematophagous (Kelley & Atz 1964; Goulding 1979, 1980; Machado & Sazima 1983; Winemiller & Yan 1989; de Pinna 1998; Zuanon & Sazima 2004; Fernández & Schaefer 2009). The family has a wide distribution in the Neotropical freshwater drainages (de Pinna 1998) of Central and South America (Wosiacki 2004) from Costa Rica to Patagonia, occurring on both versants of the Andes, and even in a few insular freshwater environments (de Pinna & Wosiacki 2003; Fernández & Schaefer 2005).

Eight trichomycterid subfamilies are currently recognized: Copionodontinae, Glanapteryginae, Sarcoglanidinae, Stegophilinae, Trichogeninae, Trichomycterinae, Tridentinae, and Vandelliinae. Only two publications with explicit cladistics analyses tested the interrelationships among all these subfamilies, being one based on morphological data (Datovo & Bockmann 2010) and another on combined nuclear and mitochondrial genes (Ochoa *et al.* 2017). The present phylogenomic analysis assembled a dataset of ultraconserved DNA elements (UCEs) and their flanking regions representing over 851 loci from 150 taxa including the outgroup (about and 41% of species diversity of the family). A new well-supported hypothesis of relationships for the Trichomycteridae emerged from this analysis, which serve to explore macroevolutionary dynamics across the tree, as well as to identify changes in speciation and extinction rates and its relationship with body size and trophic evolution.

### *1.2 Material and methods*

#### *1.2.1 Taxon sampling*

Tissues samples and voucher species used in this project were deposited in the collection of Laboratório de Biologia e Genética de Peixes UNESP, Botucatu, Brazil (LBP), Instituto de Pesquisas da Amazonia, Manaus, Brazil (INPA), and The Academy of Natural Sciences of Drexel University (ANSP). The table 1.1 synthesize pertinent data from all the samples belonging to ingroup and outgroup. Our analysis includes representatives of the all eight subfamilies and from 26 genera and 132 species of Trichomycteridae. The outgroup includes species of the: Nematogenyidae (*Nematogenys inermis*), Callichthyidae (*Corydoras elegans*, *Corydoras gossei* and *Hoplosternum littorale*), Scoloplacidae (*Scoloplax dicra*, *Scoloplax distolothrix*) Astroblepidae (*Astroblepus grixalvii* and *Astroblepus* sp.), Loricaiidae

(*Lasiancistrus saetiger*, *Falorwella oxrryncha* and *Lamonichthys filamentosus*) as representatives of the Loricarioidei and the resulting trees were rooted in the characiform *Leporinus striatus*.

### *1.2.2 UCE methods*

DNA extractions were done from approximately 25 mg of tissue using Qiagen DNeasy Tissue kits following the manufacturer's protocols, and we ran all genomic DNA extractions on an agarose gel to assess quality. We quantified 2µl of each sample using fluorometry (Qubit, Life Technologies). The samples used in the library preparation presented a concentration between 10-40 ng/µl. To prepare the libraries initially we sheared 1-2µg of DNA to 400-600 bps in length using a Diagenode Bioruptor Standard (UCD 200) with 6-8 cycles of sonication (depending on DNA quality). The DNA libraries from 150 species were prepared using the Nextera (Epicentre Biotechnologies, Inc.) library preparation protocol for solution-based target enrichment following Faircloth et al. (2012) and increasing the number of PCR cycles following the tagmentation reaction to 20 as recommended by Faircloth et al (2013). We used the Nextera library preparation protocol of *in vitro* transposition followed by PCR to prune the DNA and attach sequencing adapters (Adey et al. 2010), then used the Epicentre Nextera kit to prepare transposase-mediated libraries with insert sizes averaging 100 bp (95% CI: 45 bp) following Adey et al. (2010). The libraries were enriches using a probe set developed for application to ostariophysan fishes to generate sequences data for approximately 2500 UCE loci (Faircloth et al. in prep). We converted the DNA to Illumina sequencing libraries with a slightly modified version of the NEBNext(R) Ultra(TM) DNA Library Prep Kit for Illumina(R). After ligation of sequencing primers, libraries were amplified using KAPA HiFi HotStart ReadyMix (Kapa Biosystems) for 6 cycles using the manufacturer's recommended thermal profile and dual P5 and P7 indexed primers (see Kircher et al. 2012 (doi: 10.1093/nar/gkr771) for primer configuration). After purification with SPRI beads, libraries were quantified with the Quant-iT(TM) Picogreen(R) dsDNA Assay kit (ThermoFisher). We then enriched pools comprising 100 ng each of 8 libraries (800 ng total) using the MYbaits(R) Target Enrichment system (MYcroarray) following manual version 3.0. After capture cleanup, the bead-bound library was resuspended in the recommended solution and amplified for 10 cycles using a universal P5/P7 primer pair and KAPA HiFi reagents. After purification, each captured library pool was quantified with PicoGreen, and combined with all other pools in projected equimolar ratios prior to sequencing. Sequencing was performed across two Illumina HiSeq paired-end 100 bp lanes using v4 chemistry.

### *1.2.3 Phylogenetic inference*

We used the software Phyluce v1.5.0 (Faircloth 2016) to the analysis of UCE's. The first step was to clean the data of adapter contamination, low quality bases and sequences containing ambiguous bases, using the program illumiprocessor, included in the Phyluce software. We assembled reads and generated consensus contigs for each species using ABySS (version 2.0.2) (Simpson *et al.* 2009) with a kmer value equal to 55. ABySS is the most accurate assembler which runs read-based error correction prior to assembly resulting in more accurate contigs.

Following assembly, we proceeded to identify those contigs that were UCE loci and align species-specific contigs to the set probes/UCEs used for enrichment. These processes were realized with Python program (phyluce_assembly_match_contigs_to_probes.py) integrating LASTZ (Harris 2007) a pairwise aligner to match contigs and UCE loci. During the matching, the program creates a relational data base of matches to UCE loci by taxon. We removed reciprocal and non-reciprocal duplicates UCE loci and create a database of UCE loci recovered. The monolithic FASTA files were used to generate the alignments using MAFFT (version 7.130b) and we trimmed resulting alignments using the algorithm implemented by the seqcap_align.py script within phyluce. Every alignment was cleaned from the locus name using phyluce_align_remove_locus_name_from_nexus_lines and Gblocks. From the trimmed alignments, we created an incomplete matrix with 50, 75, and 85% completeness in order to evaluate the role of missing data in our matrices, tree topology and clade support values. For each matrix we prepared a concatenated alignment in PHYLIP format and every matrix was analyzed using maximum likelihood (ML) algorithm in RAxML v8.2.X (Stamatakis 2014) to compare the topologies with different levels of completeness.

The best-fitting partitioning scheme was obtained using the Bayesian Information Criterion and hcluster search in Partitionfinder v1.1.1 (Lanfear *et al.* 2012) and the best scheme grouping together loci having the same substitution model was used in subsequent analyses. The phylogenetic analysis was performed using maximum likelihood inference in RAxML v. 7.2.6 (Stamatakis, 2010) with "GTRGAMMA" option. A posteriori bootstrapping analysis were conducted with RAxML's autoMRE tool indicated that trees converged after 50 replicates, we reconciled the best fitting ML tree with the bootstrap replicates. Bayesian inference were performed in ExaBayes version 1.5 (Aberer, Kobert & Stamatakis 2014) with 1'000,000 iterations (2 chains; bur-in:25%) with parameters in default.

### *1.2.4 Time Calibrated tree in BEAST*

Divergence times estimates were performed in BEAST v 1.8.0 (Drummond *et al.* 2012) using a reduced matrix, of 90% completeness, with a total 66,845 pb and uncorrelated lognormal clock and birth and death speciation process. We ran two independent analyses of 50,000,000 generations each. To verify effective sampling of all parameters and to assess convergence of independent chains, we examined output log files in Tracer v.1.6 (Rambaut *et al.* 2014). After removing 25% of samples as burn-in independent runs were combined and a maximum clade-credibility (MCC) tree was constructed using TreeAnnotator v1.8.0 (Drummond *et al.* 2012). We offset the minimum ages of three nodes across the phylogeny using a combination of fossil and secondary priors. The fossil was described from the Monte Hermoso Formation in Argentina (Bogan & Agnolin 2009; Tomassini *et al.* 2013), it was placed under a log normal distribution with a mean of 4.5 Myr and standard deviation of 1.5, allowing for the origin of the subfamily Trichomycterinae. Secondary priors were placed under a normal distribution on the root, with the origin of Siluriforms reported by Betancur-R et al (2015) of 150 Myr and the search was conducted among the interval of 136.3-163.7 Myr using the lower and upper quantiles of 2.5%, respectively. The other point was in the origin of Trichomycteridae about 106 million years ago (Myr) as was estimated by Betancur-R et al. (2017). We implanted a normally distributed prior with mean of 106 and standard deviation of 7. The search was conducted among the interval of 92.28-119.7 Myr using the lower and upper quantiles of 2.5%, respectively.

### *1.2.5 Analyses of speciation/extinction and body size rates*

To estimate the number of distinct evolutionary regimes across our phylogenetic tree, we used the Bayesian Analysis of Macroevolutionary mixture (BAMM). This Bayesian approach uses reversible jump Markov chain Monte Carlo (RJMCMC) sampling to explore shifts in macro-evolutionary regimes assuming they occur across the branches of a phylogeny under a compound Poisson process, and explicitly accommodates diversification rate variation through time and among lineages. BAMM is both time sensitive and diversity-dependent, allowing rate shifts to occur anywhere on a branch based on the posterior tree density (Rabosky 2014). Speciation and extinction were inferred using the 'speciation-extinction' module, with correction for differential sampling across genera.

The body size evolution was inferred using the continuous 'trait' module. The sampling fraction of each genus was determined by comparing the number sampled with the

number of species reported in data base as Fishbase (http://www.fishbase.org/search.php), Catalogue of Fishes (CAS) (http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp) and Siluriformes data (http://silurus.acnatsci.org/ACSI/taxa/Families.html). Data on standard length (LS) were obtained mainly from morphological descriptions and the reporters in the Check List of Freshwater Fishes of South and Central America (Reis, Kullander & Ferraris 2003), the specimens identified just to genus level were directly measure, all measurements were log-transformed.

Priors for BAMM were generated using the R package BAMM-tools v.2.0.2 (Rabosky *et al.* 2014a) by providing the MCC tree from BEAST and total species numbers of the family. Two independent MCMC chains of 10'000,000 generations were run in BAMM and convergence was assessed by computing the effective sample sizes of log likelihoods, as well as the number of shift events present in each sample using the R package coda v. 0.16-1 (Plummer et al., 2006). After removing 10% of trees as burn-in, we analyzed the BAMM output using BAMMtools (Rabosky *et al.* 2014a) to estimate summary statistics, such as phylorate plots (showing rates and rate shifts in diversification), 95% credible sets of rate shifts configuration and average rates of speciation and extinction.

### *1.2.6 Ancestral reconstruction of feeding modes*

For the ancestral character reconstruction analysis, we obtained information about the trophic level from species description and www.fishbase.org. We considered six trophic categories according to description, algivorous, which consume mainly periphyton and phytoplankton; lepidophagous, feeding mucus and scale; hematophagous, parasite species feeding blood; carrion feeders; insectivorous, consume aquatic insects and small arthropods; and omnivorous consume aquatic and terrestrial insects, plant and organic material. To reconstruct the ancestral feeding modes we estimated stochastic character mapping using the function "make.simmap" and the best fit discrete model of evolution equal rates (ER) , symetric rates (SYM), all rates different (ARD) to data as it is implemented in the R packages Phytools (Revell 2017) and Geiger (Harmon *et al.* 2008).

## *1.3 Results*

### *1.3.1 Phylogenomic inferences for the Trichomycteridae family*

Sequencing produced a total of 176 million reads with a mean of 1'216,052 per sample from 143 taxa. Using ABySS, we assembled the DNA reads into a mean of 62,859 contigs (95CI, min = 1,813, max = 497,245) per sample, having an average length of 194 pb (Table 1.2). Contigs matching no UCEs and UCE loci matching multiple contigs were subsequently removed, thus an average of 1,290 unique contigs matching UCE loci from each species.

The gene trees inferred from the individual locus alignments of the matrices with 50% (1383 loci), 75% (915 loci), and 85% (669 loci) complete alignments exhibit identical topologies and strong node support. Phylogenetic trees estimated from 50% and 85% matrices are shown in the sl Figures 1.3 and 1.4 respectively. The phylogeny in the figure 1 correspond with the results of maximum likelihood and Bayesian estimation using the 75% complete matrix composes by 851 loci having a mean length of 162 bp (2.71 CI) per alignment, and a total of 160,440 bp of aligned sequences.

We recover a strong-supported phylogeny of Trichomycteridae that is identical across maximum likelihood and Bayesian analysis with a strong statistical support in most nodes (i.e., 100% bootstrap support or posterior probabilities of 1.0) and few cases of nodes receiving moderate or low support represent by gray circle in the Figure 1.1 (bootstrap support between 50 and 75%, posterior probabilities between 0.5 and 0.8). The phylogenetic hypothesis was partially congruent with previous morphological and molecular hypothesis of the relationships among trichomycterids (Eigenmann 1918; Myers 1944; Baskin 1973; Fernández & Schaefer 2009), with some differences in the relationships among subfamilies and genera.

Monophyly of the Trichomycteridae and the subfamilies Copionodontinae (100% of the genera sampled), Stegophilinae (81.8% of the genera sampled), Vandelliinae (50% of the genera sampled), Tridentinae *sensu stricto* (Baskin, 1973; 75% of the genera sampled) and Trichomycterinae sensu Datovo & Bockmann (2010) and Ochoa et al. (2017) are supported by maximum values of bootstrap (100%) and posterior probabilities (p=1). Our analysis of UCE data provide strong evidence for the monophyly of the clade B, composed by Copionodontinae and Trichogeninae (clade B, by Datovo & Bockmann 2010) as the sister group to all remaining Trichomycterids (100% bootstrap support and posterior probabilities of 1.0). Copionodontinae

and the genus *Copionodon* (*C. pecten, C. orthiocarinathus* and *C.* sp. n) are both recovered as monophyletic.

Clade C is recovered with a basal dichotomy between the Trichomycterinae and TSVSG clade. Our phylogenomic data provide strong evidence for the monophyly of Trichomycterinae (bootstrap=100%, posterior probabilities p=1) as defined by the morphological study of Datovo & Bockmann (2010) and the recent multilocus analysis of Ochoa et al (2017). This definition of the subfamily includes *Ituglanis* and *Scleronema* and excludes *Potamoglanis*. The latter genus was recently erected (Henschel *et al.* 2017) to include miniature species group previously allocated in *Trichomycterus* and often referred to as the *Trichomycterus hasemani* group. Our study confirms the non-monophyly of *Trichomycterus*, the largest genus of the Trichomycteridae that concentrates 72% of the family diversity. In our topology, an undescribed trichomycterine (currently in analysis by DoNascimiento) from the coast in Venezuela is placed as sister to all remaining trichomycterines, which are grouped into two major lineages. The first lineage is further subdivided into six main clades. Four of these clades appear as successive sister taxa: the first clade in diversify includes *Trichomycterus cachirensis*, *T. sandovali*, and the monotypic *Eremophilus mutisii*, all from the Magdalena basin (Clade D1'); the second joins *T. guianensis* and *T.* cf *guianensis* from the Essequibo basin (Clade D2); the third, *T. trasandianus* and *T.* aff. *spilosoma* from Magdalena basin and Dos bocas river in Ecuador repectively (Clade D2'); and the fourth *T. striatus*, *T. ruitoquensis*, *T. banneaui*, and *T.* sp. 1 from the Magdalena basin (clade D2''). A group of five undescribed species of *Trichomycterus* from Paraná-Paraguay basin forms a monophyletic group that is sister to Chilean Clade E, composed by *Bullockia maldonadoi*, *T. aerolatus*, and *T. chiltoni*. Finally, the monophyletic *Ituglanis* is located at the apical portion of this major trichomycterine lineage. The sampled species of the genus are grouped into four main clades: one containing the species from the Tocantins basin (*I. goya*, *I. ramiroi*, and *I.* sp. 1); other with species from coastal Atlantic drainages (*I. boitata*, *I. parahybae*, and *I.* sp. Ribeira) and an undescribed species from the Amazon basin (*I.* sp. 2); the third and fourth clades combine species from the Amazon and La Plata system, being *Ituglanis* cf *amazonicus*, *I. eichhorniarum* plus two undescribed species (*I.* sp. 3 and *I.* sp. 4) in one clade and *I. amazonicus*, *I. parkoi*, *I. herberti* and an undescribed species (*I.* sp. 5) in the other.

The second major trichomycterine lineage includes two successive basal clades, being one composed by *T. punctulatus* (Central Andean Pacific slopes) and *T.* cf. *knerii* (Orinoco), and another by *T.* cf. *oroyae* and *T. quechuorum* (both from Amazonas High Andes) (Clade D1).

Remaining taxa are grouped into a large clade that includes *Scleronema* and all species of *Trichomycterus* from the La Plata, Northeastern Atlantic, and Southeastern Atlantic provinces. Clade D4 clusters *Scleronema* (*S. minutum*) and several species of *Trichomycterus* from southeastern Brazil (*T. inhering, T. balios*, *T. poikilos*, *T. perkos*, *T. davisi*, *T. zonatus*, *T. stawiarski* and *T. cubataonis*). Clade D5 contains three main subclades: the first includes *T. nigroauratus*, *T. pradensis*, *T. albinotatus*, *T. alternatus*, *T.* cf. *auroguttatus*, *T. mimosensis*, and *T. immaculatus*; the second *T. brasiliensis, T.* cf. *brasiliensis, T. pirabitira*, *T. candidus,* plus four undescribed species (*T.* sp. n. Grande, *T.* sp. SF, *T.* sp. 6, and *T.* sp. 7); the third *T. reinhardti*, *T. pauciradiatus*, *T. piratymbara, T. septemradiatus*, *T.* cf. *septemradiatus*, and one undescribed species (*T.* sp. 8).

The TSVSG clade includes the subfamilies Tridentinae, Stegophilinae, Vandelliinae, Sarcoglanidinae and Glanapteryginae. In our analysis, we included five representatives of the three currently recognized glanapterygine genera, including the most generalized *Listrura* (*L. camposi* and *L. picinguabae*) and the highly derived psammophilic *Typhlobelus* (*T. guacamaya*) and *Pygidianops* (*P. slender*, and *P.* sp); only *Glanapteryx* could not be sampled. For Sarcoglanidinae we included half of the genera (*Sarcoglanis simplex*, *Stauroglanis gouldingi* and *Microcambeva barbata*), lacking *Stenolicmus*, *Malacoglanis* and *Ammoglanis*. Additionally, a new undescribed genus seemingly belonging to the Glanaperyginae (Trichomycteridae n. gen.; de Pinna & Datovo; pers. comm.) was incorporated to our study. The resulting hypothesis recovers the monophyly of Tridentinae, Stegophilinae and Vandelliinae with exception of Glanapteryginae and Sarcoglanidinae. Representatives of both subfamilies from coastal Atlantic drainages (Southeastern South America) are grouped in one clade and those from the Amazon and Orinoco into another group along with *Potamoglanis* from Amazon and Paraguay (Northwestern South America). The Southeastern lineage is at the base of the TSVSG clade and includes Trichomycteridae n. gen., *Microcambeva barbata* (Sarcoglanidinae) and the two species of *Listrura* (Glanapteryginae). The Northwestern clade clusters *Potamoglanis hasemani*, *Stauroglanis gouldingi, Sarcoglanis simplex, Typhlobelus guacamaya* and the two *Pygidianops* species. This clade is the sister to the so-called Vandelliinae-group, a node with strong support that includes the Tridentinae, Stegophilinae, and Vandelliinae, with the last two subfamilies appearing as sister taxa. Our results not support the recently allocation of *Potamoglanis hasemani* in the Tridentinae *contra* Henschel et al (2017) and resolve *Tridens* sp. as sister to the clade formed by *Tridensimilis brevis* and *Tridentopsis pearsoni*.

With a representation of nine among the 11 genera of the Stegophilinae (only *Schultzichthys* and *Apomatoceros* are missing), the internal relationships of the subfamily is well resolved and mostly in agreement with a recent morphological phylogeny of the group (DoNascimiento 2015). Our hypothesis divides the Stegophilinae in two major groups. The largest group contains *Homodiaetus* (*Ho. passarellii* and *Ho. anisitsi*) at the base and two subclades: one composed by monotypic *Megalocentor echthrus* and *Henonemus* (*He. intermedius, He. punctatus*, and *He.* sp. 1) and the second by *Pareiodon* as sister of *Pseudostegophilus* + *Acanthopoma*. The latter genus is monotypic and included into a non-monophyletic *Pseudostegophilus* (*P. haemomyzon, P. nemurus, P. paulensis*, and *P.* sp.). The second major stegophiline group clusters *Ochmacanthus* (*O. reinhardti, O.* sp. 1, *O.* sp. 2, and *O.* sp. 3) as sister group of the clade composed by *Stegophilus panzeri* and the monotypic *Haemomaster venezuelae*.

Two of the four vandelliine genera were included in our analysis, *Paracanthopoma* and *Vandellia*. Monophyly of both genera and the whole subfamily are strongly supported, but species-level interrelationships showed low support.

### *1.3.2 Speciation and extinction rates in Trichomycteridae*

Divergence times were very similar with the reported by Ochoa et al (2017), dating the diversification of Trichomycteridae family during the Lower Cretaceous about 107.52 Myr (93.9-120.61, 95% HPD) (Figure 1.5) around the time of the continental separation between Africa and South America. The oldest split within Trichomycteridae was estimated at 66.63 Myr (43.76-90.5, 95% HPD) and established the clade Copionodontinae+Trichogeninae subfamilies. Subsequently, the clade C composes by the remaining trichomycterids diversified in two big clades, the first group including Tridentinae, Stegophilinae and Vandelliinae, as well as, the representative species from Sarcoglanidinae and Glanapteryginae; and the second group represented by Trichomycterinae subfamily, which diversified during the Paleocene (41.97 Myr, 28.62-55.02, 95% HPD).

BAMM analyses strongly supported a diversity-dependent speciation process across Trichomycteridae with a net diversification rate of 0.131 species/Myr (0.105-0.164, 95% HPD) and extinction rate of 0.041 species/Myr (0.009-0.088, 95%HPD). The highest speciation rates are seen in Trichomycterinae node (0.214 species/Myr) and the sister group Copionodontinae+Trichogeninae (0.087species/Myr). These two clades also show the highest extinction rates (0.045 species/Myr) (Table 1.3). The TSVSG clade exhibits the lowest

speciation and extinction rates within the family. The changes in speciation rates (cool colors=slow, warm=fast) along each branch of the Trichomycteridae phylogeny can be observed in the figure 1.2A. The 95% credible set of rate shift configurations sampled with BAMM included eight distinct shifts of which the configuration with the highest probability included two shifts. The figure 1.2B shows the shifts both along the stem of Trichomycterinae with a frequency f=0.94 and f=0.058 respectively. Although the specific placements are at different times, both rate shifts occur during Oligocene and Miocene. Rate-through-time plots (Figure 1.2C) revealed a constant speciation rate until ~14Mya, at which point speciation and net diversification rate began to increase with the highest average rate (~0.065 species/My) seen at present. The figure 1.2D representing the branch-specific marginal shift probabilities, the length of each branch represents the percentage of samples from the posterior that contain a rate shift; in this case, the large branch of Trichomycterinae corroborates the probability of rate shift in this clade.

Rates of body size (SL) evolution exhibited a single background rate characterized by a low diversification for all subfamilies with exception of Trichomycterinae, where the *T. aerolatus* clade composes by *T. aerolatus*, *T. chiltoni* and *Bullockia maldonadoi* showed an increase in diversification rates (Figure 1.6). The results show nine shift configurations that account for more than 95% of samples and, all configurations show slowdowns in the family with subsequently increase in the *T. aerolatus* clade. The best shifts configurations are showed in gray, with shifts in Stegophilinae, Vandelliinae and three different shifts in the stem and recently diversified clades in Trichomycterinae.

In the ancestral reconstruction of feeding modes, we evaluated three different models for discrete comparative data and the Equal Rates (ER) model showed the best fit to data with AIC=114.3731 (Table 1.4). The ER model assume a single parameter governs all transition rates and below this model we simulated 100 stochastic character maps. The aggregate map (Figure 1.7) suggests that the ancestral feeding mode should be considered as insectivorous. Subsequently, this was followed by the acquisition of the most specialized modes as lepidophagous and hematophagous, with an increase in the omnivorous, one of the most generalized trophic habits.

## *1.4 Discussion*

### *1.4.1 Phylogenetics relationships in Trichomycteridae*

The present phylogenomic analysis recovered almost fully resolved trees with two different methods of phylogenetic inference (ML and B). The topology obtained is congruent with previous hypothesis of trichomycterid relationships based on morphology (Eigenmann 1918; Myers 1944; Baskin 1973; de Pinna 1992, 1998; Datovo & Bockmann 2010) and molecular datasets (Fernández & Schaefer 2009; Ochoa *et al.* 2017). The monophyly of the Trichomycteridae is supported by maximum values of bootstrap (BS=100%) and posterior probabilities (P=1). This result is congruent with all morphological studies, which provide a high number of unequivocal synapomorphies for the family (Eigenmann 1918; Myers 1944; Baskin 1973; de Pinna 1992, 1998; Datovo & Bockmann 2010). Our dataset support the monophyly of all subfamilies with the exception of Glanapteryginae and Sarcoglanidinae, and shows a perfect correspondence of the relative position of the early diverging branches with the most recent morphological and molecular hypothesis of the family (Datovo & Bockmann 2010; Ochoa *et al.* 2017), where the clade composed by Copionodontinae and Trichogeninae is the sister group of the clade C (Datovo & Bockmann 2010) clustering the remaining trichomycterids, represented by the TSVSG group (Tridentinae, Stegophilinae, Vandelliinae, Sarcoglanidinae, Glanapteryginae) (Costa & Bockmann 1994) and Trichomycterinae. The Copionodontinae and Trichogeninae have been considered basal lineages within the Trichomycteridae, and members of these subfamilies exhibit several morphological conditions that are intermediate between those present in remaining trichomycterids and other loricarioids (Eigenmann 1918; Myers 1944; Baskin 1973; de Pinna 1992, 1998; Datovo & Bockmann 2010).

Clade C gathers all members of the Trichomycteridae except the basal Copionodontinae and Trichogeninae. This clade is basally divided into two lineages: Trichomycterinae (sensu Datovo & Bockmann 2010) and TSVSG clade. Strong morphological and molecular evidence support the monophyly of the clade, which has been unanimously recovered in all analyses of the Trichomycteridae including the present study.

The longstanding controversy in the relationships in Trichomycterinae (Baskin 1973; de Pinna 1989, 1998), began to be elucidated with the support of its monophyletic status by morphological characters (Datovo & Bockmann 2010), as well as, the molecular evidence with the inclusion of genera *Scleronema* and *Ituglanis* in this subfamily, and the exclusion of

*Potamoglanis* (previously referred to as *Trichomycterus hasemani* group).This three trichomycterine subgroups were more explicitly proposed as aligned with the clade TSVSG, however, the relationship of *Scleronema* and *Ituglanis* with the clade TSVSG was rejected by Datovo & Bockmann (2010) who provided morphological evidence for the grouping of these genera with the remaining trichomycterines. In contrast, the inclusion of *Potamoglanis* within the TSVSG clade is supported by morphological (de Pinna 1989, 1998; Datovo & Bockmann 2010; DoNascimiento 2015) and molecular hypothesis (Henschel *et al.* 2017; Ochoa *et al.* 2017).

On the other hand, the relationship within Trichomycterinae has never been extensively surveyed by any publication based on morphological data, notwithstanding some suggestion of small putative subgroups with restrict geographic distribution have been proposed (e.g.; (de Pinna 1989; Costa 1992; Wosiacki 2002; Fernández & Osinaga 2006; Wosiacki & De Pinna 2008; Barbosa & Costa 2010; Datovo, Carvalho & Ferrer 2012). In recent molecular analysis by Ochoa et al (2017) including a substantial taxonomic sampling of the subfamily, with a total of 70 trichomycterine terminals, were identified two major lineages and six main subclades (D1, D2, D3, D4, D5, and E). The genomic analysis expands this previous sampling of trichomycterines in about 15% and the resulting subfamily tree exhibits only three most significant divergences relative to that of Ochoa et al. (2017): the inclusion of an undescribed trichomycterine from the Caribbean coast in Venezuela (previously unsampled) at the base of the whole subfamily and the non-monophyly of Clades D1 and D2. Our dataset recovers the two major clades identified in Ochoa et al (2017) with a strong node support, the first including *Eremophilus*, *Bullockia*, *Ituglanis*, and most northern species of *Trichomycterus*; and the second, composed by *Scleronema* and all remaining species of *Trichomycterus* (predominantly from southeastern South America).

Previous morphological analysis (Costa & Bockmann 1994; de Pinna 1998; Datovo & Bockmann 2010; DoNascimiento 2015) and molecular studies (Fernández & Schaefer 2009; Henschel *et al.* 2017; Ochoa *et al.* 2017) have supported the monophyly of the TSVSG clade, a lineage composed by Tridentinae, Stegophilinae, Vandelliinae, Sarcoglanidinae and Glanagapteryginae, our phylogenomic hypothesis strongly support this group as well.

One of the most important changes in the relationship of TSVSG clade was the incorporation of the clade *Potamoglanis* (formely referred to as the *Trichomycterus hasemani*-group) as sister clade of some sarcoglanidines in molecular analysis (Ochoa *et al.* 2017).

Previous hypothesis about the relationship of this clade with TSVSG group were proposed initially by De Pinna (1989), who suggested the inclusion of *Potamoglanis* in Tridentinae, based in the following characters shared for both clades: the small size and the presence of a single enormous cranial fontanela. Subsequently, DoNascimiento (2015) in a morphological analysis including 520 characters and 49 terminal taxa, allocated *Potamoglanis* at the base of the entire TSVSG clade, refuting de Pinna's (1989) hypothesis. Most recently, molecular studies by Henschel et al. (2017) recovers *Potamoglanis* as sister to tridentines and ignoring previous molecular results formally erected the genus *Potamoglanis*, as a subgroup of Tridentinae. Nevertheless, this phylogenomic hypothesis support a result similar to that of Ochoa et al. (2017) in grouping *Potamoglanis* with the sarcoglanidines and glanapterygines from Amazon and Orinoco. In light of these conflicting hypotheses of relationships for *Potamoglanis*, we consider the assignment of this group to any trichomycterid subfamily premature. In this way, for the sake of nomenclatural stability, the present paper follows the traditional concept of the Tridentinae, that is, not including *Potamoglanis.*

Within the TSVSG clade morphological studies traditionally have proposed the sister relationship between Glanapteryginae and Sarcoglanidinae, recognized as Glanapteryginae-group (Baskin 1973; Costa & Bockmann 1994; de Pinna 1998; Datovo & Bockmann 2010). However, the most recent morphological analysis (DoNascimiento 2015) and molecular phylogenies of the Trichomycteridae (Henschel *et al.* 2017; Ochoa *et al.* 2017) refuted the monophyly of the Glanapteryginae-group. DoNascimiento (2015) resolved the Sarcoglanidinae and Glanapteryginae are successive sister taxa to the Vandelliinae-group. In the topology of Henschel et al. (2017), tridentines and *Potamoglanis* are intercalated between glanapterygines (at the base of the TSVSG clade) and sarcoglanidines. The Glanapteryginae is also placed at the base of the TSVSG clade in the hypothesis of Ochoa et al. (2017). In this scheme; however, the Sarcoglanidinae is not monophyletic, with *Stauroglanis* appearing closer to vandelliines and *Sarcoglanis* to *Potamoglanis*. The present analysis, which has the largest taxonomic sampling of both subfamilies to date, obtained an even more striking result in which neither Sarcoglanidinae nor Glanapteryginae are monophyletic. Members of both subfamilies are clustered into two clades that are successive sister taxa to the Vandelliinae-group. Interestingly, sarcoglanidines and glanapterygines from the Atlantic coastal drainages (*Microcambeva, Listrura,* and trichomycterid n. gen.) are grouped into one clade, whereas the second clade gathers together members of both subfamilies from the Amazon and Orinoco (*Sarcoglanis, Stauroglanis, Typhlobelus,* and *Pygidianops*) along with *Potamoglanis* (Amazon

and Paraguay). The sarcoglanidines and glanapterygines grouped in the latter clade curiously share several reductive features, such as extreme reductions in the number of opercular odontodes, interopercular odontodes, premaxillary teeth, and pigmentation (Costa, 1994; de Pinna, 1989). The dismantling of the Glanapteryginae-group is a drastic change that obviously demands further investigation, but this result is not so surprising at all. A critical appraisal of the osteological characters listed to support various nodes of the Glanapteryginae-group tree demonstrates that several putative synapomorphies are of difficult polarization and known to exhibit some degree of homoplasy (de Pinna 1989, 1998; Costa & Bockmann 1994; de Pinna & Winemiller 2000b). Moreover, diagnoses and interrelationships among the putative basal most genera of the Glanapteryginae and Sarcoglanidinae are particularly unstable and the limits between each subfamily is increasingly blurry. For instance, new data suggest that *Ammoglanis pulex* is actually a glanapterygine, rather than a sarcoglanidine as originally described (de Pinna, 2016). Allocation of newly discovered taxa (e.g., trichomycterid n. gen.) into one or another subfamily is often difficult and possibly arbitrary (pers. obs.; de Pinna, 2016).

In spite of the new relationships showed in this study are strongly supported, decisions about taxonomic boundaries are often less than ideal due to the absence of the keys taxa in both subfamilies (*Glanapteryx*, *Ammoglanis*, *Malacoglanis* and *Stenolicmus*). However, this results insight the importance of a comprehensive taxonomic examination of Glanapteryginae and Sarcoglanidinae.

In contrast with the myological evidence presented by Datovo & Bockmann (2010) and the molecular support for the hypothesis of a tridentine-stegophiline group (Ochoa *et al.* 2017), our data recovered with a strong support the monophyly of the Vandelliinae-group as proposed by Baskin (1973) including Tridentinae as sister group of the candiru subfamilies and reinforce the molecular evidence presented by Fernandez & Schaefer (2009) to support the monophyly of Stegophilinae and Vandelliinae, a clade sharing derived conditions of the mesethmoid conua, maxillary and rictal babrbels, restricted gill openings, branchiostegal membrane lacking a free edge (Baskin 1973) and the presence of a median premaxilla (de Pinna 1998). Even though subfamilial analysis is beyond the scope of this study, our results show interesting results in the vandelliinae-group. Our study included three of the four genera of Tridentinae; only *Miuroglanis* was unavailable. According to Baskin (1973) derived characters as, a greater number of anal rays, the origin of the anal fin anterior to the dorsal origin, the ventral exposure and distinctly larger eyes support the monophyletic Tridens-group clustering *Tridens*, *Tridensimilis* and *Tridentopsis*; in so far as *Miuroglanis* shows primitive conditions in each of

these characters. As well as the following characters: fewer opercular teeth, rictal babel not visible externally, eyes face more ventrally than dorsally, Weberian capsule with an elongate neck and anal fin origin three or more vertebrae anterior to dorsal origin support the relationships within Tridens-group with *Tridensimilis* more closely related to *Tridens* than to *Tridentopsis*. Our results not support the morphological hypothesis of relationships among Tridens-group, with *Tridensimilis* more related to *Tridentopsis* than to *Tridens* involving a strong node support for this scheme of relationships.

Regarding Vandelliinae, we included only two genera of the four, missing *Plectrochilus* and *Paravandellia*. Morphological hypothesis proposed by Schmidt (1993) support the sister-group between *Paracanthopoma* and *Pletrochilus* plus *Vandellia* based on the loss of median premaxillary teeth, proximal end of premaxilla and ethmoid cornua both forked, reduced numbers of dentary teeth, and interopercular odontodes directed posterior.

Our hypothesis of interrelationships among stegophilines is almost identical to of the comprehensive revision of the subfamily published by DoNascimiento (2015). In both analyses, the Stegophiline has a basal dichotomy with a clade clustering *Haemomaster, Ochmacanthus*, and *Stegophilus* and another grouping all remaining genera. The only difference between the two topologies is the placement of the monotypic *Acanthopoma* among the species of *Pseudostegophilus* in our analysis. By contrast, DoNascimiento's (2015) tree allocate *Acanthopoma* in a basal polytomy with *Pareiodon* and a monophyletic *Pseudostegophilus*.

### *1.4.2 Diversification pattern in Trichomycteridae*

Patterns of species diversity have been shaped by both speciation and extinction throughout the history of life, and one of the key questions in evolutionary biology is to understand the temporal and spatial dynamics of these processes (Nee, Mooers & Harvey 1992; Sanderson & Donoghue 1996; Barraclough & Nee 2001; Jablonski *et al.* 2003; Ricklefs 2007). Additionally, the fossil record and molecular phylogenetic data of extant lineages can provide valuable information on the process of diversification in form of branch length and the distribution of divergence times throughout the evolutionary history of a clade (Silvestro, Schnitzler & Zizka 2011). Relevant issues in detecting significant rate shifts include incorporating extinction, phylogenetic uncertainty, phylogenetic scale, sampling density, correlation and/or causality of biotic or niche attributes driving the rate shifts (Barraclough & Nee 2001). The program BAMM, as now implemented, can address a number of these issues.

Trichomycteridae is an ideal candidate for comparative evolutionary studies considering its relatively old origins in the Lower Cretaceous, a widespread biogeographical distribution, occupation of diverse habitats, considerable morphological diversity, the presence of both species-rich and depauperate clades, and now a well-supported phylogeny.

The analysis of diversification rates in Trichomycteridae shows a high clade heterogeneity. The rates-throughout-time analysis exhibited long term speciation rates decline in the TSVSG and Copionodontinae+Trichogeninae clades with an extinction rate constant throughout its entire 66 Myr diversification until the present; in comparison, it was identified an increase in the speciation rate and evolutionary shift in Trichomycterinae, a clade is characterized by its species richness. The average rate of net diversification for Trichomycteridae is not striking (0.131 lineage/ Myr). This rate is similar to those estimated by Rabosky et al (2013) in an extensive analysis correlating diversification rates across approximately ~30.000 living species of ray-finned fishes.

In a macroevolutionary context, shift in diversification rates in a phylogeny should be positively associated with phenotypic evolution (Eldredge & Gould 1972; Pennell, Harmon & Uyeda 2013). Regardless of the underlying causal mechanisms, a growing body of empirical research suggests that species diversification and morphological evolution are frequently coupled in nature (Rabosky *et al.* 2014b). Recent studies demonstrated that rates of species diversification are highly correlated with the rate of body size evolution across the 30,000 living species of ray-finned fishes that comprise the majority of vertebrate biological diversity (Rabosky *et al.* 2013). Despite the coupling in species diversification rates and body size evolution seem to be a general feature, our phylogeny not support the correlation of different regimen in diversification rate with the evolution of body size in Trichomycteridae. Researches in mammals, cetaceans, squamates and salamanders have shown similar results, decoupling morphological diversification from speciation and suggest that the processes that give rise to the morphological diversity of a class of animals are far more free to vary than previously considered (Adams *et al.* 2009; Venditti, Meade & Pagel 2011; Rabosky & Adams 2012; Burbrink *et al.* 2012). Evolutionary hypotheses related to the evolution of miniature species have been proposed for Trichomycteridae (Weitzman & Vari 1988; de Pinna 1998); however, little is known about the relationship between morphological evolution and speciation rates in this group.

The diversification rates in Trichomycterinae are probably influenced by ecological traits as its wide geographical distribution, occupying different types of environments and generalist trophic habit. According to Rundell & Price (2009) geographic range expansions is a critical part of "successful" speciation, but it may require that recently separated species are undergo ecological or morphological divergence, before sympatry is possible (Podrabsky, Garrett & Kohl 2010). This way, one interpretation of the higher speciation rates in Trichomycterinae can be related with the progressive colonization of a new regions and generalist trophic habit. According to ancestral reconstruction of feeding modes, Trichomycterinae exhibits a more generalist trophic habit with insectivorous and omnivorous species, that in combination with its wide geographical distribution and ecological diversity could have contributed in the increase of diversification rates. In comparison, clades with more specialized feeding (algivorous, hematophagous, lepidophagous and carrion feeders) have major constraint in its response to environmental changes. Most ecological studies indicate that specialists are more sensitive to extinction under environmental changes, as consequence of small size of local populations, an often restricted geographic range, and a limited utilization of resources and habitats (Colles, Liow & Prinzing 2009).

In summary, our analysis showed that the diversification rates in Trichomycteridae are heterogeneous and diversity dependent, as it was evidenced in the increasing of diversification rates in Trichomycterinae. Our results do not show a direct association between speciation rates and body size evolution, probably that Trichomycteridae was predisposed to diversify in ways that do not involve adaptive divergence as might result from ecological specialization and allopatric speciation. Our results, also highlight the importance of more exploratory analysis at morphological and ecological level may provide refinement on the placement of and processes leading to significant shifts in speciation rates within Trichomycteridae.

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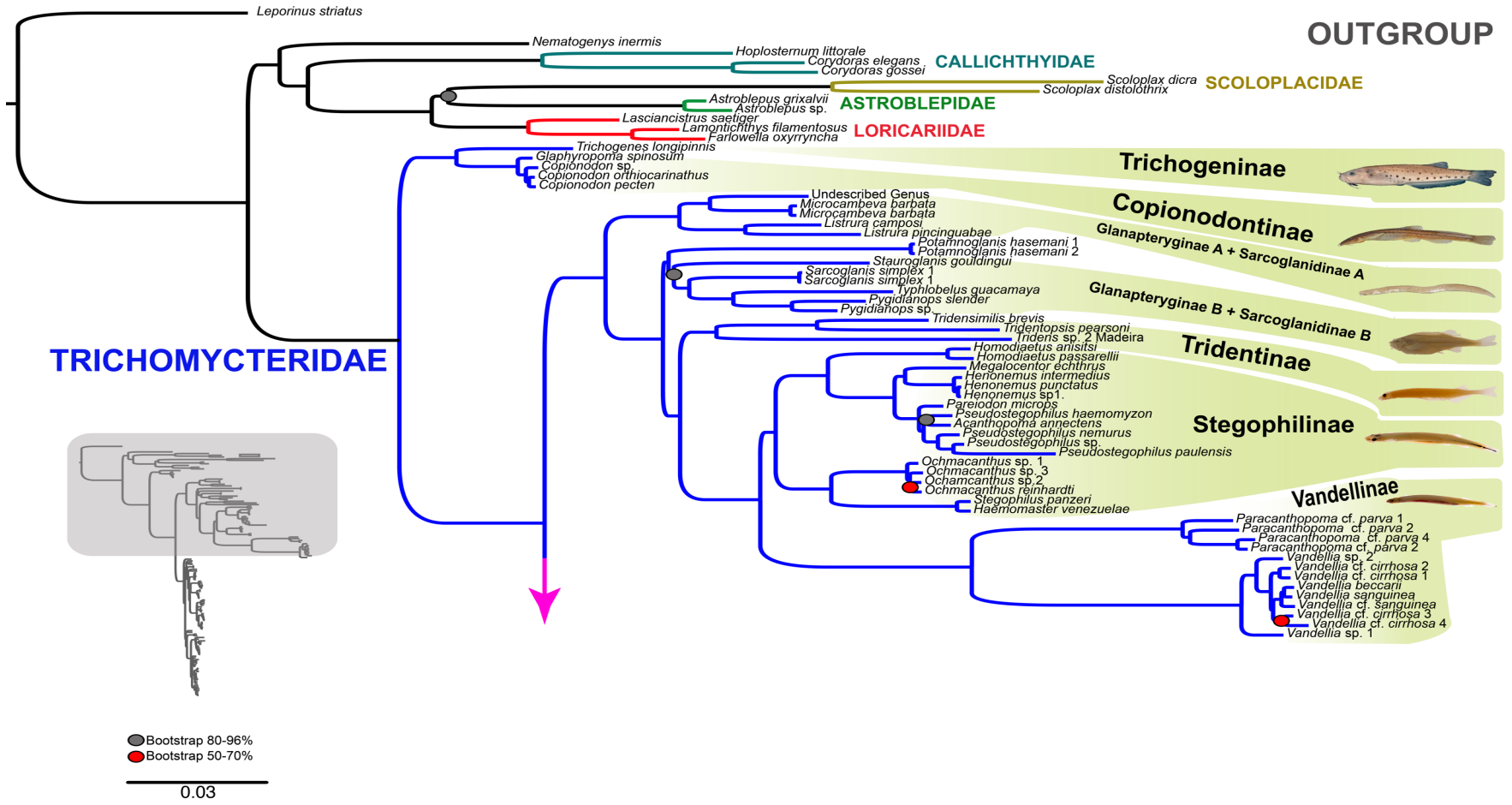


**Figure 1.1**. Maximum-likelihood and Bayesian phylogeny inferred from a 75% complete supermatrix containing data from 143 taxa from which we enriched and assembled (AByss) ultraconserved element loci. The posterior probabilities for all nodes were equal to B = 1.0. Bootstrap support values <100% are indicated by red (50-70%) and gray circles (80-96%).

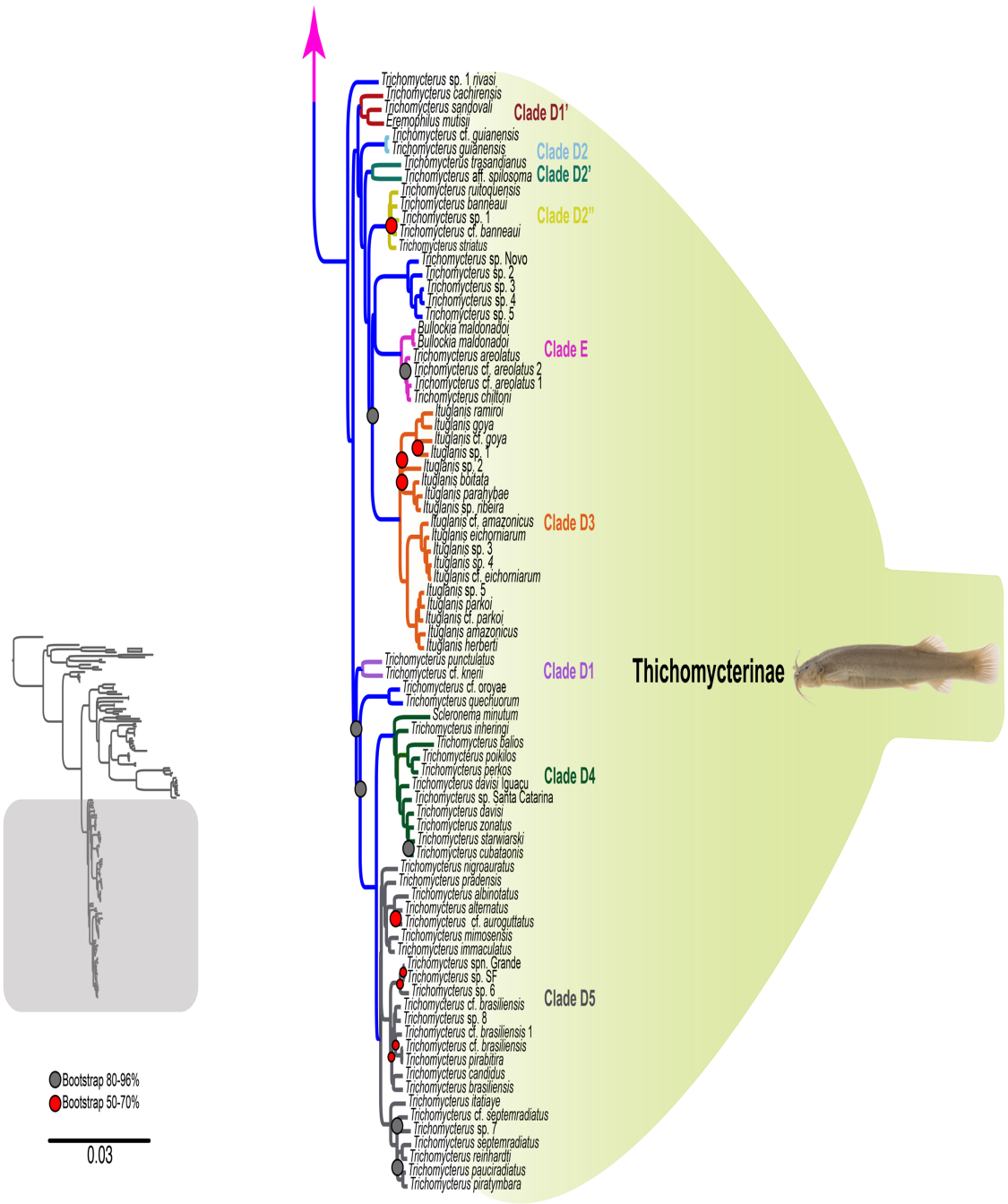


**Figure 1.1 continue**. Maximum-likelihood and Bayesian phylogeny inferred from a 75% complete supermatrix containing data from 143 taxa from which we enriched and assembled (AByss) ultraconserved element loci. The posterior probabilities for all nodes were equal to B = 1.0. Bootstrap support values <100% are denoted by red and gray circle

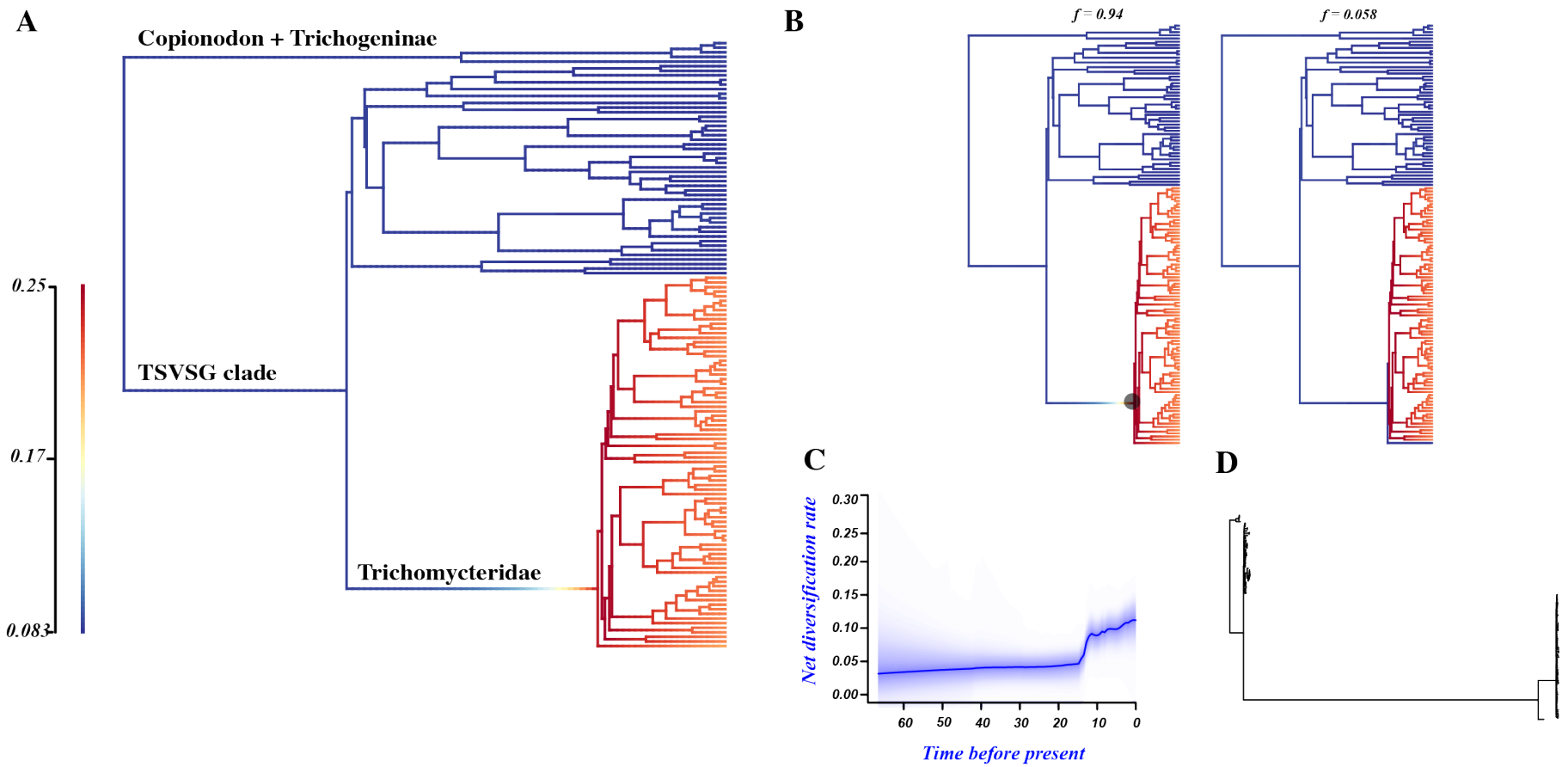


**Figure 1.2.** A) Dated phylogenetic tree of Trichomycteridae, with rates of speciation inferred using BAMM (warmer colours=faster rates; scale bar on left represents speciation rate per My); B) 95% credibility shift configurations with their frequencies for the evolution across a phylogeny of Trichomycteridae; C) net speciation rate through time plot; and D) Marginal shift probabilities.

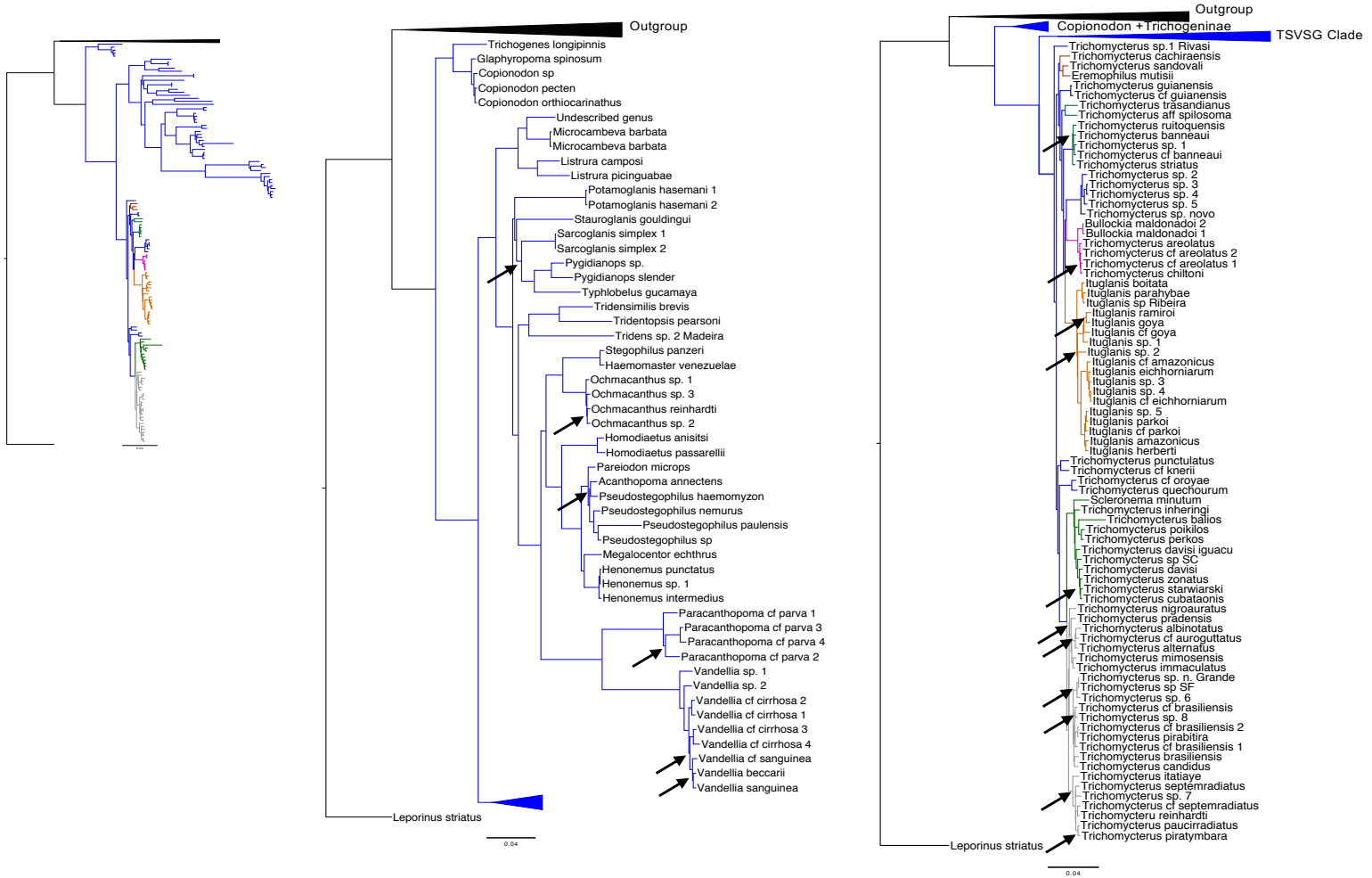


**Figure 1.3**. Maximum-likelihood tree inferred from a 50% complete supermatrix containing data from 143 taxa. Bootstrap values <100 are denoted by black arrow

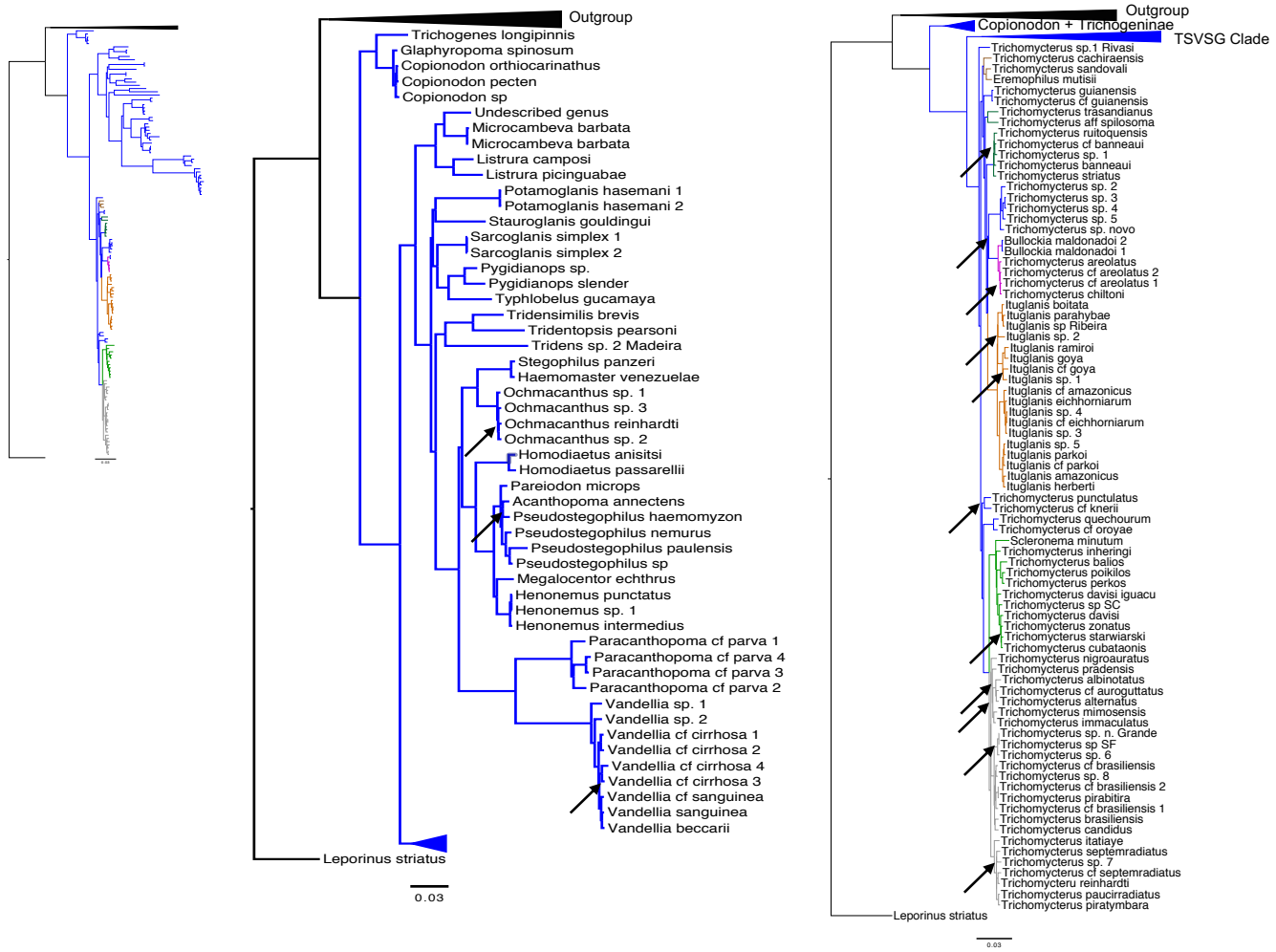


**Figure 1.4**. Maximum-likelihood tree inferred from 85% complete supermatrix containing data from 143 taxa. Bootstrap values <100 are denoted by black arrows

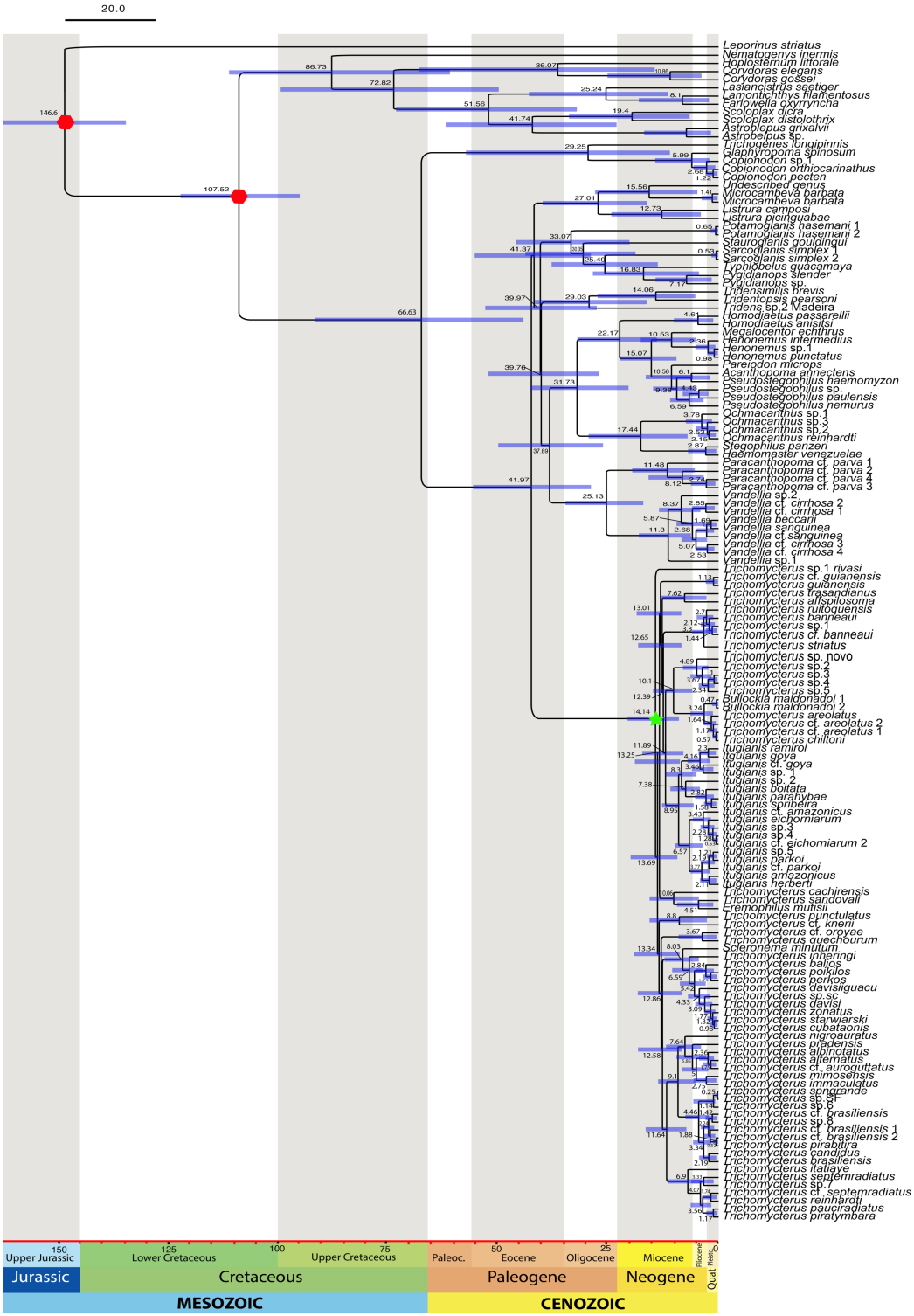


**Figure 1.5**. Maximum clade credibility tree of Trichomycteridae obtained from BEAST analysis. Mean divergence time estimates shown with 95% highest posterior density (HPD; blue bars). Red circles representing second calibration points and fossil in Trichomycterinae is indicated by green star.

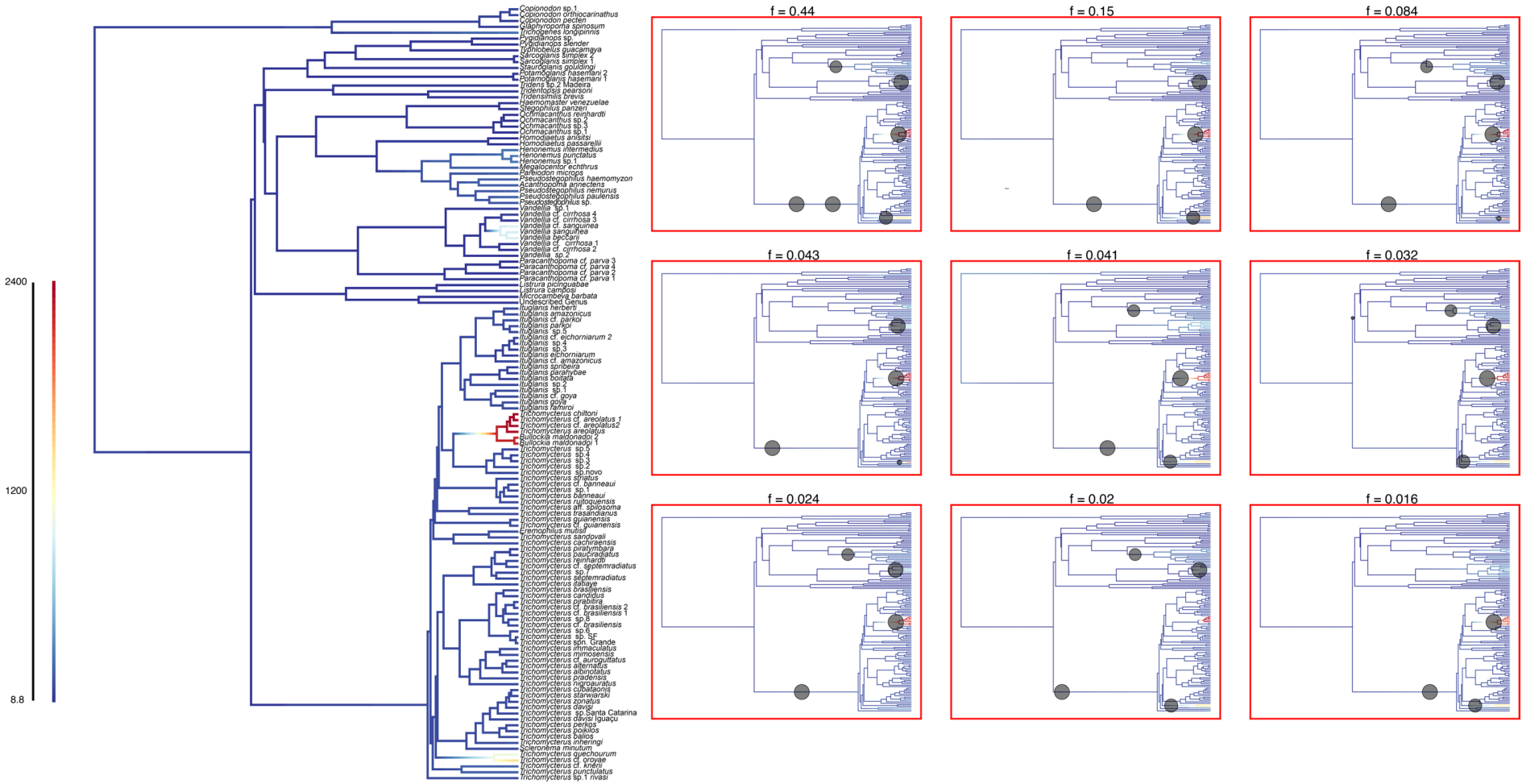


**Figure 1.6**. A) The best shifts configurations for body size in Trichomycteridae; B) 95% credibility shift configurations (gray cirlces) with their frequencies for the evolution of body size across a phylogeny of Trichomycteridae

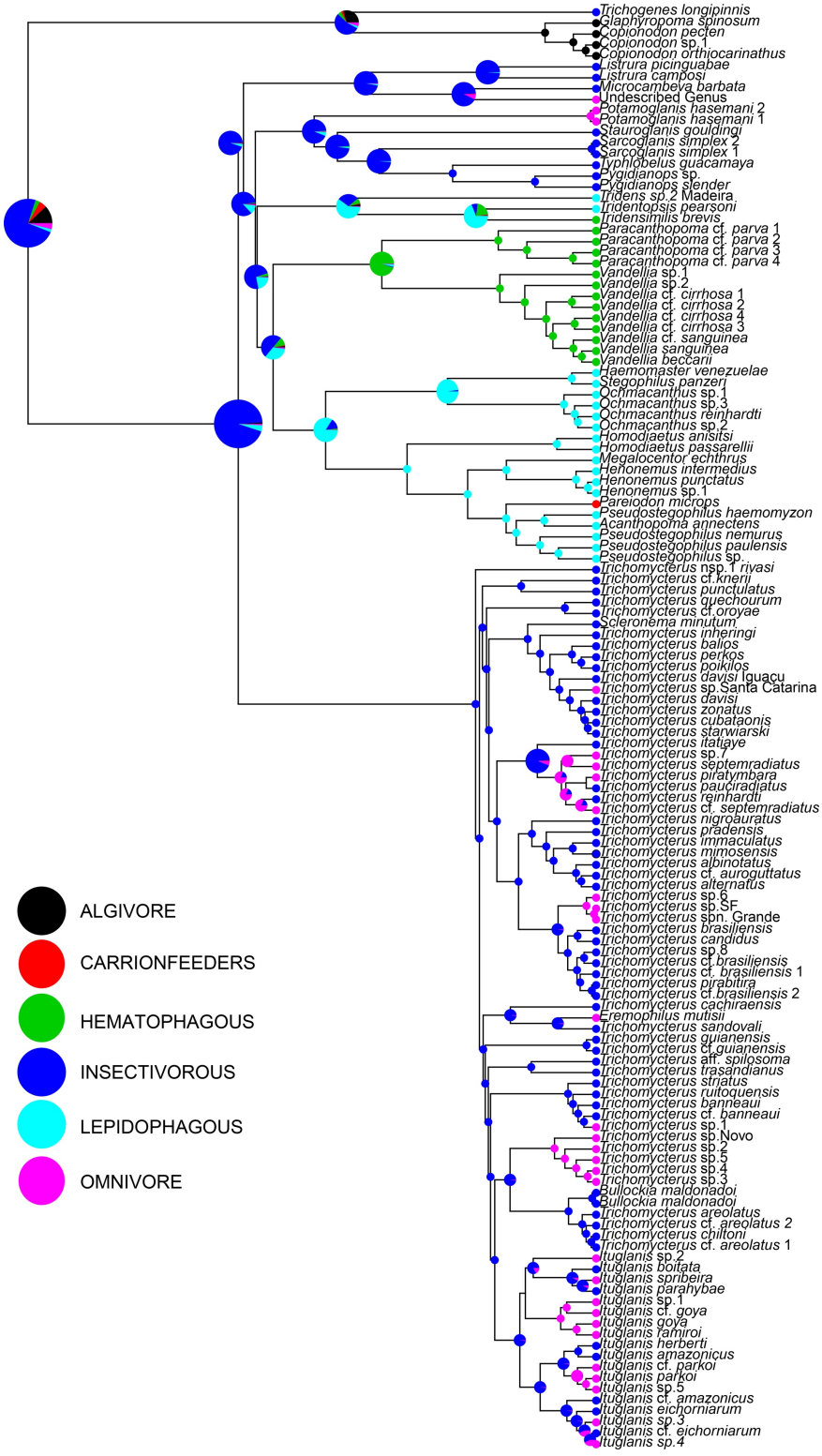


**Figure 1.7**. Pie chart showing the posterior probabilities of feeding modes states on Trichomycteridae phylogeny.

**Table 1.1**. Taxonomic sampling, catalog number, voucher number and geographic distribution of the samples included in this study.

| Subfamily | *Genus* | *Species* | Catalog number | Voucher | River | Basin | Country |
|---|---|---|---|---|---|---|---|
| Trichogeninae | *Trichogenes* | *Trichogenes longipinnis* | LBP3862 | 22411 | Cachoeira do amor | Atlantico | Brazil |
| Copionodontinae | *Glaphyropoma* | *Copionodon orthiocarinathus* | LBP17354 | 17354 | Mucujê | Paraguaçu | Brazil |
| | *Copionodon* | *Copionodon pecten* | LBP17357 | 38993 | Mucujê | Paraguaçu | Brazil |
| | | *Copionodon sp1* | LBP17361 | 59311 | Mucujê | Paraguaçu | Brazil |
| | | *Glaphyropoma spinosum* | LBP17359 | 17359 | Gruta dos torres | Paraguaçu | Brazil |
| Glanapteryginae | *Listrura* | *Listrura camposi* | LBP7438 | 35362 | Riacho sem nome | Ribeira do Iguape | Brazil |
| | | *Listrura picinguabae* | LBP3864 | 22423 | Afluente rio da Fazenda | Ribeira do Iguape | Brazil |
| | *Pygidianops* | *Pygidianops "slender"* | ANSP190505 | 2115 | Asita | Orinoco | Venezuela |
| | | *Pygidianops sp* | ANSP190504 | 2113 | Asita | Orinoco | Venezuela |
| | *Typhlobelus* | *Typhlobelus guacamaya* | ANSP190503 | 2114 | Cuao | Orinoco | Venezuela |
| | *New genus* | *Undescribed genus* | LBP16842 | 69447 | Fau | Ribeira do Iguape | Brazil |
| Sarcoglanidinae | *Microcambeva* | *Microcambeva barbata* | LBP21985 | 21985 | Aldeia Velha | Paraíba do Sul | Brazil |
| | | *Microcambeva barbata* | LBP21985 | 21985 | Aldeia Velha | Paraíba do Sul | |
| | *Sarcoglanis* | *Sarcoglanis simplex 1* | ANSP179212 | 872 | Ireng | Takutu | Guyana |
| | | *Sarcoglanis simplex 2* | ANSP180021 | 2201 | Takutu | Takutu | Guyana |
| | *Stauroglanis* | *Stauroglanis gouldingi* | LBP3159 | 19301 | Negro | Amazonas | Brazil |
| Tridentinae | *Potamoglanis* | *Potamoglanis hasemani* | LBP4483 | 24450 | Negro | Amazonas | Brazil |
| | *Tridensimilis* | *Tridensimilis brevis* | LBP13940 | 13940 | Tapajos | Amazonas | Brazil |
| | *Tridens* | *Tridens sp. 2 Madeira* | LBP12070 | 12070 | Madeira | Amazonas | Brazil |
| | *Tridentopsis* | *Tridentopsis pearsoni* | LBP13944 | 13944 | Branco | Amazonas | Brazil |
| Stegophilinae | *Acanthopoma* | *Acanthopoma annectens* | ANSP181146 | 890 | Amazonas | Amazonas | Perú |
| | *Haemomaster* | *Haemomaster venezuelae* | INPA43789 | 10704 | Xingu | Amazonas | Brazil |
| | *Homodiaetus* | *Homodiaetus anisitsi* | LBP13194 | 55163 | Camaquã | Lagoa dos Patos | Brazil |
| | | *Homodiaetus passarellii* | LBP2502 | 16505 | Macacu | Atlantico | Brazil |
| | *Henonemus* | *Henonemus intermedius* | LBP2394 | 16454 | Araguaia | Amazonas | Brazil |

| Subfamily | *Genus* | *Species* | Catalog number | Voucher | River | Basin | Country |
|---|---|---|---|---|---|---|---|
| | | *Henonemus punctatus* | LBP4146 | 23679 | Jurua | Amazonas | Brazil |
| | | *Henonemus sp 1* | ANSP197334 | 12772 | Jarauçu | Amazonas | Brazil |
| | *Megalocentor* | *Megalocentor echthrus* | ANSP199997 | 4732 | Itaya | Amazonas | Perú |
| | *Pareiodon* | *Pareiodon microps* | ANSP191783 | 4707 | Amazonas | Amazonas | Brazil |
| | *Pseudostegophilus* | *Pseudostegophilus haemomyzon* | ANSP198977 | 13134 | Paraguai | Paraná | Brazil |
| | | *Pseudostegophilus nemurus* | LBP1581 | 11769 | Apure | Apure | Venezuela |
| | | *Pseudostegophilus paulensis* | LBP6738 | 6738 | Das Garças | Araguaia | Brazil |
| | | *Parastegophilus sp* | LBP5133 | 26235 | Tiete | Paraná | Brazil |
| | *Ochmacanthus* | *Ochmacanthus sp.1* | ANSP191769 | 4748 | Itaya | Amazonas | Brazil |
| | | *Ochmacanthus sp. 2* | LBP4351 | 24088 | Igarapé do cajual | Amazonas | Brazil |
| | | *Ochmacanthus sp.3* | ANSP197613 | 11339 | Itaya | Amazonas | Brazil |
| | | *Ochmacanthus reinhardti* | LBP10987 | 50481 | Lajeado | Amazonas | Brazil |
| | *Stegophilus* | *Stegophilus panzeri* | LBP8619 | 43462 | Tapajos | Amazonas | Brazil |
| Vandelliinae | *Paracanthopoma* | *Paracanthopoma* cf *parva 1* | LBP2498 | 15495 | Araguaia | Amazonas | Brazil |
| | | *Paracanthopoma* cf *parva 2* | ANSP199868 | 4948 | Nanay | Amazonas | Perú |
| | | *Paracanthopoma* cf *parva 3* | ANSP198315 | 12408 | Nanay | Amazonas | Perú |
| | | *Paracanthopoma* cf *parva 4* | LBP5245 | 26466 | Jari | Amazonas | Brazil |
| | *Vandellia* | *Vandellia beccarii* | LBP10155 | 47557 | Apure | Orinoco | Venezuela |
| | | *Vandellia* cf *cirrhosa 1* | ANSP191329 | 3936 | Ventuari | Orinoco | Venezuela |
| | | *Vandellia* cf *cirrhosa 2* | LBP5342 | 26973 | Ventuari | Orinoco | Venezuela |
| | | *Vandellia* cf *cirrhosa 3* | ANSP197186 | 11246 | Tamshiyacu | Amazonas | Perú |
| | | *Vandellia* cf *cirrhosa 4* | LBP2477 | 16450 | Araguaia | Amazonas | Brazil |
| | | *Vandellia* cf *sanguinea* | LBP2477 | 16449 | Araguaia | Amazonas | Brazil |
| | | *Vandellia sanguinea* | LBP15976 | 66164 | Xingu | Amazonas | Brazil |
| | | *Vandellia sp.1* | LBP1631 | 11765 | Araguaia | Amazonas | Brazil |
| | | *Vandellia sp.2* | LBP14854 | 57887 | Amazonas | Amazonas | Perú |
| Trichomycteridae | *Bullockia* | *Bullockia maldonadoi* | LBP3112 | 3112 | La Laja | Bio Bio | Chile |

| Subfamily | *Genus* | *Species* | Catalog number | Voucher | River | Basin | Country |
|---|---|---|---|---|---|---|---|
| | | *Bullockia maldonadoi* | LBP3112 | 19793 | La Laja | Bio Bio | Chile |
| | *Eremophilus* | *Eremophilus mutisii* | ANSP11306 | no voucher | Magdalena | Magdalena | Colombia |
| | *Ituglanis* | *Ituglanis amazonicus* | LBP2442 | 16211 | Araguaia | Amazonas | Brazil |
| | | *Ituglanis amazonicus 2* | LBP11003 | 50532 | Madeira | Amazonas | Brazil |
| | | *Ituglanis boitata* | LBP14546 | 60865 | Jacui | Lagoa dos Patos | Brazil |
| | | *Ituglanis eichhorniarum* | LBP4686 | 24825 | Paraguay | Paraguay | Brazil |
| | | *Ituglanis* cf *eichhorniarum* | LBP10777 | 49859 | Paraguay | Paraguay | Brazil |
| | | *Ituglanis goya* | LBP17137 | 68599 | Dos Couros | Tocantins | Brazil |
| | | *Ituglanis* cf *goya* | LBP17131 | 68592 | Das Almas | Tocantins | Brazil |
| | | *Ituglanis herberti* | LBP676 | 8028 | Pirai | Paraná | Brazil |
| | | *Ituglanis parahybae* | LBP10730 | 49719 | Macabu | Paraíba do Sul | Brazil |
| | | *Ituglanis parkoi* | LBP14153 | 59188 | Tapajos | Amazonas | Brazil |
| | | *Ituglanis* cf *parkoi* | LBP7995 | 37376 | Tapajos | Amazonas | Brazil |
| | | *Ituglanis ramiroi* | LBP15293 | 63261 | Rio São Bernardo | Tocantins | Brazil |
| | | *Ituglanis sp.1* | LBP19465 | 77969 | Das Brancas | Tocantins | Brazil |
| | | *Ituglanis sp. 2* | LBP16129 | 66849 | Tapajos | Amazonas | Brazil |
| | | *Ituglanis sp 3* | LBP12960 | 55688 | Rio Cuiaba | Paraguai | Brazil |
| | | *Ituglanis sp 4* | LBP7667 | 36475 | Corrego João Dias | La Plata | Brazil |
| | | *Ituglanis sp 5* | INPA11584 | 11584 | Xingu | Amazonas | Brazil |
| | | *Ituglanis sp ribeira* | LBP7416 | 35678 | Batatau | Ribeira do Iguape | Brazil |
| | | *Trichomycterus albinotatus* | LBP6326 | 29802 | Paraiba do sul | Paraíba do Sul | Brazil |
| | | *Trichomycterus alternatus* | LBP1014 | 10261 | Chopoto | São francisco | Brazil |
| | | *Trichomycterus areolatus* | LBP3118 | 19819 | Maichin | Maichin | Chile |
| | | *Trichomycterus areolatus 1* | LBP3113 | 3113 | La Laja | Bio Bio | Chile |
| | | *Trichomycterus areolatus 2* | LBP3118 | 19820 | Maichin | Maichin | Chile |
| | | *Trichomycterus cf auroguttatus* | LBP8374 | 40446 | Paraiba do sul | Paraíba do Sul | Brazil |
| | | *Trichomycterus balios* | LBP607 | 7358 | Tainhas | Taquari-Antas | Brazil |

| Subfamily | *Genus* | *Species* | Catalog number | Voucher | River | Basin | Country |
|---|---|---|---|---|---|---|---|
| | | *Trichomycterus brasiliensis* | LBP10675 | 49540 | São Francisco | São francisco | Brazil |
| | | *Trichomycterus cf brasiliensis* | LBP10276 | 47976 | Grande | La Plata | Brazil |
| | | *Trichomycterus cf brasiliensis 1* | LBP8060 | 37829 | Grande | La Plata | Brazil |
| | | *Trichomycterus cf brasiliensis* | LBP6247 | 29199 | Grande | La Plata | Brazil |
| | | *Trichomycterus banneaui* | LBP19847 | 77973 | Magdalena | Magdalena | Colombia |
| | | *Trichomycterus* cf *banneaui* | LBP19537 | 77971 | Magdalena | Magdalena | Colombia |
| | | *Trichomycterus cachirensis* | LBP19832 | 77957 | Galvanes | Magdalena | Colombia |
| | | *Trichomycterus candidus* | LBP11630 | 58011 | Paraná | Paraná | Brazil |
| | | *Trichomycterus chiltoni* | ANSP180474 | 940 | Andalien | Andalien | Chile |
| | | *Trichomycterus cubataonis* | LBP3123 | 19690 | Itapocu | Atlantico | Brazil |
| | | *Trichomycterus davisi* | LBP6383 | 29744 | Preto | La Plata | Brazil |
| | | *Trichomycterus davisi_iguaçu* | LBP1222 | 10598 | Iguaçu | Atlantico | Brazil |
| | | *Trichomycterus cf. guianensis* | ANSP179111 | 918 | Orokang | Orokang | Guyana |
| | | *Trichomycterus guianensis* | LBP17444 | 69015 | Potaro | Essequibo | Guyana |
| | | *Trichomycterus inheringi* | LBP4512 | 24563 | Paranapiacaba | Paraná | Brazil |
| | | *Trichomycterus immaculatus* | LBP8351 | 40419 | Doce | São francisco | Brazil |
| | *Trichomycterus* | *Trichomycterus itatiaye* | LBP16356 | 62282 | Paraiba do sul | Paraíba do Sul | Brazil |
| | | *Trichomycterus cf knerii* | LBP18717 | 18717 | Meta/caño Guamal | Orinoco | Colombia |
| | | *Trichomycterus mimosensis* | LBP8290 | 38358 | Jequitinhonha | Jequitinhonha | Brazil |
| | | *Trichomycterus nigroauratus* | LBP6301 | 29341 | Itagaçaba | Paraíba do Sul | Brazil |
| | | *Trichomycterus cf. oroyae* | LBP3255 | 3255 | Chontabamba | Amazonas | Perú |
| | | *Trichomycterus pauciradiatus* | LBP16323 | 61977 | São Francisco | São francisco | Brazil |
| | | *Trichomycterus pradensis* | LBP8291 | 38359 | Jequitinhonha | Jequitinhonha | Brazil |
| | | *Trichomycterus pirabitira* | LBP18381 | 72641 | Grande | La Plata | Brazil |
| | | *Trichomycterus piratymbara* | LBP9004 | 42138 | Grande | La Plata | Brazil |
| | | *Trichomycterus perkos* | LBP17033 | 66403 | Uruguay | Uruguay | Brazil |
| | | *Trichomycterus poikilos* | LBP14693 | 61154 | Carreiro | Uruguay | Brazil |

| Subfamily | *Genus* | *Species* | Catalog number | Voucher | River | Basin | Country |
|---|---|---|---|---|---|---|---|
| | | *Trichomycterus punctulatus* | ANSP180733 | 905 | Pisco | Pisco | Perú |
| | | *Trichomycterus quechourum* | ANSP180572 | 912 | Mapacho | Mapacho | Perú |
| | | *Trichomycterus sp.1 rivasi* | ANSP191470 | 2083 | | Peninsula de Paria | Venezuela |
| | | *Trichomycterus reinhardti* | LBP16302 | 61942 | São Francisco | São francisco | Brazil |
| | | *Trichomycterus ruitoquensis* | LBP19838 | 77956 | Magdalena | Magdalena | Colombia |
| | | *Trichomycterus sandovali* | LBP19833 | 77946 | Don Juan cave | Magdalena | Colombia |
| | | *Trichomycterus septemradiatus* | LBP5939 | 28065 | São Domingos | Grande | Brazil |
| | | *Trichomycterus* cf. *septemradiatus* | LBP6550 | 31670 | Das Velhas | São francisco | Brazil |
| | | *Trichomycterus aff spilosoma* | LBP19339 | 77975 | Dos Bocas | Atlantico | Ecuador |
| | | *Trichomycterus sp. 1* | LBP19834 | 77958 | Magdalena | Magdalena | Colombia |
| | | *Trichomycterus sp. 2* | LBP7674 | 36488 | Afluente rio Coxipo-Açu | La Plata | Brazil |
| | | *Trichomycterus sp. 3* | LBP7669 | 36484 | Afluente rio da Casca | Paraguai | Brazil |
| | | *Trichomycterus sp. 4* | LBP10236 | 47789 | Corrego sem nome | La Plata | Brazil |
| | | *Trichomycterus sp. 5* | LBP7640 | 36427 | Afluente rio Aricá-Mirim | La Plata | Brazil |
| | | *Trichomycterus sp. 6* | LBP11648 | 58088 | Bonito | Paraná | Brazil |
| | | *Trichomycterus sp. 8* | LBP9001 | 42123 | Grande | La Plata | Brazil |
| | | *Trichomycterus sp.7* | LBP11631 | 58082 | Paraná | Paraná | Brazil |
| | | *Trichomycterus sp N grande* | LBP10282 | 47992 | Araguari | La Plata | Brazil |
| | | *Trichomycterus sp. SF* | LBP11842 | 58147 | Córrego da Agua Santa | São francisco | Brazil |
| | | *Trichomycterus sp. Novo* | LBP8563 | 43332 | Sepotuba | Paraguay | Brazil |
| | | *Trichomycterus starwiarski* | LBP16165 | 66905 | Cachoeira | Paraguay | Brazil |
| | | *Trichomycterus striatus* | LBP19846 | 77969 | Magdalena | Magdalena | Colombia |
| | | *Trichomcyterus sp. Santa Catarina* | LBP3121 | 19686 | Itapocu | Atlantico | Brazil |
| | | *Trichomycterus trasandianus* | LBP19845 | 77964 | Magdalena | Magdalena | Colombia |
| | | *Trichomycterus zonatus* | LBP2653 | 17409 | Ribeira do Iguape | Ribeira do Iguape | Brazil |
| | *Scleronema* | *Scleronema minutum* | LBP3310 | 19841 | Arroio dos Corrientes | Atlantico | Brazil |
| Nematogenyidae | *Nematogenys* | *Nematogenys inermis* | LBP3105 | 3105 | Andalien | Pacifico | Chile |

| Subfamily | *Genus* | *Species* | Catalog number | Voucher | River | Basin | Country |
|---|---|---|---|---|---|---|---|
| Callichthyidae | *Corydoras* | *Corydoras elegans* | LBP57701 | 57701 | Amazonas | Amazonas | Perú |
| | | *Corydoras gossei* | LBP544 | 7171 | Aquarum | NA | Brazil |
| | *Hoplosternum* | *Hoplosternum littorale* | LBP466 | 7282 | Paranapanema | Paranapanema | Brazil |
| Scoloplacidae | *Scoloplax* | *Scoloplax dicra* | LBP11001 | 50522 | Lajeado | Amazonas | Brazil |
| | | *Scoloplax distolothrix* | LBP1938 | 14340 | Itiquira | Piquiri | Brazil |
| Loricaridae | *Farlowella* | *Farlowella oxyrryncha* | LBP1558 | 11509 | Das mortes | Amazonas | Brazil |
| | *Lamontichthys* | *Lamontichthys filamentosus* | LBP162 | 4093 | Branco | Amazonas | Brazil |
| | *Lasiancistrus* | *Lasiancistrus saetiger* | LBP9156 | 42517 | Guamá | Amazonas | Brazil |
| Astroblepidae | *Astroblepus* | *Astroblepus grixalvii* | ANSP188920 | 188920 | Dormilon | Magdalena | Colombia |
| | | *Astroblepus sp* | LBP19844 | 77952 | Frio | Magdalena | Colombia |
| Characidae | *Leporinus* | *Leporinus striatus* | LBP3180 | 16871 | Paranapanema | Paraná | Brazil |

**Table 1.2**. Summary values describing the number of reads, number of trimmed reads, number of contigs assembled, total base pair of contigs, number of UCE contigs, mean length of all contigs and their average coverage for samples.

| Subfamily | Genus | Specie | Catalogue number | Voucher | Raw reads | Number of trimmed reads | contigs assembled | total bp Contigs | UCE contigs | mean length | mean coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Trichogeninae | *Trichogenes* | *Trichogenes longipinnis* | LBP3862 | 22411 | 1,392,637 | 2,575,084 | 17,048 | 3,448,340 | 1,713 | 202 | 348 |
| Copionodontinae | *Copionodon* | *Copionodon orthiocarinathus* | LBP17354 | 17354 | 1,378,441 | 2,612,582 | 39,446 | 9,228,869 | 1,652 | 234 | 275 |
| | | *Copionodon pecten* | LBP17357 | 38993 | 1,041,597 | 1,406,788 | 31,772 | 7,005,358 | 1,486 | 220 | 208 |
| | | *Copionodon sp1* | LBP17361 | 59311 | 1,976,223 | 2,554,783 | 186,304 | 26,666,207 | 1,473 | 143 | 395 |
| | *Glaphyropoma* | *Glaphyropoma spinosum* | LBP17359 | 17359 | 1,333,380 | 4,460,737 | 34,490 | 8,001,172 | 1,669 | 232 | 266 |
| Glanapteryginae | *Listrura* | *Listrura camposi* | LBP7438 | 35362 | 648,076 | 2,544,499 | 3,018 | 623,977 | 1,140 | 207 | 129 |
| | | *Listrura picinguabae* | LBP3864 | 22423 | 856,430 | 1,389,668 | 11,777 | 2,867,691 | 1,118 | 243 | 171 |
| | *Pygidianops* | *Pygidianops "slender"* | ANSP190505 | 2115 | 2,288,690 | 4,191,971 | 132,642 | 27,265,969 | 1,317 | 206 | 572 |
| | | *Pygidianops sp* | ANSP190504 | 2113 | 2,997,209 | 5,484,465 | 56,537 | 8,877,014 | 1,349 | 157 | 749 |
| | *Typhlobelus* | *Typhlobelus guacamaya* | ANSP190503 | 2114 | 1,127,940 | 2,060,441 | 72,254 | 12,695,780 | 1,407 | 176 | 281 |
| | *New genus* | *Undescribed genus* | LBP16842 | 69447 | 828,128 | 1,532,438 | 68,373 | 11,388,730 | 1,389 | 167 | 207 |
| Sarcoglanidinae | *Microcambeva* | *Microcambeva barbata* | LBP21985 | 21985 | 5,100,735 | 223,637 | 5,608 | 977,666 | 825 | 174 | 1020 |
| | | *Microcambeva barbata* | LBP21985 | 21985 | 3,562,133 | 335,526 | 4,072 | 654,841 | 675 | 161 | 712 |
| | *Sarcoglanis* | *Sarcoglanis simplex 1* | ANSP179212 | 872 | 3,016,719 | 1,610,059 | 57,220 | 11,916,517 | 1,396 | 208 | 603 |
| | | *Sarcoglanis simplex 2* | ANSP180021 | 2201 | 3,473,869 | 2,009,103 | 497,245 | 70,173,159 | 1,400 | 141 | 868 |
| | *Stauroglanis* | *Stauroglanis gouldingi* | LBP3159 | 19301 | 2,089,313 | 2,095,335 | 13,507 | 2,873,730 | 1,359 | 213 | 522 |
| Tridentinae | *Potamoglanis* | *Potamoglanis hasemani* | LBP4483 | 24450 | 627,557 | 2,062,904 | 4,592 | 775,094 | 1,002 | 169 | 125 |
| | *Tridensimilis* | *Tridensimilis brevis* | LBP13940 | 13940 | 701,445 | 1,340,964 | 3,847 | 762,926 | 995 | 198 | 140 |
| | *Tridens* | *Tridens sp 2 madeira* | LBP12070 | 12070 | 943,854 | 1,809,411 | 6,255 | 1,163,687 | 1,087 | 186 | 188 |
| | *Tridentopsis* | *Tridentopsis pearsoni* | LBP13944 | 13944 | 1,111,153 | 2,120,882 | 15,931 | 4,433,203 | 995 | 278 | 222 |
| Stegophilinae | *Acanthopoma* | *Acanthopoma annectens* | ANSP181146 | 890 | 1,057,893 | 2,038,992 | 13,587 | 3,300,709 | 1,146 | 243 | 211 |
| | *Haemomaster* | *Haemomaster venezuelae* | INPA43789 | 10704 | 304,176 | 477,062 | 40,028 | 5,376,855 | 776 | 134 | 76 |
| | *Homodiaetus* | *Homodiaetus anisitsi* | LBP13194 | 55163 | 764,449 | 2,365,757 | 10,183 | 2,681,007 | 1,017 | 263 | 152 |
| | | *Homodiaetus passarellii* | LBP2502 | 16505 | 2,299,067 | 3,084,996 | 40,593 | 7,122,989 | 1,197 | 175 | 459 |
| | *Henonemus* | *Henonemus intermedius* | LBP2394 | 16454 | 1,454,620 | 4,056,525 | 187,224 | 26,300,367 | 1,314 | 140 | 363 |
| | | *Henonemus punctatus* | LBP4146 | 23679 | 927,559 | 1,340,525 | 10,057 | 2,450,397 | 1,100 | 244 | 185 |
| | | *Henonemus sp 1* | ANSP197334 | 12772 | 685,221 | 860,615 | 84,521 | 12,202,197 | 1,215 | 144 | 171 |
| | *Megalocentor* | *Megalocentor echthrus* | ANSP199997 | 4732 | 562,676 | 2,807,759 | 7,201 | 2,050,417 | 1,042 | 285 | 112 |

| Subfamily | Genus | Specie | Catalogue number | Voucher | Raw reads | Number of trimmed reads | contigs assembled | total bp Contigs | UCE contigs | mean length | mean coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Pareiodon* | *Pareiodon microps* | ANSP191783 | 4707 | 1,370,195 | 1,515,489 | 201,547 | 27,317,226 | 1,206 | 136 | 342 |
| | *Pseudostegophilus* | *Pseudostegophilus haemomyzon* | ANSP198977 | 13134 | 1,518,665 | 1,327,537 | 211,852 | 28,697,332 | 1,219 | 135 | 379 |
| | | *Pseudostegophilus nemurus* | LBP1581 | 11769 | 1,222,563 | 2,230,258 | 13,105 | 3,046,401 | 1,172 | 232 | 244 |
| | | *Pseudostegophilus paulensis* | LBP6738 | 6738 | 676,403 | 1,756,399 | 36,342 | 7,420,777 | 632 | 204 | 169 |
| | | *Parastegophilus sp* | LBP5133 | 26235 | 747,154 | 1,136,511 | 37,644 | 6,938,236 | 1,238 | 184 | 186 |
| | *Ochmacanthus* | *Ochmacanthus sp.1* | ANSP191769 | 4748 | 343,894 | 3,892,538 | 35,355 | 6,147,496 | 1,151 | 174 | 85 |
| | | *Ochmacanthus sp. 2* | LBP4351 | 24088 | 261,461 | 3,235,129 | 19,900 | 4,090,599 | 1,146 | 206 | 65 |
| | | *Ochmacanthus sp.3* | ANSP197613 | 11339 | 474,056 | 1,197,170 | 37,744 | 6,602,644 | 1,167 | 175 | 118 |
| | | *Ochmacanthus reinhardti* | LBP10987 | 50481 | 1,598,114 | 2,820,795 | 21,066 | 4,207,716 | 1,111 | 200 | 319 |
| | *Stegophilus* | *Stegophilus panzeri* | LBP8619 | 43462 | 80,458 | 2,566,246 | 9,798 | 1,831,814 | 874 | 187 | 20 |
| Vandelliinae | *Paracanthopoma* | *Paracanthopoma* cf *parva 1* | LBP2498 | 15495 | 884,100 | 2,756,814 | 10,063 | 2,687,698 | 1,056 | 267 | 176 |
| | | *Paracanthopoma cf parva 2* | ANSP199868 | 4948 | 891,343 | 652,418 | 119,528 | 16,755,661 | 1,058 | 140 | 222 |
| | | *Paracanthopoma cf parva 3* | ANSP198315 | 12408 | 702,217 | 1,088,389 | 86,406 | 11,695,581 | 862 | 135 | 175 |
| | | *Paracanthopoma cf parva 4* | LBP5245 | 26466 | 1,292,400 | 819,318 | 201,315 | 27,209,497 | 1,008 | 135 | 323 |
| | *Vandellia* | *Vandellia beccarii* | LBP10155 | 47557 | 2,254,971 | 4,203,329 | 360,078 | 49,481,006 | 1,007 | 137 | 563 |
| | | *Vandellia* cf *cirrhosa 1* | ANSP191329 | 3936 | 248,802 | 1,131,701 | 55,199 | 8,654,328 | 977 | 157 | 62 |
| | | *Vandellia* cf *cirrhosa 2* | LBP5342 | 26973 | 1,177,681 | 2,201,698 | 199,875 | 26,894,537 | 931 | 135 | 294 |
| | | *Vandellia* cf *cirrhosa 3* | ANSP197186 | 11246 | 621,096 | 1,131,701 | 55,199 | 8,654,328 | 977 | 157 | 155 |
| | | *Vandellia* cf *cirrhosa 4* | LBP2477 | 16450 | 1,915,987 | 3,582,938 | 302,433 | 41,269,147 | 957 | 136 | 478 |
| | | *Vandellia sanguinea* | LBP15876 | 66164 | 1,057,925 | 2,030,908 | 11,004 | 2,933,690 | 981 | 267 | 211 |
| | | *Vandellia* cf *sanguinea* | LBP2477 | 16449 | 1,363,141 | 2,509,744 | 213,645 | 28,773,741 | 928 | 135 | 340 |
| | | *Vandellia sp.1* | LBP1631 | 11765 | 969,340 | 1,799,338 | 152,915 | 20,695,816 | 906 | 135 | 242 |
| | | *Vandellia sp.2* | LBP14854 | 57887 | 618,112 | 1,187,003 | 7,731 | 2,165,604 | 895 | 280 | 123 |
| Trichomycteridae | *Bullockia* | *Bullockia maldonadoi* | LBP3112 | 3112 | 37,904 | 657,831 | 22,292 | 5,860,066 | 559 | 9 | 263 |
| | | *Bullockia maldonadoi* | LBP3112 | 19793 | 37,904 | 2,649,930 | 22,292 | 5,860,066 | 1,430 | 263 | 9 |
| | *Eremophilus* | *Eremophilus mutisii* | ANSP11306 | no voucher | 740,262 | 2,716,966 | 19,198 | 4,061,518 | 1,268 | 212 | 148 |
| | *Ituglanis* | *Ituglanis amazonicus* | LBP2442 | 16211 | 1,662,540 | 525,319 | 6,238 | 1,459,121 | 1,465 | 234 | 332 |
| | | *Ituglanis* cf *amazonicus* | LBP11003 | 50532 | 470,105 | 1,761,924 | 29,591 | 6,578,231 | 1,424 | 222 | 117 |
| | | *Ituglanis boitata* | LBP14546 | 60865 | 489,305 | 451,062 | 11,278 | 3,684,050 | 1,409 | 327 | 97 |
| | | *Ituglanis eichorniarum* | LBP4686 | 24825 | 563,365 | 848,287 | 5,927 | 1,326,752 | 1,350 | 224 | 140 |
| | | *Ituglanis* cf *eichorniarum* | LBP10777 | 49859 | 666,096 | 1,597,887 | 4,401 | 944,765 | 1,291 | 215 | 140 |
| | | *Ituglanis goya* | LBP17137 | 68599 | 568,860 | 1,139,418 | 4,007 | 810,916 | 1,226 | 202 | 120 |
| | | *Ituglanis* cf *goya* | LBP17131 | 68592 | 797,657 | 1,530,163 | 5,061 | 1,008,900 | 1,362 | 199 | 159 |

| Subfamily | Genus | Specie | Catalogue number | Voucher | Raw reads | Number of trimmed reads | contigs assembled | total bp Contigs | UCE contigs | mean length | mean coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Ituglanis herberti* | LBP676 | 8028 | 995,900 | 1,667,541 | 8,437 | 1,906,700 | 1,453 | 226 | 248 |
| | | *Ituglanis parahybae* | LBP10730 | 49719 | 291,808 | 1,648,909 | 21,252 | 4,881,748 | 1,387 | 230 | 72 |
| | | *Ituglanis parkoi* | LBP14153 | 59188 | 2,135,206 | 1,023,343 | 7,504 | 1,750,612 | 1,517 | 233 | 427 |
| | | *Ituglanis* cf *parkoi* | LBP7995 | 37376 | 862,848 | 631,989 | 8,102 | 1,807,925 | 1,469 | 223 | 215 |
| | | *Ituglanis ramiroi* | LBP | 63261 | 6,585,555 | 1,275,546 | 15,010 | 4,532,530 | 1,384 | 301 | 131 |
| | | *Ituglanis sp.1* | LBP19465 | 77969 | 985,292 | 1,885,919 | 6,719 | 1,294,185 | 1,406 | 193 | 197 |
| | | *Ituglanis sp. 2* | LBP16129 | 66849 | 772,515 | 1,278,393 | 4,817 | 1,034,808 | 1,317 | 215 | 154 |
| | | *Ituglanis sp 3* | LBP12960 | 55688 | 563,051 | 1,089,983 | 13,944 | 4,064,166 | 1,363 | 291 | 112 |
| | | *Ituglanis sp 4* | LBP7667 | 36475 | 782,805 | 1,515,489 | 5,075 | 1,143,554 | 1,250 | 255 | 156 |
| | | *Ituglanis sp 5* | INPA11584 | 11584 | 958,916 | 1,229,746 | 114,665 | 15,968,864 | 1,221 | 139 | 239 |
| | | *Ituglanis sp ribeira* | LBP7416 | 35678 | 1,661,673 | 1,083,727 | 134,999 | 19,823,318 | 1,506 | 147 | 415 |
| | *Trichomycterus* | *Trichomycterus albinotatus* | LBP6326 | 29802 | 365,885 | 674,307 | 51,856 | 8,339,980 | 1,331 | 161 | 91 |
| | | *Trichomycterus alternatus* | LBP1014 | 10261 | 603,061 | 4,039,139 | 22,807 | 5,264,756 | 1,338 | 231 | 120 |
| | | *Trichomycterus areolatus* | LBP3118 | 19819 | 1,490,115 | 1,205,504 | 10,271 | 2,014,622 | 1,390 | 196 | 298 |
| | | *Trichomycterus areolatus 1* | LBP3113 | 3113 | 1,443,507 | 1,839,591 | 23,356 | 5,270,323 | 1,315 | 226 | 288 |
| | | *Trichomycterus areolatus 2* | LBP3118 | 19820 | 590,467 | 2,610,414 | 56,204 | 10,115,138 | 1,488 | 180 | 147 |
| | | *Trichomycterus cf auroguttatus* | LBP8374 | 40446 | 1,655,287 | 2,040,601 | 13,795 | 2,590,739 | 1,415 | 188 | 331 |
| | | *Trichomycterus balios* | LBP607 | 7358 | 1,561,481 | 2,873,117 | 148,164 | 24,047,770 | 658 | 162 | 390 |
| | | *Trichomycterus brasiliensis* | LBP10675 | 49540 | 442,866 | 4,995,362 | 76,645 | 11,272,069 | 1,371 | 147 | 110 |
| | | *Trichomycterus cf brasiliensis* | LBP10276 | 47976 | 899,050 | 1,483,970 | 72,163 | 12,871,185 | 1,575 | 178 | 224 |
| | | *Trichomycterus cf brasiliensis 1* | LBP8060 | 37829 | 803,865 | 1,479,875 | 65,695 | 11,761,674 | 1,579 | 179 | 200 |
| | | *Trichomycterus cf brasiliensis* | LBP6247 | 29199 | 754,397 | 1,390,876 | 61,606 | 11,320,545 | 1,446 | 184 | 188 |
| | | *Trichomycterus banneaui* | LBP19847 | 77973 | 694,605 | 2,686,894 | 17,098 | 5,167,636 | 1,502 | 302 | 138 |
| | | *Trichomycterus cf banneaui* | LBP19537 | 77971 | 1,333,632 | 674,307 | 197,022 | 28,482,105 | 1,507 | 145 | 333 |
| | | *Trichomycterus cachirensis* | LBP19832 | 77957 | 1,680,434 | 1,916,448 | 17,427 | 2,959,710 | 1,470 | 170 | 336 |
| | | *Trichomycterus candidus* | LBP11630 | 58011 | 452,601 | 1,089,983 | 43,481 | 7,975,089 | 1,434 | 183 | 113 |
| | | *Trichomycterus chiltoni* | ANSP180474 | 940 | 382,924 | 2,058,398 | 36,678 | 6,703,674 | 1,319 | 183 | 95 |
| | | *Trichomycterus cubataonis* | LBP3123 | 19690 | 1,078,378 | 2,040,601 | 11,082 | 2,100,468 | 1,305 | 190 | 215 |
| | | *Trichomycterus davisi* | LBP6383 | 29744 | 669,117 | 1,275,546 | 53,699 | 10,271,566 | 1,541 | 191 | 167 |
| | | *Trichomycterus davisi_iguaçu* | LBP1222 | 10598 | 1,103,289 | 2,991,667 | 10,731 | 2,058,015 | 1,334 | 192 | 220 |
| | | *Trichomycterus cf. guianensis* | ANSP179111 | 918 | 1,087,515 | 2,488,653 | 138,447 | 20,834,370 | 973 | 150 | 271 |
| | | *Trichomycterus guianensis* | LBP17444 | 69015 | 715,143 | 1,484,105 | 56,432 | 10,519,907 | 1,494 | 186 | 178 |

| Subfamily | Genus | Specie | Catalogue number | Voucher | Raw reads | Number of trimmed reads | contigs assembled | total bp Contigs | UCE contigs | mean length | mean coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Trichomycterus inheringi* | LBP4512 | 24563 | 1,117,378 | 2,058,795 | 85,341 | 15,013,811 | 1,573 | 176 | 279 |
| | | *Trichomycterus immaculatus* | LBP8351 | 40419 | 1,089,097 | 1,479,875 | 15,530 | 2,584,142 | 1,323 | 166 | 217 |
| | | *Trichomycterus itatiaye* | LBP16356 | 62282 | 1,402,440 | 3,161,125 | 144,521 | 22,564,878 | 1,561 | 156 | 350 |
| | | *Trichomycterus cf knerii* | LBP18717 | 18717 | 1,690,435 | 1,885,919 | 15,816 | 2,785,265 | 1,504 | 176 | 338 |
| | | *Trichomycterus mimosensis* | LBP8290 | 38358 | 774,427 | 1,390,876 | 16,978 | 2,784,389 | 1,395 | 164 | 154 |
| | | *Trichomycterus nigroauratus* | LBP6301 | 29341 | 1,106,475 | 2,261,658 | 66,895 | 12,587,528 | 1,503 | 188 | 276 |
| | | *Trichomycterus cf oroyae* | LBP3255 | 3255 | 1,554,984 | 1,603,577 | 31,694 | 6,480,863 | 1,345 | 204 | 310 |
| | | *Trichomycterus pauciradiatus* | LBP16323 | 61977 | 1,224,740 | 2,328,959 | 35,791 | 8,160,897 | 1,468 | 228 | 244 |
| | | *Trichomycterus pradensis* | LBP8291 | 38359 | 1,357,778 | 1,479,875 | 15,530 | 2,584,142 | 1,323 | 166 | 271 |
| | | *Trichomycterus pirabitira* | LBP18381 | 72641 | 861,240 | 2,366,092 | 298,816 | 42,003,062 | 1,634 | 141 | 215 |
| | | *Trichomycterus piratymbara* | LBP9004 | 42138 | 1,377,890 | 2,924,535 | 157,282 | 23,928,325 | 1,575 | 152 | 344 |
| | | *Trichomycterus perkos* | LBP17033 | 66403 | 1,302,643 | 928,397 | 23,360 | 6,411,312 | 1,493 | 274 | 271 |
| | | *Trichomycterus poikilos* | LBP14693 | 61154 | 1,231,080 | 4,203,329 | 21,414 | 5,771,094 | 1,496 | 270 | 246 |
| | | *Trichomycterus punctulatus* | ANSP180733 | 905 | 2,164,904 | 2,328,959 | 284,126 | 39,327,526 | 1,566 | 138 | 541 |
| | | *Trichomycterus quechuorum* | ANSP180572 | 912 | 1,464,625 | 1,530,163 | 156,790 | 23,691,896 | 1,565 | 151 | 366 |
| | | *Trichomycterus sp 1 rivasi* | ANSP191472 | | 1,142,982 | 2,128,871 | 21,430 | 3,818,842 | 1,575 | 178 | 285 |
| | | *Trichomycterus reinhardti* | LBP16302 | 61942 | 2,510,997 | 2,366,092 | 273,480 | 40,001,257 | 1,609 | 146 | 627 |
| | | *Trichomycterus ruitoquensis* | LBP19838 | 77956 | 1,305,363 | 1,139,418 | 9,139 | 1,957,364 | 1,474 | 214 | 261 |
| | | *Trichomycterus sandovali* | LBP19833 | 77946 | 1,536,372 | 1,532,438 | 15,932 | 2,732,428 | 1,467 | 172 | 307 |
| | | *Trichomycterus septemradiatus* | LBP6550 | 31670 | 1,211,748 | 2,499,669 | 113,381 | 17,892,589 | 1,524 | 158 | 302 |
| | | *Trichomycterus* cf *septemradiatus* | LBP5939 | 28065 | 1,985,598 | 3,414,540 | 223,057 | 33,163,841 | 1,625 | 149 | 496 |
| | | *Trichomycterus aff spilosoma* | LBP19339 | 77975 | 1,700,107 | 1,848,568 | 9,183 | 1,914,819 | 1,541 | 209 | 340 |
| | | *Trichomycterus sp. 1* | LBP19834 | 77958 | 1,190,324 | 2,261,658 | 9,300 | 1,913,990 | 1,436 | 206 | 238 |
| | | *Trichomycterus sp. 2* | LBP7674 | 36488 | 711,735 | 1,371,402 | 3,846 | 878,124 | 1,169 | 228 | 142 |
| | | *Trichomycterus sp. 3* | LBP7669 | 36484 | 847,450 | 1,610,059 | 4,107 | 945,660 | 1,220 | 230 | 169 |
| | | *Trichomycterus sp. 4* | LBP10236 | 47789 | 938,905 | 1,820,364 | 4,526 | 1,034,616 | 1,286 | 228 | 187 |
| | | *Trichomycterus sp. 5* | LBP7640 | 36427 | 907,045 | 1,756,399 | 4,523 | 1,083,550 | 1,297 | 239 | 181 |
| | | *Trichomycterus sp. 6* | LBP11648 | 58088 | 22,586 | 2,509,744 | 4,004 | 529,410 | 238 | 132 | 5 |
| | | *Trichomycterus sp.7* | LBP11631 | 58082 | 353,564 | 3,687,832 | 52,727 | 8,108,585 | 1,293 | 154 | 88 |
| | | *Trichomycterus sp. 8* | LBP9001 | 42123 | 598,743 | 1,839,831 | 24,277 | 5,662,529 | 1,464 | 233 | 119 |

| Subfamily | Genus | Specie | Catalogue number | Voucher | Raw reads | Number of trimmed reads | contigs assembled | total bp Contigs | UCE contigs | mean length | mean coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Trichomycterus sp N grande* | LBP10282 | 47992 | 1,132,526 | 2,120,882 | 157,478 | 23,398,836 | 1,495 | 149 | 283 |
| | | *Trichomycterus sp. SF* | LBP11842 | 58147 | 1,851,805 | 2,201,698 | 247,635 | 35,742,865 | 1,583 | 144 | 462 |
| | | *Trichomycterus sp. Novo* | LBP8563 | 43332 | 996,707 | 1,131,701 | 4,901 | 1,080,687 | 1,393 | 221 | 199 |
| | | *Trichomycterus starwiarski* | LBP16165 | 66905 | 1,130,993 | 2,162,482 | 10,760 | 2,059,180 | 1,352 | 191 | 226 |
| | | *Trichomycterus striatus* | LBP19846 | 77968 | 999,887 | 1,892,886 | 9,006 | 1,887,141 | 1,360 | 210 | 199 |
| | | *Trichomycterus sp. Santa Catarina* | LBP3121 | 19686 | 1,005,828 | 2,087,560 | 72,374 | 13,237,795 | 1,535 | 183 | 251 |
| | | *Trichomycterus trasandianus* | LBP19845 | 77964 | 870,996 | 1,603,577 | 131,483 | 19,142,491 | 1,427 | 146 | 217 |
| | | *Trichomycterus zonatus* | LBP2653 | 17409 | 784,231 | 2,873,117 | 19,519 | 5,719,428 | 1,416 | 293 | 156 |
| | *Scleronema* | *Scleronema minutum* | LBP3310 | 19841 | 653,668 | 822,056 | 44,453 | 8,408,115 | 1,400 | 189 | 163 |
| Nematogenyidae | *Nematogenys* | *Nematogenys inermis* | LBP3105 | 3105 | 2,317,481 | 4,441,190 | 27,426 | 6,955,648 | 1,797 | 254 | 463 |
| Callichthyidae | *Corydoras* | *Corydoras elegans* | LBP57701 | 57701 | 986,424 | 1,833,170 | 106,632 | 18,265,913 | 1,537 | 171 | 246 |
| | | *Corydoras gossei* | LBP544 | 7171 | 1,004,325 | 1,921,329 | 55,169 | 9,062,728 | 1,268 | 164 | 200 |
| | *Hoplosternum* | *Hoplosternum littorale* | LBP466 | 7282 | 1,361,537 | 2,602,840 | 23,589 | 6,928,099 | 1,668 | 294 | 272 |
| Scoloplacidae | *Scoloplax* | *Scoloplax dicra* | LBP11001 | 50522 | 1,914,123 | 3,626,939 | 22,777 | 5,650,579 | 1,215 | 248 | 382 |
| | | *Scoloplax distolothrix* | LBP1938 | 14340 | 760,101 | 1,377,449 | 81,451 | 12,292,952 | 1,164 | 151 | 190 |
| Loricaridae | *Farlowella* | *Farlowella oxyrryncha* | LBP1558 | 11509 | 1,270,154 | 2,441,790 | 26,904 | 6,737,612 | 1,523 | 250 | 254 |
| | *Lamontichthys* | *Lamontichthys filamentosus* | LBP162 | 4093 | 829,123 | 1,596,203 | 15,479 | 4,780,201 | 1,554 | 309 | 165 |
| | *Lasiancistrus* | *Lasiancistrus saetiger* | LBP9156 | 42517 | 1,214,762 | 2,248,118 | 95,886 | 16,016,903 | 1,731 | 167 | 303 |
| Astroblepidae | *Astroblepus* | *Astroblepus grixalvii* | ANSP188920 | 188920 | 676,307 | 1,227,559 | 61,155 | 8,675,650 | 1,008 | 142 | 169 |
| | | *Astroblepus sp* | LBP19844 | 77952 | 854,404 | 2,309,522 | 13,905 | 3,809,942 | 1,436 | 274 | 170 |
| Characidae | *Leporinus* | *Leporinus striatus* | LBP3180 | 16871 | 3,820,230 | 3,742,443 | 31,451 | 5,073,864 | 1,397 | 161 | |
| | | *Total* | | | 176,327,536 | 290,552,635 | 9,114,586 | 1,462,651,710 | 187,372 | 28130 | |
| | | *average* | | | 1,216,052 | 2,003,811 | 62,859 | 10,087,253 | 1,292 | 194 | |

**Table 1.3**. Estimated speciation and extinction rates (with 95%CI) for selected crown nodes in Trichomycteridae based on BAMM analysis.

| Clade | Speciation | | Extinction | |
|---|---|---|---|---|
| | **mean** | **5-95%CI** | **mean** | **95% CI** |
| Trichomycteridae | 0.131 | 0.105-0.164 | 0.041 | 0.009-0.088 |
| TSVGS | 0.085 | 0.058-0.124 | 0.038 | 0.003-0.098 |
| Trichomycterinae | 0.214 | 0.171-0.268 | 0.045 | 0.004-0.123 |
| Copionodontinae+Trichogeninae | 0.087 | 0.055-0.141 | 0.045 | 0.003-0.125 |

**Table 1.4**. AIC results for character model: equal rates (ER), symmetric rates (SYM), all-rates-different (ARD) using six trophic levels.

| | **Equal rates** | **Symetric rates** | **All rates different** |
|---|---|---|---|
| Trophic level | **114.3731** | 116.4464 | 139.7781 |

*"Every species has come into existence coincident both in time and space with a pre-esxiting closely allied species"*

*Alfred Russel Wallace*

# Chapter 2

## A phylogenomic perspective on the historical biogeography of Trichomycteridae inferred from target enrichment of DNA ultraconserved elements.

# A phylogenomic perspective on the historical biogeography of Trichomycteridae inferred from target enrichment of DNA ultraconserved elements

**By**

**Luz Eneida Ochoa Orrego**

## Abstract

The high diversity of freshwater fishes in the Neotropical region reflects its complex geological and biological history. Different biogeographic patterns have been observed in the freshwater ichthyofauna on the Brazilian crystalline shield and the Atlantic coastal drainages, however, the mechanism that have modeled its distribution are poorly understood. Here, we focus on the biogeography history of Trichomycteridae, a primary group of catfishes widely distributed from Costa Rica to Patagonia, on both versants of the Andes. Based in a strong supported phylogeny from 874 ultraconserved elements (UCEs), we estimated a timetree using fossil information and secondary calibrations points to estimate lineage ages. Combining the time-tree and information of the species distribution we reconstructed the historical distribution of lineages using the R module BioGeoBEARS under extinction and cladogenesis (DEC) model and an augmented model including founder effect speciation (DEC+*j*, DIVA+*j* and BayArea+*j*). Biogeographic inference suggests that the most recent common ancestor of the Trichomycteridae dates from Lower Cretaceous and it was widely distributed in the Amazon and Atlantic regions. A combination of global and local palaeogeographical events modeled the biogeographic patterns of Trichomycteridae and are consistent with the establishment of the major drainages of the Neotropics. The distribution of non-monophyletic regional assemblages and species inhabiting more than one basin reveals the crucial role of headwater-capture events in the diversification and biogeographic pattern of this family.

## Keywords

Trichomycterids, South America, freshwater fishes, headwater-capture, evolution.

### *2.1 Introduction*

South America harbors the greatest biodiversity on Earth, containing five of the world's biodiversity 'hot spots' (Bayona *et al.* 2007). The distinct taxonomic composition of the Neotropical ichthyofauna reflects its ancient history of geological and biological isolation, several studies have indicated a long evolutionary history dating back to the final separation of the South American and Africa continents (e.g. Siluriformes and Characiformes) (Lundberg *et al.* 1998; Reis *et al.* 2003). Different hypotheses based on several processes have been proposed to explain the origin of tropical species richness and patterns of distribution in South America (Hubert & Renno 2006). In this way, the diversification processes in the Neotropical freshwater fishes cannot be restricted to a particular time interval or mechanism (Rull 2011), but rather to a succession of major geological events that have modified both continents and oceans (Graham 2009; Cavallotto, Violante & Hernández-Molina 2011; Lavina & Fauth 2011; Folguera *et al.* 2011). A special combination of geological, climatological, biogeographic and extinction processes has modeled the high species richness of the modern aquatic system (Albert, Petry & Reis 2011).

In a paleogeographical context, the most prominent patterns in the biogeography of Neotropical freshwater fishes can be traced to geological and climatic events in the past ca. 100 Myr (Lundberg *et al.* 1998). Geomorphological processes of vicariance and geo-dispersion occurred in this period were the primary forces in the species diversification in the Neotropical region. Climate changes and associated glaciations, as well as dynamic palaeobasins and shifting shorelines due to marine transgressions, have profoundly impacted the continent, creating complex scenarios for species diversification (Colinvaux *et al.* 1996; Antonelli & Sanmartin 2011; Compagnucci 2011). The Cretaceous -Tertiary (K/T) boundary represents a significant transformation in global biodiversity, involving mass extinctions, subsequent adaptive radiations, and a substantial turnover in the taxic composition of regional biotas (e.g., (Alroy 1996; Alegret *et al.* 2002; Hansen, Kelley & Haasl 2004; Kiessling & Baron-Szabo 2004; Lockwood 2004; Roelants *et al.* 2007). The marine incursions and regressions subsequently to K/T event also played a critical role in shaping the Amazonian biota (Bates, Hackett & Cracraft 1998; Lovejoy, Bermingham & Martin 1998; Hubert & Renno 2006; Haffer 2008). The last event of great marine incursion in the Atlantic coastal drainages, prior to the final establishment of the Amazon, was previously dated between 15 and 10 Myr and it was postulated to lead to a 150-m marine high stand (Haq, Hardenbol & Vail 1987; Räsänen *et al.*

1992; Hoorn *et al.* 1996). Sea level fluctuations have been identified as promotors of dispersion between neighboring rivers via delta connections or coastal marshes (Hoorn 1993; Monsch 1998; Hernández *et al.* 2005; Rebata H. *et al.* 2006; Hoorn *et al.* 2010).

Different geological forces affecting the distribution of freshwater fishes in the Neotropics were additionally identified. Ribeiro (2006) reported megadome uplifts and vertical movements between rifted block and the erosive retreat of the South American eastern continental margin. This process gave rise to taphrogenic basins that captured surrounding headwaters and originated a complex hydrography. As consequence of these processes, three different biogeographic patterns in the freshwater fauna can be identified: Pattern A, associated mainly with the cladogenetic events at familial or subfamilial level; Pattern B, involving sister-group relationships between genera; and Pattern C, in the species level, which echoes the most recent vicariant events between the upland crystalline shield rivers and the adjacent coastal drainages.

One of the paramount episodes of the geological history of South America was the Andean uplift in the late Oligocene to early Miocene (about 23 Ma) (Hoorn *et al.* 2010). This event had a profound impact in the hydrological history of the continent, it changed the Amazonian landscape by the rearrangement of its drainage patterns and the creation of a vast influx of sediments into the basin. For instance, the Quechua 1 and 2 orogenies, for example, produced large volumes of sediment discharge into the area of the modern Western Amazon, contributing to the formation of the Early to Middle Miocene Pebas Formation, and later to the Late Miocene Acre Formation. More generally, the precipitous rise of the Central and Northern Andes in the middle to late Neogene exerted profound effects on the formation of modern river basins across all of tropical South America (Albert & Reis 2011). The formation of inter-basin arches had exerted pronounced influences on fish biogeography and aquatic habitats. Although many arches rise just a few tens or hundreds of meters above the surrounding landforms, they constrained the watercourses flow for hundreds to thousands of kilometers over millions of years. For example, during the late Miocene (c. 10 Ma) the Vaupes arch in eastern Colombia rose and separed the modern Orinoco and Amazon basins (Cooper *et al.* 1995; Harris & Mix 2002; Rousse *et al.* 2003) at the same time, the high sedimentary processes of the Western Amazon, contributed in breaching of the Purús High and rose of the Fitzcarrald Arch, resulting in drainages compartmentalization in southwestern Amazon during the Pliocene (c. 4 Ma)

(Westaway 2006; Espurt *et al.* 2007). In combination, both uplifts interrupted the ancient flow of the Proto-Amazon river from headwaters in Bolivia, or even further south which had continued unbroken for more than 100 Myr, and resulted in the formation of the modern water-sheds and outflows (Albert & Reis 2011). As consequence of the rise of the Michicola Arch in eastern Bolivia, the headwaters of Proto-Amazon were captured by the nascent of Paraná basin (Lundberg *et al.* 1998).

Over the past decade, several biogeographic studies of different lineages of Neotropical freshwater fishes were performed with the aim of understanding the processes driving the current distribution patterns based in phylogenies (Albert & Carvalho 2011; Lujan *et al.* 2011; Roxo *et al.* 2014; Tagliacollo *et al.* 2015). Nevertheless, detailed and robust phylogenetic hypotheses for most of the South American fishes are still needed in order to achieve a comprehensive picture of the origin and maintenance of Neotropical biodiversity. Most recently, a new phylogenetic hypotheses for the catfish family Trichomycteridae was proposed based in molecular data (Ochoa *et al.* 2017). New insights into the relationships of this diverse group were offered, especially at subfamilial level in which a high correspondence between topology and geographical distribution was detected. Nevertheless, the mechanisms that have modeled these distributions have never been tested. The Trichomycteridae is considered one of the most widely distributed group of primary catfishes, extending from Costa Rica to Patagonia, on both versants of the Andes, and even in a few insular freshwaters environments (de Pinna & Wosiacki 2003; Fernández & Schaefer 2005). In terms of elevational range, trichomycterids are found from rivers and lakes in the Andes at 4500 m asl to lowland rivers of the Atlantic coast of Brazil (Arratia & Menu-Marque 1984; Fernández & Vari 2000; Fernández & Schaefer 2003).

According to Stiassny & Pinna (1994), a common pattern observed in freshwater fishes is the restricted distribution and depauperate number of species in basal taxa in comparison with their sister-groups. This pattern can be observed in the Trichomycteridae, with the basal copionodontines and trichogenines being restricted to drainages of eastern coast of Brazil. The cladogenesis separating both subfamilies from the remaining trichomycterids are of great antiquity dating from 84.66 Myr. Despite of this long evolutionary history, *Trichogenes longipinnis* was considered for many years to be a unique relict, without any congener until the recent description of *Trichogenes claviger* from a high-altitude stream of the rio Itapemirim

system, an isolated Atlantic drainage in the State of Espírito Santo, southeastern Brazil. The sister group of *Trichogenes* is the subfamily Copionodontinae (de Pinna 1992; Bichuette, De Pinna & Trajano 2008; Datovo & Bockmann 2010), other relictual group of fishes, endemic to the Chapada Diamantina, an isolated plateau in the northeastern Brazilian State of Bahia, drained by the headwaters of the rio Paraguaçu and *ca*. 1200 km distant in straight line from the known range of *T. longipinnis*.

In this study, we investigate the biogeographic history of the Trichomycteridae based on a time calibrated phylogeny generated from phylogenomic data. The ancestral geographic distribution on the family is reconstructed with the aim to: 1) determine which geomorphological processes influenced the distribution of trichomycterids species in the context of the major geological and climatic events of South America.

## *2.2 Material and methods*

### *2.2.1 Taxon sampling and Species distribution*

We sampled 132 specimens of the Trichomycteridae family and 11 specimens from the outgroup. The tissues samples and voucher species used in this study are deposited in the collection of Laboratório de Biologia e Genética de Peixes UNESP - Botucatu (LBP) and in the collection of The Academy of Natural Sciences of Drexel University (ANSP). The table 2.1 shows the respective information for each sample used in this study. Species distributions was drawn from revisionary studies (e.g. Reis *et al.* 2003), syntheses of museum specimen data (LBP, MZUSP, ANSP) and online catalog of fishes (Eschmeyer & Fong 2017).

### *2.2.2 Phylogeny construction*

DNA extraction were done from approximately 25 mg of tissue using Qiagen DNeasy Tissue kits following the manufacturer's protocols, and we ran all genomic DNA extractions on an agarose gel to assess quality. We quantified 2µl of each sample using fluorometry (Qubit, Life Technologies). The samples used in the library preparation presented a concentration between 10-40 ng/µl. To library preparation we sheared 1-2µg of DNA to 400-600 bps in length using a Diagenode Bioruptor Standard (UCD 200) with 6-8 cycles of sonication (depending on DNA quality). The DNA libraries from 150 species were prepared using the Nextera (Epicentre Biotechnologies, Inc.) library preparation protocol for solution-based target enrichment following Faircloth et al. (2012) and increasing the number of PCR cycles following the

tagmentation reaction to 20 as recommended by Faircloth et al (2013). We used the Nextera library preparation protocol of *in vitro* transposition followed by PCR to prune the DNA and attach sequencing adapters (Adey *et al.* 2010), then used the Epicentre Nextera kit to prepare transposase-mediated libraries with insert sizes averaging 100 bp (95% CI: 45 bp) following Adey et al. (2010). The libraries were enriched using a probe set developed for application to ostariophysan fishes to generate sequences data for approximately 2500 UCE loci (Hoeksema et al. in prep). We converted the DNA to Illumina sequencing libraries with a slightly modified version of the NEBNext(R) Ultra(TM) DNA Library Prep Kit for Illumina(R). After ligation of sequencing primers, libraries were amplified using KAPA HiFi HotStart ReadyMix (Kapa Biosystems) for 6 cycles using the manufacturer's recommended thermal profile and dual P5 and P7 indexed primers (see Kircher et al. 2012 (doi: 10.1093/nar/gkr771) for primer configuration). After purification with SPRI beads, libraries were quantified with the Quant-iT(TM) Picogreen(R) dsDNA Assay kit (ThermoFisher). We then enriched pools comprising 100 ng each of 8 libraries (800 ng total) using the MYbaits(R) Target Enrichment system (MYcroarray) following manual version 3.0. After capture cleanup, the bead-bound library was resuspended in the recommended solution and amplified for 10 cycles using a universal P5/P7 primer pair and KAPA HiFi reagents. After purification, each captured library pool was quantified with PicoGreen, and combined with all other pools in projected equimolar ratios prior to sequencing. Sequencing was performed across two Illumina HiSeq paired-end 100 bp lanes using v4 chemistry.

We used the software Phyluce v1.5.0 (Faircloth 2016) for the analysis of UCE's. We used the program Illumiprocessor included in the Phyluce software to clean the data from adapter contamination, low quality bases and sequences containing ambiguous bases. Then, we assembled reads and generated consensus contigs for each species using ABySS (version 2.0.2) (Simpson *et al.* 2009) with a kmer value equal to 55. ABySS is the most accurate assembler which runs read-based error correction prior to assembly resulting in more accurate contigs.

Following assembly, we proceeded to identify those contigs that were UCE loci and align species-specific contigs to the set probes/UCEs used for enrichment. These processes were realized with Python program (phyluce_assembly_match_contigs_to_probes.py) integrating LASTZ (Harris 2007) a pairwise aligner to match contigs and UCE loci. During the matching, the program creates a relational data base of matches to UCE loci by taxon. We removed reciprocal and non-reciprocal duplicates UCE loci and created a database of UCE loci recovered. The monolithic FASTA file were used to generate the alignments using MAFFT

(version 7.130b) and we trimmed resulting alignments using the algorithm implemented by the seqcap_align.py script within phyluce; every alignment was cleaned from the locus name using phyluce_align_remove_locus_name_from_nexus_lines and Gblocks. From the trimmed alignments, we created an incomplete matrix with 50, 75, and 85% completeness in order to evaluate the role of missing data in our matrices and tree topology and clade support values. For each matrix we prepared a concatenated alignment in PHYLIP format and every matrix was analyzed using maximum likelihood (ML) algorithm in RAxML v8.2.X (Stamatakis 2014) to compare the topologies with different levels of completeness.

The best-fitting partitioning scheme was obtained using the Bayesian Information Criterion and hcluster search in Partitionfinder v1.1.1 (Lanfear *et al.* 2012) and the best scheme grouping together loci having the same substitution model was used in subsequent analyses. The phylogenetic analysis was performed using maximum likelihood inference in RAxML v. 8 (Stamatakis 2014) with "GTRGAMMA" option. A posteriori bootstrapping analysis were conducted with RAxML's autoMRE tool indicated that trees converged after 50 replicates, we reconciled the best fitting ML tree with the bootstrap replicates. Bayesian inference were performed in ExaBayes version 1.5 (Aberer *et al.* 2014) with 1'000,000 iterations (4 chains; bur-in:25%).

### *2.2.3 Divergence time estimates*

We estimated divergence times of the trichomycterid lineages using a relaxed molecular clock approach implemented in BEAST v 1.8.0. We used a reduced matrix of 90% completeness with a total 66,845 pb. We used three calibration points, one fossils and two second calibrations reported in previous studies: (1) The diversification time of the Siluriformes reported by (Betancur-R, Orti, & Pyron, 2015) of 150 My was implemented in the root with a normal distribution and the search was conducted among the interval of 136.3-163.7 Ma using the lower and upper quantiles of 2.5%, respectively. (2) The second point was in the origin of the Trichomycteridae about 106 million years ago (Ma) as estimated by Betancur-R et al. (2017). We implanted a normally distributed prior with mean of 106 and standard deviation of 7. The search was conducted among the interval of 92.28-119.7 Ma using the lower and upper quantiles of 2.5%, respectively. (3) The third calibration point was implemented using the only known fossil for the family Trichomycteridae described from the Monte Hermoso Formation in Argentina by Bogan & Agbolin (2009). Based on biostratigraphy, (Tomassini *et al.* 2013) estimated the upper and lower boundaries of the Monte

Hermoso Formation to be 4.5/5 and 5.3 Myr, respectively. We implemented a log-normal prior offset and mean of 4.5 Mayr with standard deviation of 1.5 for the origin of the subfamily Trichomycterinae. The search for the second calibration point was conducted within the interval of 4.4-32.13 Myr using the lower and upper quantiles of 2.5%, respectively. The analysis was run for 100 million generations and sampled every 10,000th generation.

### *2.2.4 Biogeographic methods: Ancestral range inference*

To infer the biogeographic history of the Trichomycteridae, species were categorized and coded for presence/absence in six broad geographical areas: (A) Trans-Andean, (B) Orinoco-Guiana, (C) Amazonas, (D) South Pacific drainages, (E) La Plata basin, and (F) Atlantic coastal drainages (Figure 2.1). We divided the palaeogeographic history in three times intervals corresponding to large-scale palaeogeogrpahical events that have influenced the evolutionary history and the distribution range of freshwater fishes (Table 2.2). The area-dispersal matrix was constructed following Tagliacollo et al (2015) using the spatial areas ($km^2$) of the geographical units with modified linear distances between the center middle points (GPS coordinates) of the geographical units (Table 2.3).

Ancestral areas of the Trichomycteridae were estimated using the dispersal-extinction-cladogenesis (DEC) (Ree & Smith 2008) of geographical range evolution. DEC models are composed of two (DEC) or three (DECj) parameters including: 1) dispersal (D), where species expand ancestral ranges by adding new geographical units, 2) extinction (E), where species reduce ancestral ranges by extirpation of geographical units and 3) 'jump' events (j), where j specifies the weight of 'jumping' events beyond and ancestral range (Matzke 2013). We test the six models through of three analytical procedures implemented in the R package BiogeoBears 0.2.1(Matzke 2013): DIVA-like (Ronquist 1997), DEC (Ree *et al.* 2005; Ree & Smith 2008) and BayArea-like (Landis *et al.* 2013) allowed a free variation of the parameters *d* (dispersal) and *e* (extinction) at the branches and a fixed matrix for range variation at cladogenesis. The analyses were compared by Akaike information criterion weights (AICwt) to provide a sense of the relative probability of the models.

## *2.3 Results*

### *2.3.1 Phylogenomic inference of Trichomycteridae family*

We sequenced a total of 176 million reads pairs with a mean of 1,216,559 per sample from 143 taxa, we assembled a mean of 62,859 (95CI, min = 462, max = 497,245) contigs per sample having an average length of 194 pb. The 75% complete matrix was composed by 851 loci having a mean length of 162 bp (2.71 CI) per alignment, totaling 160,440 bp of aligned sequence. We used this incomplete matrix for subsequent analyses with RAxML. Our analysis using UCEs include representatives of the eight subfamilies, 28 genera and 129 species of Trichomycteridae. In the outgroup were included species of the families Nematogenyidae, Callichthyidae, Scoloplacidae, Loricaridae and Astroblepidae as representatives of the superfamily Loricarioidei and the resulting trees were rooted in the species *Leporinus striatus*.

Data set recovered a well-supported phylogeny of the Trichomycteridae with a strong statistical support (e.g., 100% bootstrap support or posterior probabilities of 1.0) and few clades were a low bootstrap values (<80%) (Figure 2.2 and Figure 2.3). Our hypothesis supports the monophyletic status of clade composed by Copionodontinae and Trichogeninae as sister group of the remaining trichomycterids, which includes TSVSG clade and Trichomycterinae. The monophyly of the Copionodontinae, Trichogeninae, Vandelliinae, Stegophilinae, Tridentinae and Trichomycterinae is supported, with the exception of the Sarcoglanidinae, Glanapteryginae, and the putative clade formed by the two subfamilies (= Glanapteryginae-group). Interestingly, this new topology is congruent with the geographic distribution of the involved taxa. The sarcoglanidines and glanapterygines from the Atlantic coastal drainages (*Microcambeva barbata, Listrura camposi, L. picinguabae*, and Trichomycteridae n. gen., referred as Glanapteryginae A + Sarcoglanidinae A) are grouped into one clade, species distributed in the Amazon and Orinoco (*Sarcoglanis simplex, Stauroglanis gouldingi, Typhlobelus guacamaya, Pygidianops slender*, and *P.* sp. denominated Glanapteryginae B + Sarcoglanidinae B) were grouped into a separate lineage with *Potamoglanis hasemani* as sister group (Figure 2.2). Our analysis supports the sister group between Stegophilinae and Vandelliinae and this clade as sister group of Tridentinae. Also, the monophyly of the Trichomycterinae is recovered, as well the two large clades proposed by Ochoa et al (2017), one including all species from Atlantic coastal drainages and Upper Paraná (*Scleronema* and part of *Trichomycterus*; D4+D5) and another including primarily Amazonian and trans-Andean taxa (*Bullockia*, *Eremophilus*, *Ituglanis* and part of *Trichomycterus*; clades D1+D2+E+D3)

(Figure 2.3). However, the clades D1 and D2 are not recovered as monophyletic groups. Different relationships were observed in the clade D1, where the sister clade composes by *Trichomycterus* cf *knerii* and *Trichomycterus punctulatus* is recovered as a differentiated lineage (D1') not related with *Eremophilus mutisii* and *Trichomycterus sandovali*. In the same way, species included in the clade D2 by Ochoa et al (2017) were grouped in three differentiated lineages referred in this study as clade D2 (*Trichomycterus guianensis*, *Trichomycterus* cf. *guianensis*), clade D2' (*Trichomycterus. Trasandianus, Trichomycterus ruitoquensis* and *Trichomycterus spilosoma*) and clade D2'' (*Trichomycterus straminus* and *Trichomycterus banneaui*).

### *2.3.2 Diversification time of Trichomycteridae*

Divergence time estimates suggest a lower Cretaceous origin of the Trichomycteridae with a mean crown age of 107.52 Myr (93.9-120.61, 95% HPD), and the earliest divergence for the family of 66.63 Myr (43.76-90.5, 95% HPD) with the diversification of the Copionodontinae and Trichogeninae during the early Paleocene. The clade C (TSVSG clade plus Trichomycterinae) diversified approximately 41.97 Myr (43.76-90.5, 95% HPD) in the Lower Eocene. Within this clade spltis at subfamily level dates from Paleogene, mainly in the Lower Eocene (41.37 Myr) and Early Oligocene (33.07 Myr). The origin of the Vandelliinae-group (Tridentinae, Stegophilinae and Vandelliinae) was 39.76 Myr and the diversification time between Stegophilinae and Vandelliinae was 37.89 Myr (Figure 2.4).

In a general context, the diversification processes at genus level are relatively recent, dating from Neogene with elevated divergent events in the Miocene extending until Pleistocene. Regarding the Trichomycterinae distinct geographic clades diversified during the middle Miocene, with the earliest to diverge (13.25 Myr) wa represented by *Trichomycterus* sp. n. from Paria peninsula in Venezuela. The subsequent cladogenesis corresponds to the largest dichotomy of trichomycterines and is estimated in 14 Myr. This was closely followed by the clade composed by *Trichomycterus guianensis* and *Trichomycterus* cf *guianensis* (13.01 Myr). The three separate clades consisting of Trans-Andean species diversified between 14.14 to 12.65 Myr. The highest diversity of trichomycterines is concentrated in South and Southeastern Brazil and these lineages diversified diversified during the last 12.86 – 0.52 Myr (Figure 2.4).

### *2.3.3 Ancestral range inference*

The BayArea-like + *j* model was selected as the most appropriate model for the *Trichomycteridae* data (AICwt = 1.0) when compared with the other models implemented in BioGeoBEARS (Table 2.4). In the BayArea model the ancestral ranges are inherited identically following two ways: on the first, every area has an equal rate of colonization or extinction; in the second rates of colonization are distance dependent (Landis *et al.* 2013). The prevalence of BayArea-like + *j* model (*LnL* = -243.8) suggests an important role for dispersion (*d* = 0.038), extinction (*e* = 0.027) and the "founder event" or "jump dispersal event" (Paulay & Meyer 2002; Templeton 2008), according to the values obtained for the free parameter *j* (*j* = 0.16). The founder event is also termed speciation through long-distance dispersal (Head 2012) or allopatric mode II speciation (Wiley 1981; Maguire & Stigall 2008; Lomolino *et al.* 2020). In founder-event speciation, a small number of individuals, sometimes even a single individual, take part in a rare, long-distance colonization event which founds a population which is instantly genetically isolated from the ancestral population (Matzke 2014).

As a general pattern, the Amazon (C) and Atlantic drainages (F) are the most likely ancestral areas for most ancient trichomycterid node (Figure 2.4). The time calibrated phylogeny with optimized ancestral geographical ranges indicates that the most recent common ancestor (MRCA) of the Trichomycteridae was possibly broadly distributed through these two large ichthyofaunal provinces. The origin of the Trichomycteridae dates from the lower Cretaceous (107.52 Myr) and this estimation is in agreement with the divergence times previously proposed for the family (Ochoa *et al.* 2017). The clade composed by the Trichogeninae and Copionodontinae was the first lineage to diversify (66.63 Myr) during the Paleogene, in the Early Tertiary, a time consistent with the K/T extinction events (ca. 66 Myr). Distribution of MRCA of Trichogeninae and Copionodontinae was likely restricted to the Atlantic coastal drainages (F) (Figure 2.5).

MRCA of the large clade C containing the remaining trichomycterids (TSVSG+Trichomycterinae) possibly had the same distribution than the trichomycterid ancestral, that is, through the Amazon (C) and Atlantic coastal drainages (F). Subsequently, in the TSVSG clade our reconstruction shows that MRCA of Glanapterygiste-group and Vandelliinae-group moved along Orinoco-Guyana (B) during the Paleocene about 35-25 Myr (Figure 2.5). More recently, during the Oligocene some lineages (*Homodiaetus* and *Pseudostegophilus*) dispersed to La Plata basin (E) and the Atlantic region (F). Despite of the

southeastern clade of Glanapetryginae A + Sarcoglanidinae A composes by *Listrura pincinguabae*, *Listrura camposi*, *Microcambeva barbata* and undescribed genus; shared the trichomycterid ancestral, area through the Amazon (C) and Atlantic coastal drainages (F), the reconstruction suggests an ancient dispersal to Atlantic coastal drainages (F) about 30 Myr during the Paleocene.

The ancestor to the group that contains the trichomycterins was present in the Amazon (C) and Trans-Andean (A) drainages. In this group, the early lineages to diversify were dispersing from the ancestral area (AC) to Orinoco-Guyana (B) (Figure 4). Across the Pliocene and Pleistocene, many lineages dispersed to La Plata (E), Atlantic coastal drainages (F) and South Pacific drainages (D). According to the reconstruction, multiples synchronized dispersal events to different regions have occurred in the last 10 Ma. Amazon basin seems to be the most important region in the extension of geographical range in this group due to its historical connection with La Plata and Trans-Andean basin.

## *2.4 Discussion*

### *2.4.1 Phylogenetic relationships of Trichomycteridae*

Data set recovered almost fully resolved trees with different methods of phylogenetic inference. Our hypothesis recovered the monophyletic status of six subfamilies of Trichomycteridae including Trichomycterinae subfamily except Glanapteryginae and Sarcoglanidinae, and it shows a perfect correspondence of the relative position of the early diverging branches with the most recent morphological and molecular hypothesis of the family (Datovo & Bockmann 2010; Ochoa *et al.* 2017), where the clade compose by Copionodontinae and Trichogeninae is the sister group of the clade C (Datovo & Bockmann 2010) clustering the remaining trichomycterids, represented by the TSVSG group (Tridentinae, Stegophilinae, Vandelliinae, Sarcoglanidinae, Glanapteryginae) (Costa & Bockmann 1994) and the lineage clustering the trichomycterines species.

The monophyly of TSVSG clade is very strong supported, as well as it had been proposed in previous morphological (Costa & Bockmann 1994; de Pinna 1998; Datovo & Bockmann 2010) and molecular studies (Fernández & Schaefer 2009). Internal relationship among subfamilies in this clade are partially congruent with the last molecular hypothesis based in mitochondrial and nuclear genes (Ochoa *et al.* 2017). Our hypothesis supports the

Glanapteryginae A + Sarcoglanidinae A group, as the sister clade of the group, clustering Stegophilinae, Vandelliinae, Tridentinae and Glanapteryginae B + Sarcoglanidinae B.

Despite the monophyletic status of Glanapteryginae and Sarcoglanidinae, as well as, the close relationships between both subfamilies have been supported by morphological characters (Costa & Bockmann 1994) many of these are considered of difficult polarization and exhibit a considerable degree of homoplasy (de Pinna, 1998; De Pinna, 1989; de Pinna & Winemiller, 2000). Synapomorphies proposed to define the monophyletic status of Sarcoglanidinae also have been observed in Glanapteryginae (de Pinna & Starnes 1990) with exception of *Listrura* (Costa & Bockmann 1994). Additionally, the sister clade proposed between both subfamilies have been supported by morphological traits that also occurs in other taxa in trichomycteridae as miniaturization, reduction of the opercular-interopercular ondontodes and the number of anal fin rays.

The longstanding controversy in the relationships in Trichomycterinae (Baskin 1973; de Pinna 1989, 1998), began to be elucidated with the support of its monophyletic status by morphological characters (Datovo & Bockmann 2010) and molecules (Ochoa *et al.* 2017).

Our results support the inclusion of *Scleronema* and *Ituglanis* in this subfamily and the exclusion of '*Trichomycterus hasemani'*. Recently, Henschel et al (2017) based in molecular and morphological characters allocated the *'T. hasemani'* group into Tridentinae and elevated a new genus *Potamoglanis* to include *T. hasemani*, *T. johnsoni* and *T. anhanga*. Our results not support the Henschel hypothesis and partially support the relationship proposed by Ochoa et al (2017). Due to, our study only included *T. hasemani*, we consider necessary to analyze more species to future decision at nomenclature level.

Different monophyletic subunits associated mainly with the geographic distribution we recovered in the phylogenetic reconstruction to *Trichomycterus*. The genomic data support the main clades identified in Ochoa et al (Ochoa *et al.* 2017) within Trichomycterinae with a strong node support. Despite of *Trichomycterus* was erected to place the forms lacking those specialization presents in the remaining genera in Trichomycterinae, according to De Pinna (1998) it does not preclude the existence of very large monophyletic subunits within the genus such as it was observed in this hypothesis.

### *2.4.2 Biogeographical signature of river capture events*

Many factors influence species range, including various geological, climatical, ecological and chance events. Both the diversity of factors influencing the geographic range of a species and the uncertainty regarding their relative importance have motivated pursuit of biogeographic inference through parametric methods (Landis *et al.* 2013). The probabilistic models include parameters representing processes thought to impact the geographic distribution of species. here we evaluated several popular models implemented in BioGeoBEARS to determine which model best fits the geographical and phylogenetic data of Trichomycteridae.

Results of biogeographical range analyses provide some clarification of the possible model by which present-day distribution patterns of Trichomycteridae as a whole emerged, with the BayArealike + *j* model being most likely, suggesting that widespread sympatry, dispersal and cladogenesis within overlapping ranges were potentially more significant in the emergence of current-day biogeographical patterns than vicariant events.

Despite divergence in allopatry is a common pattern in almost all species-rich groups of aquatic taxa in Tropical South America (Green, van Veller & Brooks 2002; Puebla 2009), an interesting exception to this pattern is the generally sympatric distribution of closely related species among fishes restricted to the channels of Amazonian rivers (Albert *et al.* 2011). The modern distribution of riverine species could have resulted from sympatric speciation, or perhaps from allopatric speciation with postspeciational range expansion (e.g., Barraclough & Vogler 2000). Deep river channels constitute an exceptional habitat from a biogeographic perspective, in supporting a highly diverse and specialized fauna in a very small spatial area, and also being highly interconnected (Albert *et al.* 2011).

On the other hand, the model indicates that the parameter '*j'* or "jump dispersal event" have a significantly influence in the biogeography pattern of Trichomycteridae. According to Matzke (2014), this parameter refers to a rare long-distance dispersal event that find a new population that is sufficiently genetically isolated that it rapidly becomes a new phylogenetic lineage. In this context, speciation and dispersal events are either literally coincident, or they coincide closely enough that they are reasonably modeled as a joint process in a phylogenetic model. In this case the jump dispersal events modeled by the parameter '*j*' could be related with the river capture events.

The ancestral reconstruction shows a clear signature of river capture events with the presence of non-monophyletic regional species assemblages coupled with the presence of many species inhabiting more than one basin. In the stegophilins clade including *Homodiaetus*, *Henonemus*, *Megalocentor*, *Pareiodon*, *Acanthopoma* and *Pseudostegophilus* possible river capture events between Amazon and La Plata basin can be responsible by the distribution pattern currently observed. The MRCA of genus *Homodiaetus* and other stegophilins was distributed in Orinoco-Guiana and Amazon basin at 31.73 Ma before its dispersion to La Plata and Atlantic drainages. At the same time the Paraguay Basin expanded northward by capture of headwaters of the Proto-Amazonas-Orinoco basin, subsequently the emergence of the Michicola Arch as a surface topographic feature hydrologically separated the area of the modern Upper Paraguay from downstream portions of the Sub-Andean river system, forming a watershed divide between the emerging La Plata and Amazon basins (Carvalho & Albert 2011).

Similar pattern can be observed in *Pseudostegophilus*, its MRCA was widely distributed in the Orinoco-Guiana and Amazon regions and probably its dispersion to La Plata basin could correspond with a stream-capture events identified between the La Plata and adjacent basins in the western Amazon over the last ten million of years (Lundberg *et al.* 1998). Biogeographical studies in *Gymnotus* (Albert & Crampton 2005; Lovejoy *et al.* 2010), *Pseudoplatystoma* (Torrico *et al.* 2009), *Aphyocharax* (Tagliacollo *et al.* 2012), Serranochromis (Musilová *et al.* 2013), Otothyrinae (Roxo *et al.* 2014) and Pimelodidae (Tagliacollo *et al.* 2015) have identified the river capture signature in the distribution of that groups.

On the other hand, the ancestral reconstruction shows that several river capture events have modeled the biogeographical distribution in *Ituglanis* and *Trichomycterus* the most diverse genus of Trichomycterinae, widely distributed in the coastal drainages. In this region, the reactivation of different old faults subsequently to the break-up of Gondwana (Almeida & Carneiro 1998) and the continued erosive retreat processes, led to capture various rivers of the upland crystalline shield, primarily along rift systems, to become Atlantic Ocean tributaries, originating a complex hydrography, sometimes represented by interconnected lake systems, where different portions of adjacent river basins become isolated and merged (Albert & Crampton 2010). This predictable barrier displacement produces complex and reticulated, but also predicable, patterns of taxon–area relationships (Albert & Carvalho 2011; Roxo *et al.* 2014) with non-monophyletic regional (basin-wide) species assemblages coupled with the

presence of many species inhabiting more than one basin as such can be observed in *Ituglanis* and Trichomycterus.

These geological processes would have occurred multiple times during the formation of South-eastern Brazilian shield, giving rise to similar sister-group phylogenetic patterns. Albert and Reis (2011) suggested that between 15 and 28 million years ago several head water captures occurred in the Upper Parana basin and coastal rivers in Tremembé formation and the Ponta Grossa Arch formation (Ribeiro 2006).

### *2.4.3 Influence of vicariance events in the biogeographical distribution of trans-Andean clades*

Although our results show a strong evidence to the river capture events in the distribution patterns of Trichomycteridae, is important highlight the possible influence of vicariance events. In the case of Trichomycterinae, the MRCA was widely distributed and the phylogenetic pattern suggests that vicariant events between Amazon and Trans-Andean region may have influenced the geographical distribution of some clades.

During Early and middle Miocene, the Pebas wetland system almost separated the western cratonic river systems from Andean river systems. To the west, rivers draining the Andes moved into the system (see, e.g., Burgos 2006, Hermoza et al 2005); on its eastern side, it was fed by relative short cratonic river systems (Hoorn 1994a, 2006) and smaller lowland aquatic corridors existed between the Amazon region and the Pacific through the Ecuadorian Andes (Steinmann *et al.* 1999). Subsequently, the Pebas wetland configuration in the north was open to marine settings in the Llanos basin (e.g., Bayona *et al.* 2007). This system was a single interconnected lowland aquatic ecosystem that initially also included the Magdalena Basin (Hoorn et al. 1995; Lundberg et al. 1998; Gómez et al. 2003, 2005).

In this way, the aquatic corridors shaped in the north allowed the dispersal processes of some trans-Andean species of *Trichomycterus*. Tectonic events that interrupted the course of the Sub-Andean river system following the Late Middle Miocene (c. 12–8 Ma) rise of the Eastern Cordillera and Merida Andes in modern Colombia and Venezuela (Cooper *et al.* 1995; Villamil 1999) promoving the diversification in these species. Different uplifts reorganized the whole drainage pattern of northern South America, among other things separating the modern Orinoco and Amazon basins at the Vaupes Arch c. 10 Ma (Hoorn 1994b; Gregory-Wodzicki

2000), resulting in the formation of the modern eastward drainage of the Amazon c. 11 Ma (Dobson, Dickens & Rea 2001; Figueiredo *et al.* 2009).

A particular biogeographic pattern was observed in the clade composed by the *T. aerolatus*, *B. maldonadoi*, *T. chiltoni* and some undetermined species of *Trichomycterus* (*T.* sp novo, *T.* sp 2, *T.* sp 3, *T.* sp 4, *T.* sp 5) from Paraguay basin. The reconstruction shows that cladogenetic events were very recent and can be related with three events: 1) the vicariant effect of the uplift of Andean mountains, 2) the Late Pliocene hydrogeographic changes mainly associate with the Pantanal foreland basin that captured some western tributaries of the upper Paraná and upper Tocantins (Menezes *et al.* 2008) and 3) the sea level changes in the continental shelf in the central region of Chile where prolonged episodes of seawater inundation (marine transgression), and marine regression modified the available habitat with strong reduction of effective populations sizes increasing the levels of genetic isolation, as it was recently evidenced in phylogeography studies of *Trichomycterus areolatus* (Unmack et al. 2009).

In the same way the most recent vicariant events in the upper rio Tietê (the area including the headwaters of the coastal rio Ribeira de Iguape) and the adjacent upland tributaries of the Paraná basin (the Iguaçu and Paranapanema rivers) (Ribeiro 2006) may have influenced the geographical patterns of clades distributed in the southeastern of Brazil. This process promoted a faunal interchange between the coastal drainages and the upland adjacent Paraná and São Francisco, which is evidenced in the frequency of shared species (Bizerril 1994). *Trichomycterus davisi* from upper rio Iguaçu is also reported in the neighboring Ribeira de Iguape and Paranapanema rivers (Ingenito, Duboc & Abilhoa 2004).

The continued erosive retreat of the eastern margin of the platform along the margins of the Serra do Mar escarpments, was also responsible for transferring additional ancestral stocks from upland to lowland river systems, with such groups undergoing subsequent diversification in both upland and coastal drainages (Ribeiro 2006) as it can be observed in the relationships in *Trichomycterus* and *Ituglanis*.

### *2.4.4 Ancestral reconstruction and its correspondence with biogeographic patterns in the eastern of Brazil*

The ancestral reconstruction indicated that the most recent common ancestor of the Trichomycteridae was widely distributed through Amazon and Atlantic drainages. The MRCA

of the Trichogeninae-group was likely confined to the Atlantic coastal region, whereas remaining trichomycterids are widely distributed throughout almost the entire Neotropical area. This finding supports the biogeographic pattern previously identified for the coastal drainages by Stiassny & Pinna (1994) and subsequently defined by Ribeiro (2006) as Pattern A, in which ancient clades exhibits a restricted distribution in the coastal drainages of eastern Brazil and a low species richness when compared to their sister-lineages that typically present highest alpha-diversity and wide geographic distribution. Cladogenetics events of fish fauna from the Brazilian crystalline shield and coastal drainages of eastern Brazil have been associated with tectonics and erosive processes along the continental margin of eastern South America.

The megadome uplifts, rifting, vertical movements between rifted blocks, and the erosive retreat of the South American eastern continental margin were hypothesized as the main geological forces driving the distribution of freshwater fishes in this region (Ribeiro 2006).

Similar biogeographic pattern has been observed in other species where early diversifying lineages restricted to the Atlantic region are sister group of clades widely distributed. For instance, *Wertheimeria* clade is an endemic group distribuited along of the drainages in the eastern coast of Brazil. Recent phylogenetic analysis indicated that this clade is sister group of the much larger and more widely distributed Clade 2 of doradids (Arce H. *et al.* 2013). Similar pattern was also observed in the most ancestral clade of Neoplecostominae in which species of the clade B are almost exclusive to littoral drainages and its sister group or clade C is exclusive to upland rivers (Roxo *et al.* 2012).

Other biogeographic pattern identified by Ribeiro (2006) was at level of sister-groups relationships (Pattern B) between the endemic ichthyofauna of the Brazilian coastal drainages and adjacent shield systems. Within Trichomycteridae, this pattern was represented by *Microcambeva*, with species distributed in the south-eastern and coastal rivers of Brazil. Morphological hypothesis has supported the sister-group of *Microcambeva* with a clade composes by *Malacoglanis* from Caquetá river, Colombia and *Sarcoglanis*, from the Amazonian, Negro river (Costa & Bockmann 1994). However, our phylogenetic hypothesis not recover the sister clade between these lineages, consequently the geographic pattern B seems not to be exemplified by lineages of Trichomycteridae. Different relationships were observed with a high correspondence in the geographical distribution of the species. The

sarcoglanidines and glanapterygines from the Atlantic coastal drainages (*Microcambeva barbata*, *Listrura camposi*, *L. picinguabae*, and Trichomycteridae n. gen) were grouped into one clade. In comparison species of both subfamilies distributed in the Amazon and Orinoco (*Sarcoglanis simplex*, *Stauroglanis gouldingi*, *Typhlobelus guacamaya*, *Pygidianops slender*, and *P*. sp.) weregrouped in a differentiated clade.

Finally, the biogeographical analysis shows as the most recent divergent processes in Trichomycterinae species can be a strong evidence of the pattern C proposed by Ribeiro, which reflects the vicariance events between the upland crystalline shield rivers and the adjacent coastal drainages showing a high frequency of shared species. This study provides the most comprehensive analysis of the phylogenetic relationships of Trichomycteridae to date. We provide a biogeographical context for Trichomycteridae diversification and suggest historical events can aid in explaining phylogeny at different stages of divergence. The biogeographyic pattern observed in Trichomycteridae have been modeled by different geomorphological processes through the time and space. The distributions, considered in the context of the estimated diversification timing of the groups, suggest that the signature of river capture and vicariance events played a role in shaping present distribution of these species, driven by significant geological processes in river system development.

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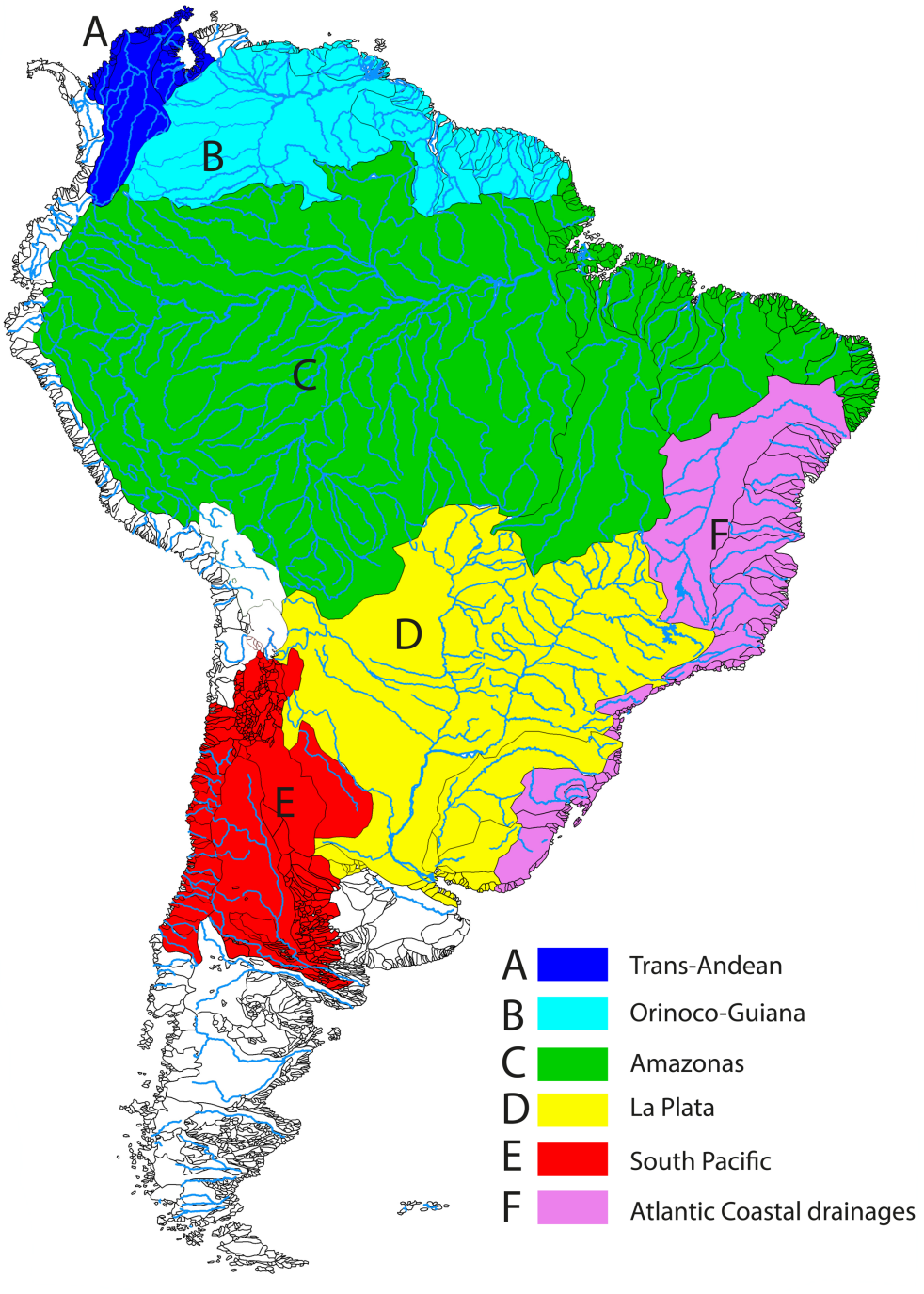


**Figure 2.1.** Map of biogeographical areas defined in this study. A-Trans Andean, B-Orinoco Guiana, C-Amazonas, D-La Plata, E-South Pacific drainages, F-Atlantic coastal drainages.

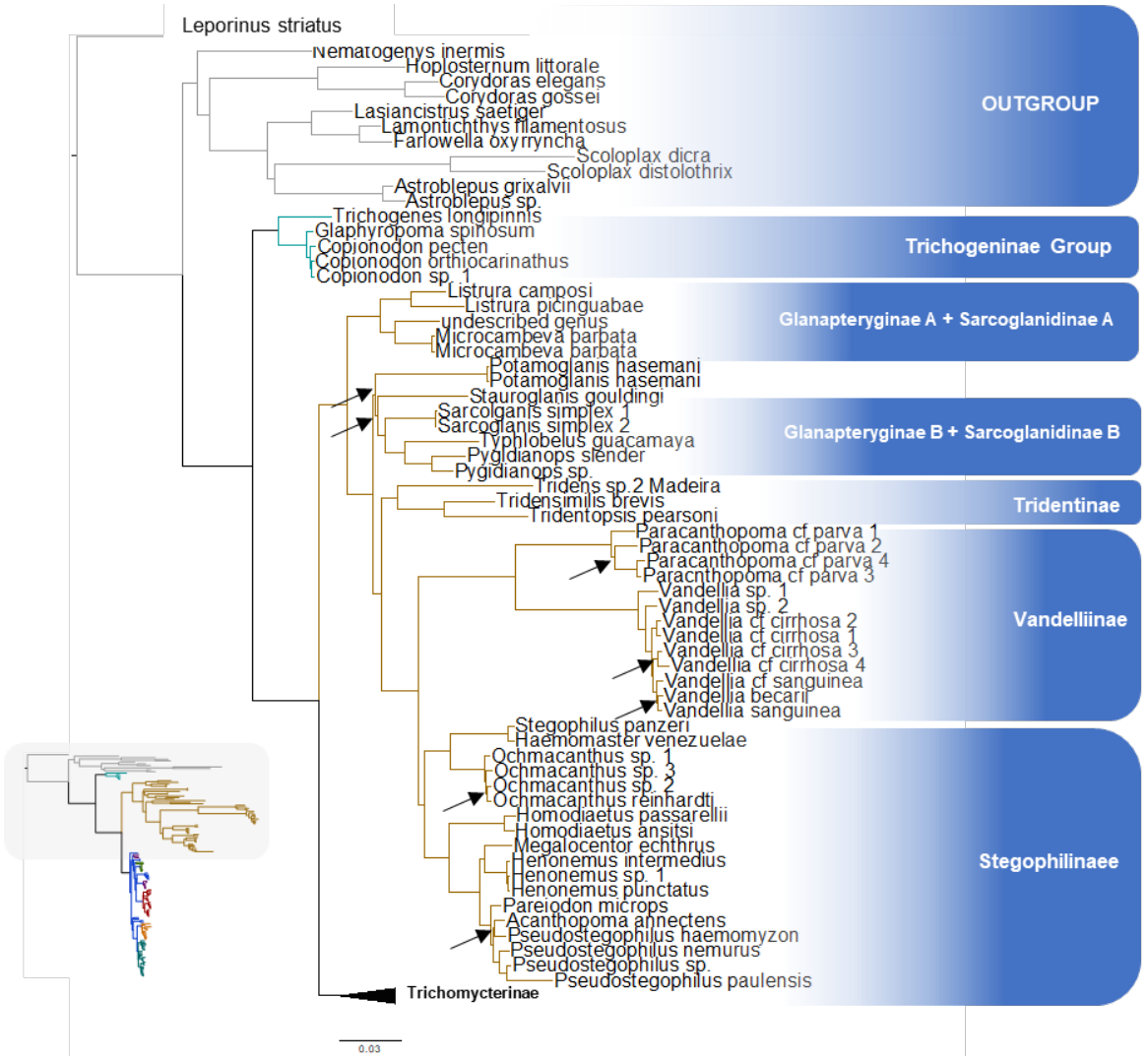


**Figure 2.2**. Maximum-likelihood and Bayesian phylogeny showing the relationships in the TSVSG clade. The posterior probabilities for all nodes were equal to B = 1.0. Bootstrap support values <100% are denoted by black arrows.

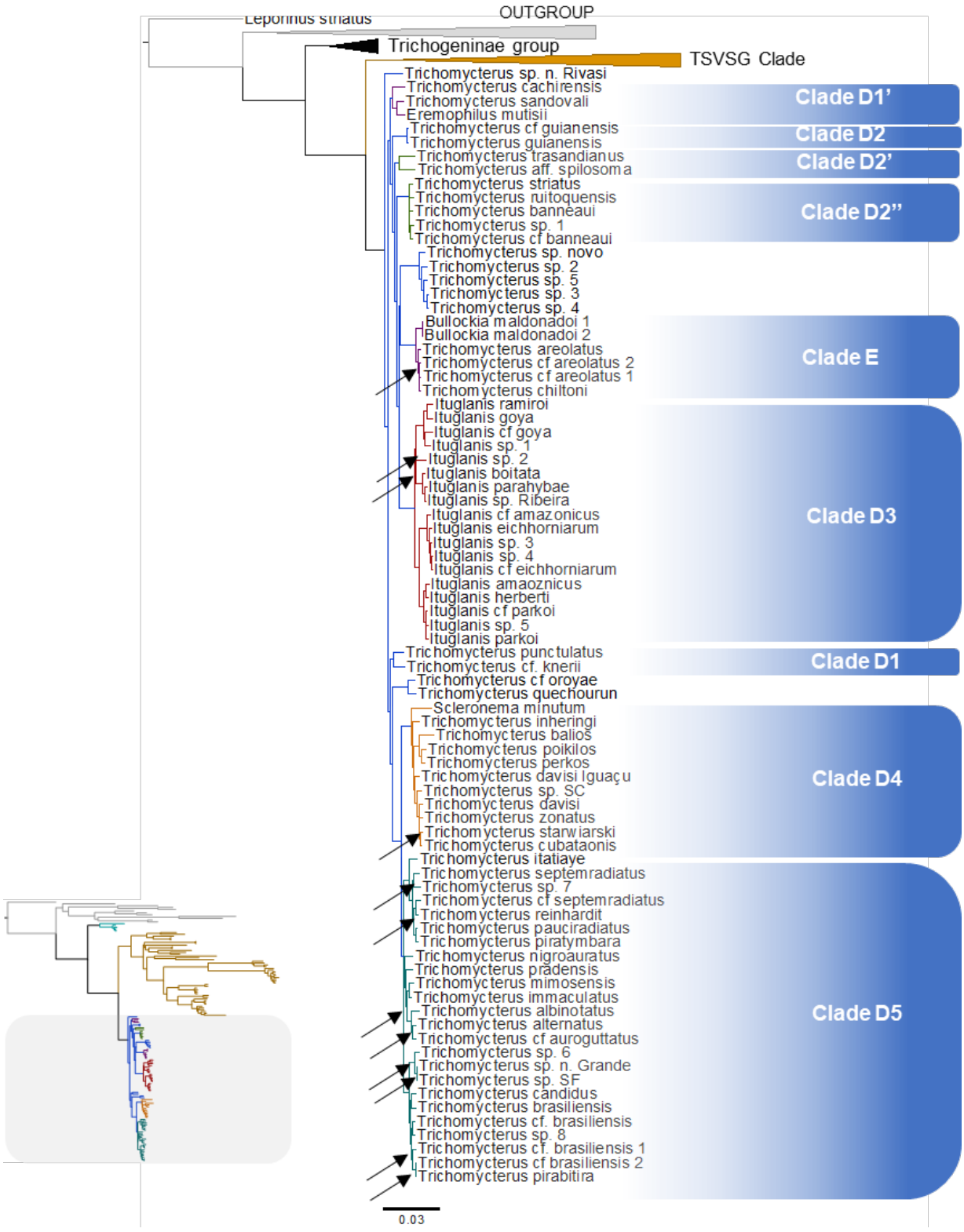


**Figure 2.3.** Maximum-likelihood and Bayesian phylogeny showing the phylogenetic relationships of Trichomycterinae. The posterior probabilities for all nodes were equal to B = 1.0. Bootstrap support values <100% are denoted by black arrows.

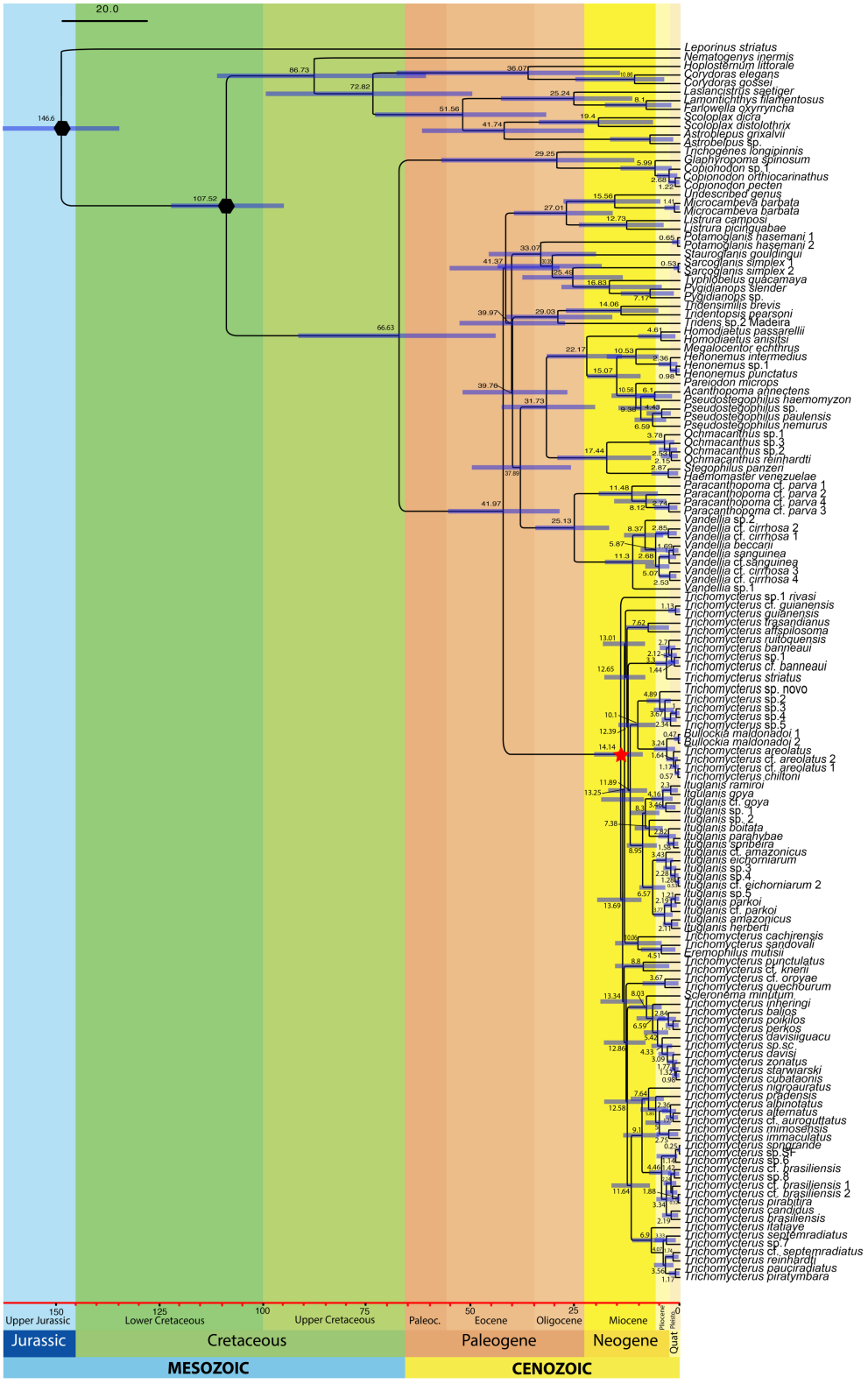


**Figure 2.4**. Time-calibrated phylogeny obtained from BEAST analysis showing mean ages for Trichomycteridae (black numbers close to nodes). Mean divergence time estimates are shown with 95% highest posterior density (HPD; blue bars).

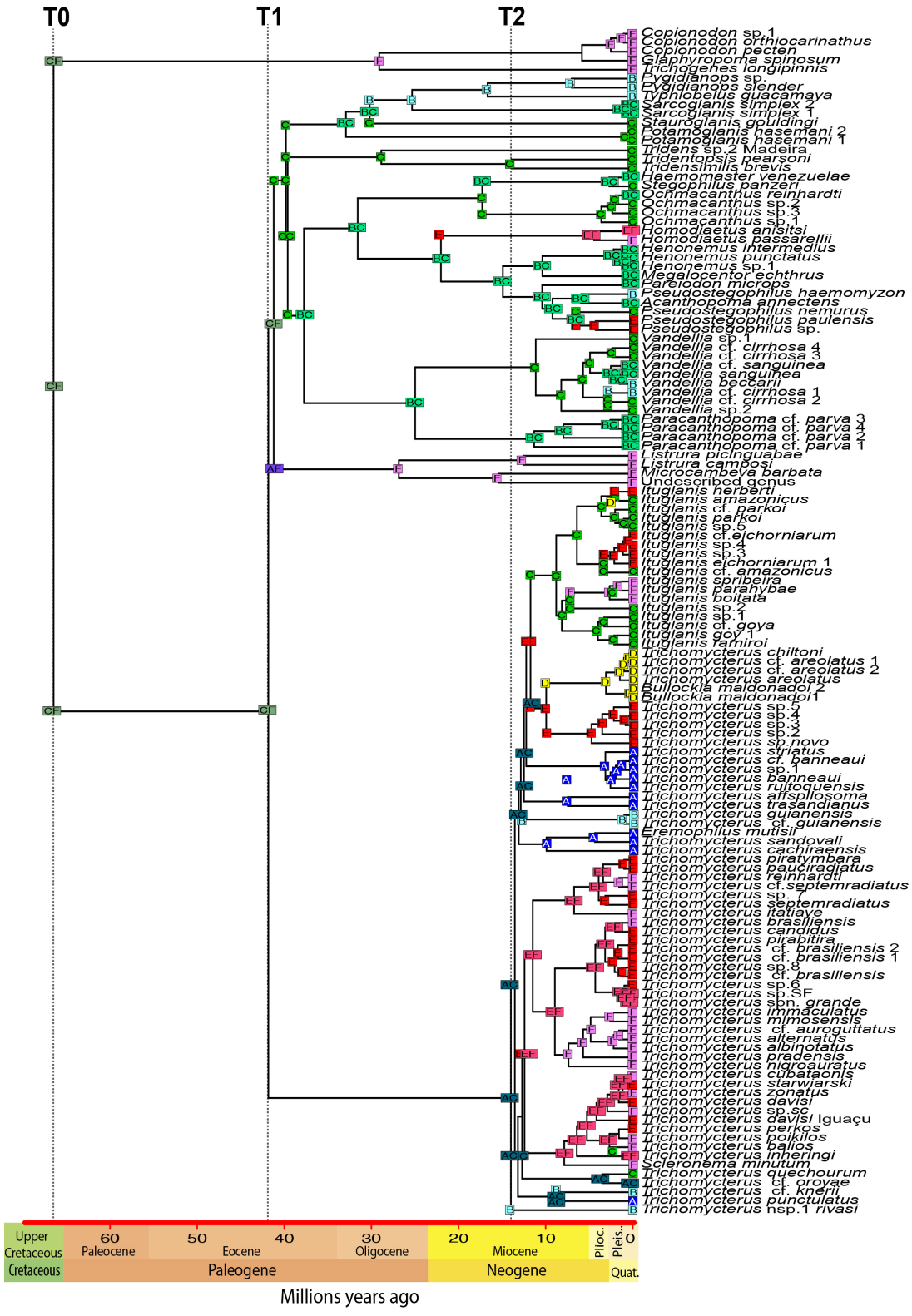


**Figure 2.5**. Chronogram with ancestral area estimates based on the BayArea-like + *j* model. Geographic regions include A-Trans Andean, B-Orinoco-Guiana, C-Amazonas, D-La Plata, E-South Pacific, F-Atlantic coastal drainages. Distribution assigned to each species are indicated before tip names.

**Table 2.1.** Geographic range of trichomycteridae species according with the defined areas in this study**:** include A-Trans Andean, B-Orinoco-Guiana, C-Amazonas, D-La Plata, E-South Pacific, F-Atlantic coastal drainages

| Species | Lote | Voucher | River | Basin | Country | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Copionodon_orthiocarinathus | LBP17354 | 17354 | Mucujê | Paraguaçu | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Copionodon_pecten | LBP17361 | 59311 | Mucujê | Paraguaçu | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Copionodon_sp1 | LBP17361 | 59310 | Mucujê | Paraguaçu | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Glaphyropoma_spinosum | LBP17359 | 17359 | Gruta dos torres | Paraguaçu | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichogenes_longipinnis | LBP3862 | 22411 | Cachoeira do amor | Atlantico | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Undescribed_genus | LBP16842 | 69447 | Fau | Ribeira do Iguape | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Listrura_camposi | LBP7438 | 35362 | Riacho sem nome | Ribeira do Iguape | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Listrura_picinguabae | LBP3864 | 22423 | Ribeira do Iguape | Ribeira do Iguape | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Potamoglanis_hasemani1 | LBP4483 | 24450 | Negro | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Potamoglanis_hasemani2 | LBP4198 | 23971 | Negro/Igarape Boiboi | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Sarcoglanis_simplex1 | ANSP179212 | 872 | Ireng | Takutu | Guyana | 0 | 1 | 1 | 0 | 0 | 0 |
| Sarcoglanis_simplex2 | ANSP180021 | 2201 | Takutu | Takutu | Guyana | 0 | 1 | 1 | 0 | 0 | 0 |
| Typhlobelus_guacamaya | ANSP190503 | 2114 | Cuao | Orinoco | Venezuela | 0 | 1 | 0 | 0 | 0 | 0 |
| Pygidianops_slender | ANSP190505 | 2115 | Asita | Orinoco | Venezuela | 0 | 1 | 0 | 0 | 0 | 0 |
| Pygidianops_sp | ANSP190504 | 2113 | Asita | Orinoco | Venezuela | 0 | 1 | 0 | 0 | 0 | 0 |
| Stauroglanis_gouldingi | LBP3159 | 19301 | Negro | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Microcambeva_barbata | LBP21985 | 21885 | Aldeia Velha | Atlantico | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Tridens_sp2madeira | LBP12070 | 12070 | Madeira | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Tridensimilis_brevis | LBP13940 | 13940 | Tapajos | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Tridentopsis_pearsoni | LBP13944 | 13944 | Branco | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Paracanthopoma cf parva1 | LBP2498 | 15495 | Araguaia | Amazonas | Brazil | 0 | 1 | 1 | 0 | 0 | 0 |

| Species | Lote | Voucher | River | Basin | Country | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Paracanthopoma cf parva2 | ANSP199868 | 4948 | Nanay | Amazonas | Perú | 0 | 1 | 1 | 0 | 0 | 0 |
| Paracanthopoma cf parva4 | LBP5245 | 26466 | Jari | Amazonas | Brazil | 0 | 1 | 1 | 0 | 0 | 0 |
| Paracanthopoma cf parva3 | ANSP198315 | 12408 | Nanay | Amazonas | Perú | 0 | 1 | 1 | 0 | 0 | 0 |
| Vandellia_sp1 | LBP1631 | 11765 | Araguaia | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Vandellia_sp2 | LBP14854 | 57887 | Amazonas | Amazonas | Perú | 0 | 0 | 1 | 0 | 0 | 0 |
| Vandellia cf cirrhosa3 | ANSP197186 | 11246 | Tamshiyacu | Amazonas | Perú | 0 | 0 | 1 | 0 | 0 | 0 |
| Vandellia cf cirrhosa4 | LBP2477 | 16450 | Araguaia | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Vandellia cf cirrhosa2 | LBP5342 | 26973 | Jari | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Vandellia cf cirrhosa1 | ANSP191329 | 3936 | Ventuari | Orinoco | Venezuela | 0 | 1 | 0 | 0 | 0 | 0 |
| Vandellia_beccarii | LBP10155 | 47557 | Apure | Orinoco | Venezuela | 0 | 1 | 0 | 0 | 0 | 0 |
| Vandellia cf sanguinea1 | LBP2477 | 16449 | Araguaia | Amazonas | Brazil | 0 | 1 | 1 | 0 | 0 | 0 |
| Vandellia_sanguinea | LBP15976 | 66164 | Xingu | Amazonas | Brazil | 0 | 1 | 1 | 0 | 0 | 0 |
| Haemomaster_venezuelae | INPA43789 | 10704 | Xingu | Amazonas | Brazil | 0 | 1 | 1 | 0 | 0 | 0 |
| Stegophilus_panzeri | LBP8619 | 43462 | Tapajos | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ochmacanthus_sp1 | ANSP191769 | 4748 | Itaya | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ochmacanthus_reinhardti | LBP10987 | 50481 | Lajeado | Amazonas | Brazil | 0 | 1 | 1 | 0 | 0 | 0 |
| Ochmacanthus_sp3 | ANSP197613 | 11339 | Itaya | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ochmacanthus_sp2 | LBP4351 | 24088 | Igarapé do cajual | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Homodiaetus_passarellii | LBP2502 | 16505 | Macacu | Atlantico | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Homodiaetus_anisitsi | LBP13194 | 55163 | Camaquã | Lagoa dos Patos | Brazil | 0 | 0 | 0 | 0 | 1 | 1 |
| Megalocentor_echthrus | ANSP199997 | 4732 | Itaya | Amazonas | Perú | 0 | 1 | 1 | 0 | 0 | 0 |
| Henonemus_sp1 | ANSP197334 | 12772 | Jarauçu | Amazonas | Brazil | 0 | 1 | 1 | 0 | 0 | 0 |
| Henonemus_punctatus | LBP4146 | 23679 | Jurua | Amazonas | Brazil | 0 | 1 | 1 | 0 | 0 | 0 |
| Henonemus_intermedius | LBP2394 | 16454 | Araguaia | Amazonas | Brazil | 0 | 1 | 1 | 0 | 0 | 0 |

| Species | Lote | Voucher | River | Basin | Country | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pareiodon_microps | ANSP191783 | 4707 | Amazonas | Amazonas | Brazil | 0 | 1 | 1 | 0 | 0 | 0 |
| Acanthopoma_annectens | ANSP181146 | 890 | Amazonas | Amazonas | Perú | 0 | 1 | 1 | 0 | 0 | 0 |
| Pseudostegophilus_haemomyzon | ANSP198977 | 13134 | Apure | Apure | Venezuela | 0 | 1 | 0 | 0 | 0 | 0 |
| Pseudostegophilus_nemurus | LBP1581 | 11769 | Das Garças | Araguaia | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Pseudostegophilus_paulensis | LBP6738 | 6738 | Tiete | Paraná | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Pseudostegophilus_sp | LBP5133 | 26235 | Paraguai | Paraná | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_nsp1rivasi | ANSP191470 | 2083 | Peninsula de Paria |  | Venezuela | 0 | 1 | 0 | 0 | 0 | 0 |
| Trichomycterus_cachiraensis | LBP19832 | 77957 | Galvanes | Magdalena | Colombia | 1 | 0 | 0 | 0 | 0 | 0 |
| Eremophilus_mutisii | ANSP11306 | no voucher | Magdalena | Magdalena | Colombia | 1 | 0 | 0 | 0 | 0 | 0 |
| Trichomycterus_sandovali | LBP19833 | 77946 | Don Juan cave | Magdalena | Colombia | 1 | 0 | 0 | 0 | 0 | 0 |
| Trichomycterus_guianensis | LBP17444 | 69015 | Potaro | Essequibo | Guyana | 0 | 1 | 0 | 0 | 0 | 0 |
| Trichomycterus_cf guianensis | ANSP179111 | 918 | Orokang | Orokang | Guyana | 0 | 1 | 0 | 0 | 0 | 0 |
| Trichomycterus_aff spilosoma | LBP19339 | 77975 |  |  | Ecuador | 1 | 0 | 0 | 0 | 0 | 0 |
| Trichomycterus_trasandianus | LBP19845 | 77964 | Magdalena | Magdalena | Colombia | 1 | 0 | 0 | 0 | 0 | 0 |
| Trichomycterus_striatus | LBP19846 | 77968 | Magdalena | Magdalena | Colombia | 1 | 0 | 0 | 0 | 0 | 0 |
| Trichomycterus_ruitoquensis | LBP19838 | 77956 | Magdalena | Magdalena | Colombia | 1 | 0 | 0 | 0 | 0 | 0 |
| Trichomycterus_banneaui | LBP19847 | 77973 | Magdalena | Magdalena | Colombia | 1 | 0 | 0 | 0 | 0 | 0 |
| Trichomycterus_sp1 | LBP19834 | 77958 | Magdalena | Magdalena | Colombia | 1 | 0 | 0 | 0 | 0 | 0 |
| Trichomycterus cf banneaui | LBP19537 | 77971 | Magdalena | Magdalena | Colombia | 1 | 0 | 0 | 0 | 0 | 0 |
| Trichomycterus_spnovo | LBP8563 | 43332 | Sepotuba | Paraguay | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_areolatus | LBP3118 | 19819 | Maichin | Maichin | Chile | 0 | 0 | 0 | 1 | 0 | 0 |
| Trichomycterus_areolatus2 | LBP3118 | 19820 | Maichin | Maichin | Chile | 0 | 0 | 0 | 1 | 0 | 0 |
| Trichomycterus_chiltoni | ANSP180474 | 940 | Andalien | Andalien | Chile | 0 | 0 | 0 | 1 | 0 | 0 |
| Trichomycterus cf areolatus | LBP3113 | 3113 | La Laja | Bio Bio | Chile | 0 | 0 | 0 | 1 | 0 | 0 |

| Species | Lote | Voucher | River | Basin | Country | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bullockia_maldonadoi1 | LBP3112 | 3112 | La Laja | Bio Bio | Chile | 0 | 0 | 0 | 1 | 0 | 0 |
| Bullockia_maldonadoi2 | LBP3112 | 19793 | La Laja | Bio Bio | Chile | 0 | 0 | 0 | 1 | 0 | 0 |
| Ituglanis_goya | LBP17137 | 68599 | Dos Couros | Tocantins | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ituglanis_sp1 | LBP19465 | 77969 | Das Brancas | Tocantins | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ituglanis cf goya | LBP17131 | 68592 | Das Almas | Tocantins | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ituglanis_sp2 | LBP16129 | 66849 | Tapajos | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ituglanis_boitata | LBP14546 | 60865 | Jacui | Lagoa dos Patos | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Ituglanis_spribeira | LBP7416 | 35678 | Batatau | Ribeira do Iguape | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Ituglanis_parahybae | LBP10730 | 49719 | Macabu | Paraíba do Sul | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Ituglanis cf amazonicus | LBP11003 | 50532 | Madeira | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ituglanis cf eichorniarum | LBP10777 | 49859 | Paraguay | Paraguay | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Ituglanis_eichorniarum | LBP4686 | 24825 | Paraguay | Paraguay | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Ituglanis_herberti | LBP676 | 8028 | Pirai | Paraná | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Ituglanis_amazonicus | LBP2442 | 16211 | Araguaia | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ituglanis cf parkoi | LBP7995 | 37376 | Tapajos | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ituglanis_parkoi | LBP14153 | 59188 | Tapajos | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ituglanis_sp5 | INPA11584 | 11584 | Xingu | Amazonas | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Trichomycterus_punctulatus | ANSP180733 | 905 | Pisco | Pisco | Perú | 1 | 0 | 0 | 0 | 0 | 0 |
| Trichomycterus_cfknerii | LBP18717 | 18717 | Meta/caño Guamal | Orinoco | Colombia | 0 | 1 | 0 | 0 | 0 | 0 |
| Trichomycterus_cforoyae | LBP3255 | 3255 | Chontabamba | Amazonas | Perú | 1 | 0 | 1 | 0 | 0 | 0 |
| Trichomycterus_quechourum | ANSP180572 | 912 | Mapacho | Mapacho | Perú | 0 | 0 | 1 | 0 | 0 | 0 |
| Scleronema_minutum | LBP3310 | 19841 | Arroio dos Corrientes | Atlantico | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_inheringi | LBP4512 | 24563 | Paranapiacaba | Paraná | Brazil | 0 | 0 | 0 | 0 | 1 | 1 |
| Trichomycterus_balios | LBP607 | 7358 | Tainhas | Taquari-Antas | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |

| Species | Lote | Voucher | River | Basin | Country | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Trichomycterus_perkos | LBP17033 | 66403 | Uruguay | Uruguay | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_poikilos | LBP14693 | 61154 | Carreiro | Uruguay | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_davisiiguacu | LBP1222 | 10598 | Iguaçu | Atlantico | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_spsc | LBP3121 | 19686 | Itapocu | Atlantico | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_davisi | LBP6383 | 29744 | Preto | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_zonatus | LBP2653 | 17409 | Ribeira do Iguape | Ribeira do Iguape | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_cubataonis | LBP3123 | 19690 | Itapocu | Atlantico | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_starwiarski | LBP16165 | 66905 | Cachoeira | Paraguay | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_itatiaye | LBP16356 | 62282 | Paraiba do sul | Paraíba do Sul | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_sp7 | LBP11631 | 58082 | Paraná | Paraná | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_septemradiatus | LBP5939 | 28065 | São Domingos | Grande | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus cf septemradiatus | LBP6550 | 31670 | Das Velhas | São francisco | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_reinhardti | LBP16302 | 61942 | São Francisco | São francisco | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_piratymbara | LBP9004 | 42138 | Grande | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_pauciradiatus | LBP16323 | 61977 | Paraná | Paraná | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_pradensis | LBP8291 | 38359 | Jequitinhonha | Jequitinhonha | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_immaculatus | LBP8351 | 40419 | Doce | Doce | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_mimosensis | LBP8290 | 38358 | Jequitinhonha | Jequitinhonha | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_albinotatus | LBP6326 | 29802 | Paraiba do sul | Paraíba do Sul | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_cfauroguttatus | LBP8374 | 40446 | Paraiba do sul | Paraíba do Sul | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_alternatus | LBP1014 | 10261 | Chopoto | Doce | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_nigroauratus | LBP6301 | 29341 | Itagaçaba | Paraíba do Sul | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_spngrande | LBP10282 | 47992 | Araguari | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_spsf | LBP11842 | 58147 | Córrego da Agua Santa | São francisco | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |

| Species | Lote | Voucher | River | Basin | Country | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Trichomycterus_sp6 | LBP11648 | 58088 | Bonito | Paraná | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_brasiliensis | LBP10675 | 49540 | São Francisco | São francisco | Brazil | 0 | 0 | 0 | 0 | 0 | 1 |
| Trichomycterus_candidus | LBP11630 | 58011 | Paraná | Paraná | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_sp8 | LBP9001 | 42123 | Grande | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_cf brasiliensis | LBP10276 | 47976 | Grande | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_cf brasiliensis1 | LBP8060 | 37829 | Grande | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_cf brasiliensis2 | LBP6247 | 29199 | Grande | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_pirabitira | LBP18381 | 72641 | Grande | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Ituglanis_ramiroi | LBP15293 | 63261 | Rio São Bernardo | Tocantins | Brazil | 0 | 0 | 1 | 0 | 0 | 0 |
| Ituglanis_sp3 | LBP12960 | 55688 | Rio Cuiaba | Paraguai | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_sp2 | LBP7674 | 36488 | Afluente rio Coxipo-Açu | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_sp4 | LBP10236 | 47789 | Corrego sem nome | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_sp3 | LBP7669 | 36484 | Afluente rio da Casca | Paraguai | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Ituglanis_sp4 | LBP7667 | 36475 | Corrego João Dias | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |
| Trichomycterus_sp5 | LBP7640 | 36427 | Afluente rio Aricá-Mirim | La Plata | Brazil | 0 | 0 | 0 | 0 | 1 | 0 |

**Table 2.2**. Summary of time intervals used in the biogeographical analysis. // permeable barriers between areas, / impermeable barriers. A-Trans Andean, B-Orinoco-Guiana, C-Amazonas, D-La Plata, E-South Pacific, F-Atlantic coastal drainages

| Time intervals | Ages | Palaeogeographical events | Geographical barriers |
|---|---|---|---|
| T0 | 66.63 – 41.97 | Serra do Mar, Ancestral river system to western Amazonas-Orinoco. | - |
| T1 | 41.97 – 14.1 | Michicola arch separation (west Amazon and Upper Paraná basin), paleogene reactivation of Precambrian shear zones. | A, B, C//D, D//E,E//F |
| T2 | 14.1 - 0 | Uplifts Andean mountains Quechua 1 orogeny Late Miocene, separation modern Orinoco Amazonas basins at the Vaupes arc. Third deformational events of the CRSB (Continental Rift of South easter Brazil) | A/B/ C/D/E/F,D//F |

**Table 2.3**. Composition of the geographical units defined in this study based in the freshwater ecoregions (Abell et al. 2008). Spatial area sizes and respective GPS coordinates were used to construct a pairwise area-dispersal matrix between geographical units.

| Geographical unit | Ecoregion | Area ($km^2$) | GPS coordinates | Code |
|---|---|---|---|---|
| Trans-Andean | 302, 303 | 446.036 | 3º16'N 75º18'W | A |
| Orinoco-Guiana | 305, 306, 307, 308, 309, 310, 311. | 1'731,477 | 4º59'N 63º03'W | B |
| Amazonas | 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326. | 7'254,838 | 3º48'S 61º31'W | C |
| La Plata | 332, 333, 342, 343, 344, 345, 346, 347 | 2'989,542 | 24º34'S 52º36'W | D |
| South Pacific | 339, 340, 341 | 655,708 | 27º57'S 69º24'W | E |
| Atlantic coastal drainages | 327, 328, 329, 330, 331, 334, 335 | 1'337,336 | 23º58'S 46º57'W | F |

**Table 2.4**. Summary of log likelihoods for the models fit in BioGeoBEARS analysis of Trichomycteridae. Abbreviations: DEC, dispersal-extinction-cladogenesis; ln *L*, log likelihood; *K*, paramters; *d*, dispersal; *e*, extinction; *j*, jump dispersal event; AIC, Akaike information criterion; ΔAIC, delta AIC; ΔAIC*w*, delta AIC weighted.

| | **ln *L*** | ***K*** | ***d*** | ***e*** | ***j*** | **AIC** | **ΔAIC** | **ΔAIC*w*** |
|---|---|---|---|---|---|---|---|---|
| BayArea-like + *j* | -243.8 | 3 | 0.038 | 0.027 | 0.16 | 493.5 | 0 | $1 \times 10^{00}$ |
| BayArea-like | -270.1 | 2 | 0.15 | 0.05 | 0 | 544.2 | 50.7 | $1.00 \times 10^{-11}$ |
| DEC+J | -269.7 | 3 | 0.19 | 0.078 | 0.14 | 545.5 | 52 | $5.20 \times 10^{-12}$ |
| DEC | -271.3 | 2 | 0.26 | 0.1 | 0 | 546.5 | 53 | $3.20 \times 10^{-12}$ |
| DIVA-like + *j* | -347.9 | 3 | 0.052 | 0.0012 | 0.36 | 701.9 | 208.4 | 5.70 x 10-$^{46}$ |
| DIVA-like | -373.8 | 2 | 0.21 | 0.013 | 0 | 751.6 | 258.1 | $9.40 \times 10^{-57}$ |

# Supplement 1

**Multilocus analysis of the catfish family Trichomycteridae (Teleostei: Ostariophysi: Siluriformes) supporting a monophyletic Trichomycterinae.**

# Multilocus analysis of the catfish family Trichomycteridae (Teleostei: Ostariophysi: Siluriformes) supporting a monophyletic Trichomycterinae

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**Abstract**

Trichomycteridae is the second most diverse family of the order Siluriformes, its members are widely distributed through the freshwaters of Central and South America, exhibiting an exceptional ecological and phenotypic disparity. The most diverse subfamily, Trichomycterinae, represented mainly by the genus *Trichomycterus*, historically has been recognized as non-monophyletic and various characters used to unite or divide its constituents are repeatedly called into question. No comprehensive molecular phylogenetic hypothesis regarding relationships of trichomycterids has been produced, and the present study is the first extensive phylogeny for the family Trichomycteridae, based on a multilocus dataset of three mitochondrial loci and two nuclear markers (3284 bp total). Our analysis has the most comprehensive taxon-sampling of the Trichomycteridae published so far, including members of all subfamilies and a vast representation of *Trichomycterus* diversity. Analysis of these data showed a phylogenetic hypothesis with broad agreement between the Bayesian (BI) and maximum-likelihood (ML) trees. The results provided overwhelming support for the monophyletic status of Copionodontinae, Stegophilinae, Trichomycterinae, and Vandelliinae,

but not Sarcoglanidinae and Glanapteryginae. A major feature of our results including current conceptualization of Trichomycterinae, which includes *Ituglanis* and *Scleronema* but exclude the *"Trichomycterus" hasemani* group. Divergence time analysis based on DNA substitution rates suggested a Lower Cretaceous origin of the family and the divergence events at subfamilial level shaped by Paleogene events in the geohistory of South America. This hypothesis lays a foundation for an array of future studies of evolution and biogeography of the family.

**Keywords:**

Freshwater fishes, molecular phylogeny, Neotropical Region, Systematics

## 1. Introduction

Siluriformes (catfishes) is the third most speciose order of extant fishes with more than 3700 valid species (Eschmeyer et al., 2017) widely distributed in freshwaters of all continents (except Antarctica) and estuarine and marine habitats of continental shelves (de Pinna, 1998). Catfishes present an unparalleled diversity of morphological, ecological, and behavioral traits (Adriaens et al., 2010). Within the order, Trichomycteridae is the second richest family and includes species commonly known as pencil and parasitic catfishes. With about 300 valid species (Eschmeyer et al., 2017), trichomycterids are currently divided into eight subfamilies (Copionodontinae, Glanapteryginae, Sarcoglanidinae, Stegophilinae, Trichogeninae, Trichomycterinae, Tridentinae, and Vandelliinae) and 41 genera (Eschmeyer et al., 2017). Members of the family are distributed through continental freshwaters from Costa Rica to Patagonia (de Pinna and Wosiacki, 2003), with a single species occurring on the small island Gorgona island, off the Pacific coast in Colombia (Fernández and Schaefer, 2005). Species of Trichomycteridae exhibit a remarkable variety of feeding strategies, including semiparasitic hematophagy (Vandelliinae), lepidophagy and mucophagy (Stegophilinae), and occupy a wide range of habitats from subterranean waters to Andean streams and lakes up to 4500 m asl (Arratia and Menu-Marque, 1984; de Pinna and Wosiacki, 2003; Rizzato et al., 2011).

Eigenmann (1918) proposed an evolutionary tree depicting relationships among the 18 genera of trichomycterine genera known at the time, including *Nematogenys* as the most basal taxon. Baskin (1973) was the first to provide explicit cladistic support for the monophyly of the Trichomycteridae and divided all then-known trichomycterids into the Trichomycterinae-

group (Glanapteryginae, Sarcoglanidinae and Trichomycterinae) and the Vandelliinae-group (Stegophilinae, Tridentinae and Vandelliinae). Later discoveries added a clade composed of two subfamilies, Copionodontinae and Trichogeninae, as the sister to all other trichomycterids (de Pinna, 1992; de Pinna, 1998; Datovo and Bockmann, 2010). Costa and Bockmann (1994a) realigned the Glanapteryginae and Sarcoglanidinae with Baskin's Vandelliinae-group to form the so-called TSVSG clade. Subsequent studies upheld the TSVSG clade with both morphological (Datovo and Bockmann, 2010) and molecular (Fernández and Schaefer, 2009) evidence.

The monophyly of seven of the eight subfamilies of the Trichomycteridae is well supported by morphology: Copionodontinae (de Pinna, 1992), Glanapteryginae (de Pinna, 1989b), Sarcoglanidinae (de Pinna, 1989a; Costa 1994; Costa and Bockmann, 1994), Stegophilinae (Baskin, 1973; de Pinna and Britski, 1991; DoNascimiento, 2015), Trichogeninae (de Pinna et al., 2010), Tridentinae (Baskin, 1973) and Vandelliinae (Baskin, 1973; de Pinna, 1998).

Trichomycterinae is the most speciose subfamily of the Trichomycteridae with about 200 species distributed in eight genera: *Bullockia* (1 species), *Eremophilus* (1), *Hatcheria* (1), *Ituglanis* (26), *Rhizosomichthys* (1), *Scleronema* (3), *Silvinichthys* (7), and *Trichomycterus* (160+) (Eschmeyer et al., 2017). The monophyly of this subfamily remains ambiguous, as well as the synapomorphies repeatedly called into question (de Pinna, 1989a, Datovo and Bockmann, 2010; García-Melo et al., 2016). The main obstacle to understanding the relationships within Trichomycteridae is the most diverse genus, *Trichomycterus*, which has a complex taxonomic history and is a non-monophyletic assemblage that basically includes those species lacking the diagnostic characters of other trichomycterine genera (Baskin, 1973; de Pinna, 1989, 1998; Datovo and Bockmann, 2010). Despite the description of more than 70 new species in the last two decades (Eschmeyer et al., 2017), *Trichomycterus* still includes a large number of undescribed taxa. The limits and phyletic status of the whole Trichomycterinae is also controversial. While most studies agree with the exclusion of the so-called "*Trichomycterus*" *hasemani* group from the subfamily, alternative hypotheses of placement and inclusion or not of *Scleronema* and *Ituglanis* has been proposed (de Pinna, 1989; Arratia, 1990; Costa and Bockmann, 1992; 1998; Datovo and Bockmann, 2010; Dutra et al., 2012; DoNascimiento, 2015). All these issues contribute to make the phylogenetic revision of the Trichomycterinae one of the greatest challenges of catfish systematics.

Although recent work using morphology and molecules has shed much light on the phylogenetic relationships of the Trichomycteridae, disagreements persist, especially on the monophyly and composition of the two key taxa that concentrate the vast majority of the family diversity: Trichomycterinae and *Trichomycterus*. We present here a multilocus analysis of the Trichomycteridae based on the most comprehensive taxon sampling to date that includes members of all subfamilies and a vast representation of *Trichomycterus* diversity. Also, a time-calibrated molecular tree analysis was performed to hypothesize diversification dates relative to the evolutionary history of this important Neotropical lineage and key to our understanding of catfish relationships.

## 2. Material and Methods

### *2.1 Taxon sampling*

Analysis was based on a total of 94 terminals representing 18 genera of all eight subfamilies of the Trichomycteridae (Copionodontinae, Glanapteryginae, Sarcoglanidinae, Stegophilinae, Trichogeninae, Trichomycterinae, Tridentinae, and Vandelliinae). We included samples of cis- and trans-Andean species of *Trichomycterus*, the former better represented by species from the Atlantic coast of Brazil. *Nematogenys inermis*, the sole extant member of the Nematogenyidae, was chosen as the outgroup as this taxon is often hypothesized to be the sister group of the Trichomycteridae (Eigenmann, 1918, 1927; de Pinna, 1992, 1998) and is widely recognized as retaining the most primitive morphology within Loricarioidei (Eigenmann, 1918, 1927; Baskin 1973; Arratia and Huaquín, 1995; de Pinna, 1992, 1998). Data sequences were obtained from tissue samples collected by the authors. Vouchers of samples are deposited in the ichthyological collections of the Academy of Natural Sciences of Philadelphia, USA (ANSP) and the Laboratório de Biologia e Genética de Peixes, Botucatu, Brazil (LBP). Taxonomic identification of voucher specimens was validated by direct examination. Catalog numbers of vouchers and tissues used in this study are given in supplementary Table S1.

### *2.2 DNA extraction and sequencing*

DNA was extracted from tissues preserved in 95% EtOH using the DNeasy Tissue kit (Qiagen Inc.; http://www.qiagen.com) following the manufacturer's instructions. Partial

sequences of three mitochondrial (16S rRNA, cytochrome C oxidase subunit I - coi and cytochrome B - cytb) and two nuclear (myosin heavy chain 6, cardiac muscle, alpha gene - myh6 and recombination activating gene 2 - rag2) genes were amplified by polymerase chain reaction (PCR) with the primers described in Table S2. Amplifications were performed in a total volume of 12.5 µl with 1.25 µl of 10X buffer (10 mM Tris-HCl+15 mM MgCl2), 0.5 µl dNTPs (200 nM of each), 0.5 µl each 5 mM primer, 0.05 µl Platinum® Taq Polymerase (Invitrogen), 1 µl genomic DNA (10-50 ng), and 8.7 µl ddH2O. The thermo-cycler profile consisted of an initial denaturation (4 min at 95ºC) followed by 30 cycles of chain denaturation (30 s at 95ºC), primer hybridization (30-60 s at 52-54 ºC) and nucleotide extension (30-60 s at 72 ºC). All PCR products were first visually identified on 1% agarose gel and then purified using ExoSap-IT® (USB Corporation) following manufacturer instructions. The purified PCR products were sequenced using the "Big DyeTM Terminator v 3.1 Cycle Sequencing Ready Reaction Kit" (Applied Biosystems), purified again by ethanol precipitation and loaded onto an automatic sequencer 3130-Genetic Analyzer (Applied Biosystems) in the Instituto de Biociências, Universidade Estadual Paulista - UNESP, Botucatu, São Paulo, Brazil. All sequences were read twice (forward and reverse).

### *2.3 Sequences assembly and alignment*

The consensus sequences for each individual gene were assembled from chromatograms for forward and reverse sequences using Geneious software v7.1.7 (Biomatters Ltd., Auckland, New Zealand). Initially we built matrixes for every gene and they were independently aligned using the MUSCLE algorithm under default parameters (Edgar, 2004). The alignments were inspected by eye for any obvious misalignments that were then corrected. To detect potential cases of sequencing errors due to contamination or paralogy, the alignment for each gene was analyzed by maximum likelihood (ML) and rapid bootstrapping using RAxML (Stamatakis, 2006). Sequences that were found misplaced in relation to putative congeneric or conspecific specimens in the resulting gene tree were re-sequenced or eliminated from subsequent analysis. To evaluate the occurrence of substitution saturation, we used the index of substitution saturation (Iss) test as described by Xia et al. (2003) and Xia and Lemey (2009) implemented in the software Dambe 5.3.38 (Xia, 2013). Only unambiguously alignable regions were included; hypervariable, unalignable loop regions were excluded. Alignments of all loci were concatenated into a single matrix consisting of 3284 bp of the 93 terminals plus the outgroup *Nematogenys inermis*. Each gene except 16S was partitioned by gene and codon

positions to determine codon-specific models of molecular evolution in PartitionFinder v1.1.1 (Lanfear et al., 2012). The best models were chosen under the best value for the Bayesian Information Criterion (BIC) index as detailed in Table S3.

### *2.4 Phylogenetic analysis*

The phylogenetic hypotheses were inferred from two reconstruction methods using the partitioned data. First, Bayesian Inference (BI) was conducted in MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) via the CIPRES web portal (Miller et al., 2010). MrBayes was programmed to run for 15 million generations, with two runs of four independent MCMC chains (three heated, one cold), sampling one tree every one thousand generation. After a graphical analysis of the evolution of the likelihood scores, and checking for the stationarity of all model parameters, the first 4 million generations (25%) were discarded as burn-in. The remaining trees were used to calculate the consensus tree. The maximum likelihood (ML) phylogenetic reconstructions were performed using RAxML (Stamatakis, 2006) via command line with the "a" algorithm to rapid bootstrapping analysis (ML search and bootstrapping) in one step and specifying a random number seed for the parsimony estimation. The number of alternative runs was 100 and the analyses were performed under the model GTR+G.

Measures of branch support are given as posterior probability (P) and non-parametric bootstrap percentage (BS) separated by a common slash (/); asterisks represent values <0.5 (P) or <50% (BS). A topology test was performed to evaluate the degree of support for the resulting molecular phylogeny versus the previously published morphological hypothesis. We compared the ML scores of an unconstrained tree (our resulting topology) with a constrained tree enforcing the interfamilial relationships proposed by Datovo and Bockmann (2010). A Constraint tree was constructed in Mesquite (Maddison and Maddison, 2011), the confidence in the comparison of ML scores to every topology was evaluated with the Shimodaira-Hasegawa test (SH) (Shimodaira and Hasegawa, 1999), Kishino and Hasegawa test (KH) and Unbiased test (AU) using the CONSEL package.

### *2.5 Time calibrated tree*

The uncorrelated relaxed molecular clock (lognormal) was estimated using BEAST v.1.7 (Drummond et al., 2012) and all clade-age inferences are presented as 95% highest posterior density (HPD). We included two calibration points to constrain divergence dates for the 93 species of trichomycterids included in our phylogenetic tree. The first calibration point was implemented in the root of the phylogeny for the origin of Trichomycteridae about 106 million years ago (Mya) as estimated by Betancur-R et al. (2015). We implemented a normally distributed prior with mean of 106 and standard deviation of seven. The search was conducted among the interval of 92.28 - 119.7 Mya using the lower and upper quantiles of 2.5%, respectively.

The second calibration point was implemented using a log-normal prior offset and mean of 4.5 Mya and standard deviation of 1.5 for the origin of the subfamily Trichomycterinae. The only known fossil for the family Trichomycteridae was described from the Monte Hermoso Formation in Argentina by Bogan and Agnolin (2009). Based on biostratigraphy, Tomassini et al. (2013) estimated the upper and lower boundaries of the Monte Hermoso Formation to be 4.5/5 and 5.3 Mya, respectively. The search for the second calibration point was conducted within the interval of 4.4 - 32.13 Mya using the lower and upper quantiles of 2.5%, respectively. We used a macroevolutionary Birth-Death model for the diversification likelihood values and a starting tree obtained from the Bayesian inference. The analyses were conducted under different models of molecular evolution for each partition of the data matrix as evaluated by the software PartitionFinder v1.1.1 (Lanfear et al., 2012) (Table S3). The analysis was run for 10 million generations and sampled every 1000 generation. Stationarity and sufficient mixing of parameters (ESS.200) were checked using Tracer v1.5 (Rambaut and Drummond, 2007a). A consensus tree was built using TreeAnnotator v1.8.2 (Rambaut and Drummond, 2007b).

## 3. Results

### *3.1 Overall aspects of the matrix*

The concatenated matrix of the three mitochondrial and two nuclear genes consist of 3284 bp after alignment (466 for 16S; 524 for COI; 859 for Cytb; 544 for Myh6; 891 for Rag2). In the total matrix, 1278 sites were variable, 1010 were parsimony informative and 2008 were

invariant (I). The nucleotide composition of the concatenated matrix was of 28.1% thymine, 22.6% cytosine, 25.5% adenine and 23.7% guanine.

The Iss index indicated no saturation considering that the Iss.c value is greater than the Iss. For each gene, the number and percent of sequences obtained, size in base pairs (bp), number of variable and invariant sites (I), number of informative characters under parsimony, nucleotide frequency and overall mean genetic distance (S.E.) are presented in Table 1. The matrix was partitioned by gene and coding positions, except by 16S, into 13 sections. The partition scheme consisted of eight subsets and 250 parameters, and the evolutionary model for each gene and codon position evaluated in PartitionFinder are showed in the Table 2.

### *3.2 Phylogenetic hypothesis*

The tree topologies estimated by the BI and ML analyses were very similar with exception of the relationships of *Eremophilus mutisii* that in ML analysis was hypothesized as an independent lineage with a low statistical support. Although sampling at genus level was incomplete, statistical support for the monophyly of four subfamilies was high: P = 1.00, BS = 100 for Copionodontinae (2 of 2 genera sampled; clade B); P = 0.99, BS = 23 for Trichomycterinae (5 of 8 genera; clade D); P = 1.00, BS = 86 for Stegophilinae (4 of 11 genera, clade TSVSG); and P = 1.00, BS = 100 for Vandelliinae (2 of 4 genera, clade TSVSG) (Figure 2). The monophyly of the Trichogeninae (1 genus), Tridentinae (4 genera) and Glanapteryginae (4 genera) could not be tested due to the low taxonomic representation that included one genus from each subfamily, *Trichogenes longipinnis*, *Tridens* sp n2 and two species of genus *Listrura*, respectively. Our results did not support the monophyly of Sarcoglanidinae (6 genera) based on the two genera analyzed (*Sarcoglanis* and *Stauroglanis*).

The sister group relationship between Trichogenes and Copionodontinae (Fig. 2; P = 1.00, BS = 98, clade B) is strongly supported (Figure 2), as well as the placement of this clade as sister to remaining trichomycterids (Fig. 2; P = 1.00, BS = 100). The TSVSG clade was recovered as monophyletic, but with relatively low statistical support (Fig. 2; P = 0.58, BS = 57). Within the TSVSG clade, the clade composed by two glanapterygine species, *Listrura camposi* and *L. picinguabae* is the first group to diverge. The two genera of sarcoglanidines analyzed, the monotypic *Sarcoglanis* and *Stauroglanis*, were not found closely related to each other within the TSVSG clade. *Sarcoglanis* grouped with "*Trichomycterus*" *hasemani*, *Tridens*

*melanops* (Tridentinae) and members of the Stegophilinae, whereas *Stauroglanis* grouped with two genera of the Vandelliinae (*Paravandellia* and *Vandellia*).

Our analyses supported the monophyly of the Trichomycterinae exclusive of "*Trichomycterus" hasemani* with high BI support (Fig. 2; P = 0.99, BS < 50). Species of five genera (*Bullockia*, *Eremophilus*, *Ituglanis*, *Scleronema* and *Trichomycterus*) constitute a large "Clade D" (sensu Datovo and Bockmann, 2010) divided into two subclades and six main lineages. The first clade to diverge within subclade D1+D2+E+D3 (D1, P = 0.54, BS < 50; Fig. 2) clusters three trans-Andean taxa, the troglomorphic *Trichomycterus sandovali* (Magdalena basin), *T. punctulatus* (Pacific versant of Peru), and *Eremophilus mutisii* (Magdalena basin), with a species from the Meta River (Orinoco basin), *Trichomycterus* cf. *knerii*. These relationships were different in ML analysis, with *E. mutisii* as an independent lineage with a low support (BS < 50).

Clade D2 (Fig. 2; P = 1.00, BS < 50) groups species of *Trichomycterus* from the Magdalena basin (*T. banneaui*, *T. cachiraensis, T. ruitoquensis, T. straminius*, *Trichomycterus* cf. *trasandianus*) with *T* cf. *guianensis* (Essequibo basin, Guyana) and *Trichomycterus* aff. *spilosoma* (Pacific versant of Ecuador). The third clade (Fig. 2; P = 0.94, BS = 86) partially corresponds to "Clade E" of Datovo and Bockmann (2010) and grouped two trans-Andean species from Chile, *Bullockia maldonadoi* and *T. areolatus*, as sister to an undescribed cis-Andean species, *Trichomycterus* sp. 2, from upper Paraguay River basin. Clade D3 (Fig. 2; P = 1.00, BS = 100) correspond to analyzed species of *Ituglanis* from the Amazon basin (Jari, Madeira, Tapajós and Tocantins Rivers), La Plata system (Paraguay and Paraná Rivers) and smaller Atlantic Coast drainages of southern (Jacuí and Ribeira de Iguape Rivers) and southeastern (Macabú River) Brazil.

The sister group relationship between clades D4 and D5 is strongly supported (Fig. 2; P = 0.99, BS = 87) and ties *Scleronema* (lower La Plata system and Atlantic coastal drainages in Southern Brazil and Uruguay) with species of *Trichomycterus* distributed in Atlantic coastal drainages from the São Francisco River in the north to the Paraná and Uruguay rivers in the south. Clade D4 has high nodal support in both the BI and ML analysis (Fig. 2; P = 0.99, BS = 100) and places the clade formed by *S. minutum* and *Scleronema* cf. *angustirostre* (from tributaries of Laguna dos Patos system) sister of a group of species of *Trichomycterus* distributed in the Atlantic coastal drainages, including the upper São Francisco basin, Itapocu,

Jacuí, Ribeira de Iguape, Uruguay, and tributaries to the Paraná drainages (Paranapanema and Tietê Rivers).

Clade D5 (Fig. 2; P = 0.99, BS = 100) also includes species of *Trichomycterus* from Atlantic coastal drainages of eastern and southern Brazil. In this clade, the first group to diverge includes *T. itatiayae* (Paraiba do Sul, River), *T. piratymbara* (Grande-Paraná, River system), and *T. reinhardti* (Paraopeba-São Francisco River system). The second group to diverge includes *T. florensis* (Paraiba do Sul River) sister to specimens of *T. nigroauratus* from the same basin and the Grande River (Paraná basin). The third group to diverge is represented by *T. albinotatus* (Paraiba do Sul River), *T. alternatus* and *T. immaculatus* (Doce River), and two species of *Trichomycterus* from the Jequitinhonha River, *T. pradensis* and *T.* cf. *mimosensis*. The third group is sister to a clade partially corresponding to the so-called *T. brasiliensis* complex by Barbosa and Costa (2010), including species distributed in the Grande River (Paraná basin) and upper São Francisco basin.

Our topology test using a constraint tree for subfamilial relationships proposed by Datovo and Bockmann (2010), resulted in a hypothesis with low likelihood value (Table 3). However, the difference between the constraint tree and the herein proposed tree was minimal, and consequently, the topology test failed to reject the null hypothesis of morphology-based relationships within Trichomycteridae.

### *3.3 Time calibrated tree*

The mean substitution rate for the dataset estimated in BEAST was 0.0163% per My. The Trichomycteridae was estimated to have originated near the end of the Lower Cretaceous about 103.2 Mya (54.5-109.7 Mya, 95% HPD) and around the time of the continental separation between Africa and South America (ca. 100 Mya; Torsvik et al. 2008). The first split is estimated during the Middle of the Upper Cretaceous diverging in two larger groups, Copionodontinae + Trichogeninae and the clade of the remaining trichomycterids. The next split is estimated at 66.8 Mya, just before the K-T boundary, and established ancestral of the clade TSVSG clade and the subfamily Trichomycterinae. Within the TSVSG clade, the oldest glanapterygine genus *Listrura* was originated in the Paleocene and the remaining subfamilies arose in the Eocene (Sarcoglanidinae, Tridentinae, Vandelliinae) and Oligocene (Stegophilinae). Oligocene diversification within Trichomycterinae established the three major clades composed mostly of trans-Andean taxa (D1-2, E), and the three remaining strictly of

cis-Andean clades (D3-5). Much of the diversification within those major clades appears to have occurred during the Miocene (Figure 3).

## 4. Discussion

### *4.1 Phylogenetic relationships among Trichomycteridae subfamilies*

The molecular phylogenetic hypothesis supported the monophyletic status of the Copionodontinae, Stegophilinae, Trichomycterinae, and Vandelliinae, but not Sarcoglanidinae. The current conceptualization of the Trichomycterinae, which includes *Ituglanis* and *Scleronema* but excludes the *"Trichomycterus" hasemani* group (Datovo and Bockmann, 2010; see item 4.2 below), was confirmed as a monophyletic unit with high support both in both, BI and ML analyses. The topology corroborated the most recent morphological tree of the family (Datovo and Bockmann, 2010) in several important ways: Copionodontinae and Trichogenis form a monophyletic group that is sister to remaining trichomycterids, and this latter clade is divided into two main linages, the TSVSG clade and Trichomycterinae. Internal relationships for the TSVSG clade, however, differs in some significant aspects from the traditional phylogenetic hypotheses, mainly due to the recovered non-monophyly of the Sarcoglanidinae, although not all genera herein represented. The systematic positions of the two sarcoglanidines herein analyzed yielded a novel topology that remained consistent between the two analytical methods (BI and ML) with considerable statistical support. Nevertheless, the topologies advanced by previous morphological studies of inter-subfamilial relationships could not be rejected by the present molecular analysis according to the topology test (Table 3).

The monophyletic assemblage of the subfamilies Copionodontinae and Trichogeninae, identified as Clade B in Datovo and Bockmann (2010), was originally suggested by de Pinna (1998) and corroborated by Bichuette et al. (2008). These subfamilies share the derived presence of an anterior process at the anterolateral corner of hypobranchial 1, the endopterygoid ankylosed to the ventral surface of the autopalatine, and an enlarged subtemporal fossa (de Pinna, 1998). Datovo and Bockmann (2010) additionally found a myological synapomorphy for the clade consisting in the presence of the *adductor hyomandibulae* muscle, a morphological character that is functionally linked to the hollow ventral surface of the pterotic (subtemporal fossa). This clade is the sister group to all other trichomycterids and exhibits the plesiomorphic condition for several characters uniquely derived in the remaining trichomycterids (de Pinna, 1998; Datovo and Bockmann, 2010).

Therefore, the monophyly of this clade and its phylogenetic placement at the base of the Trichomycteridae is supported by both morphology and molecules hypotheses.

The group comprising all subfamilies of the Trichomycteridae with the exception of Copionodontinae and Trichogeninae (identified as Clade C in Datovo and Bockmann, 2010), was also recovered in the present molecular analysis, despite some disagreement in the composition. Results from both inferences herein analyzed, divided Clade C into two subclades, the TSVSG (Costa and Bockman, 1993) and the Trichomycterinae as redefined by Datovo and Bockmann (2010). Our hypothesis of a monophyletic group including all trichomycterines and members of the TSVSG clade corroborates previous morphological studies (de Pinna, 1992, 1998; Bockmann et al., 2004; Datovo and Bockmann, 2010) based on a wealth of anatomical support: anterior cranial fontanel partially or completely closed; sphenotic, prootic, and pterosphenoid fused; Weberian capsule with a small opening, much smaller than its lateral profile; interhyal absent; five or fewer pelvic-fin rays; dorsal caudal-fin plate with six or fewer rays; dorsal caudal-fin lobe with five or fewer branched rays; ventral caudal-fin plate with eight or fewer rays; ventral caudal-fin lobe with six or fewer branched rays; incomplete infraorbital branch of the laterosensory canal system; presence of a *protractor operculi* muscle; and *levator operculi* muscle with fibers posterodorsally oriented towards its origin.

Despite the low support values (P=0.58, BS= 57) the monophyly of the TSVSG clade is corroborated by previous studies (Costa and Bockmann, 1994; Fernández and Schaefer, 2009) and it is supported by four shared derived morphological characters: absence of a posterior process of the parasphenoid; extreme reduction or absence of the metapterygoid, interopercular patch of odontodes reduced, being nearly as long as deep; and primary section of the *dilatator operculi* passing dorsolateral to *the levator arcus palatini* (Costa and Bockmann, 1993; de Pinna, 1998; Datovo and Bockmann, 2010). A major source of incongruence between our hypothesis and previous phylogenetic arrangements lay in the internal relationships of the TSVSG clade. The main departures in the genetic analysis are the non-monophyly of the Sarcoglanidinae, with *Sarcoglanis* being most closely related to *"Trichomycterus" hasemani*, and *Stauroglanis* most closely related to Vandelliinae (*Paravandellia* and *Vandellia*). This internal arrangement of the TSVSG clade (Fig. 2) is highly supported in BI but not in ML analysis. Most morphological studies (Baskin, 1973; Costa and Bockmann, 1994; de Pinna, 1998; Datovo and Bockmann, 2010) and previous molecular analyses (Fernández and Schaefer, 2009) supported a sister group relationship between the

Sarcoglanidinae and Glanapteryginae, with the following shared derived morphological features: reduced vomer; reduced number of premaxillary teeth; quadrate with a posteriorly-directed anterodorsal process; anterior portion of the hyomandibula modified into a long process; seven or fewer anal-fin rays; and insertion of the *stegalis* (*sensu* Datovo and Vari, 2013, 2014; =A3) section of the *adductor mandibulae* onto the buccopalatal membrane.

The Sarcoglanidinae was established by Myers and Weitzman (1966) to include *Sarcoglanis simplex* and *Malacoglanis gelatinosus*. New genera were subsequently described and added: *Stauroglanis* (de Pinna, 1989), *Stenolicmus* (de Pinna and Starnes, 1990), *Microcambeva* (Costa and Bockmann, 1994), and *Ammoglanis* (Costa, 1994). The monophyletic status of this subfamily has been successively reexamined during the last two decades (Datovo and Bockmann, 2010) and only the inclusion of *Ammoglanis pulex* has been questioned (de Pinna and Winemiller, 2000). The monophyly of the Sarcoglanidinae is supported by a large set of osteological (de Pinna, 1989; Costa, 1994) and myological (Datovo and Bockmann, 2010) synapomorphies. The sister group relationship between *Sarcoglanis simplex* and "*Trichomycterus" hasemani* is unexpected and novel, since this is the first molecular analysis to include both taxa. Morphological studies have placed "*T." hasemani* sister to a clade formed by the Tridentinae, Stegophilinae, and Vandelliinae (= Vandelliinae-group), either analyzing it alone (DoNascimiento, 2015) or as part of the "*T." hasemani* group, which also includes "*T." johnsoni* and "*T." anhinga*, and *"T". wapixana* (Dutra et al., 2012; see next section). In our analysis, long-branch attraction and likely the incomplete lineage sorting may be responsible for the non-monophyly of Sarcoglanidinae, as well as the lack of support for the clade Sarcoglanidinae + Glanapteryginae. Our sampling of both subfamilies was incomplete (two of six sarcoglanidine genera and only one of four glanapterygine genera). The two sarcoglanidine genera in our analysis (*Sarcoglanis* and *Stauroglanis*) occupy rather distal positions in the subfamilial phylogeny inferred from morphology (summarized in Costa, 1994) and *Sarcoglanis simplex* has a remarkable highly specialized morphology.

Previous morphological (Baskin, 1973; de Pinna, 1998; Datovo and Bockmann, 2010) and molecular (Fernández and Schaefer, 2009) analyses hypothesized the monophyly of the Vandelliinae-group, a clade formed by the Tridentinae, Stegophilinae, and Vandelliinae. Such an arrangement is not recovered in the present study, with the Vandelliinae appearing more closely related to the Glanapteryginae and the sarcoglanidine *Stauroglanis* than to tridentines and stegophilines. Another point of disagreement corresponds to the relationship between the semiparasitic subfamilies Stegophilinae and Vandelliinae. Most studies (Baskin, 1973; de

Pinna, 1998; Fernández and Schaefer, 2009; DoNascimiento, 2015) have supported a sister relationship between those two subfamilies. Datovo and Bockmann (2010) provided myological evidence favoring and alternative hypothesis in which Tridentinae and Stegophilinae are sister taxa. These authors, however, confirm the validity of anatomical characters supporting the traditional hypothesis and concludes that the relationships between the three subfamilies could not be decisively determined based on the morphological evidence available. Our results reinforce the myological evidence presented by Datovo and Bockmann (2010) and is the first study to provide molecular support for the hypothesis of a tridentine-stegophiline group.

### *4.2 The subfamily Trichomycterinae*

Baskin (1973) was the first to suggest the non-monophyly of the Trichomycterinae. De Pinna (1989) corroborated his hypothesis based on the lack of synapomorphies for the subfamily and the possibly closer relationship of some species with other subfamilies: *Scleronema, Trichomycterus boylei* and *T. santaeritae* would be more closely related to the Sarcoglanidinae and "*T*". *hasemani* and "*T*". *johnsoni* more closely related to the Tridentinae. Whereas the former hypothesis has been rejected (Arratia, 1990; Costa and Bockmann 1993, 1994; de Pinna, 1998; Datovo and Bockmann, 2010), the latter was partially corroborated by subsequent morphological studies (Datovo and Bockmann, 2010; Dutra et al., 2012; DoNascimiento, 2015). The present analysis is the first molecular evidence corroborating the exclusion of "*T.*" *hasemani* from the Trichomycterinae, although in our topology this species is more closely related to *Sarcoglanis* (Sarcoglanidinae) than to members of the Vandelliinae-group. The so-called "*T.*" *hasemani* group (*"T". anhanga*, *"T". hasemani*, and *"T". johnsoni*, and *"T". wapixana*; Dutra et al., 2012) remains provisionally classified as *Trichomycterus* while its formal description as a new trichomycterid genus and subfamily is still underway (by W. Wosiacki and M. C. C. de Pinna; pres. comm.).

Arratia (1990) proposed four synapomorphies in supporting a monophyletic Trichomycterinae, none of which were present in "*Trichomycterus*" *hasemani* (de Pinna, 1998). Costa and Bockmann (1993) and de Pinna (1998), on the other hand, advanced that *Ituglanis* and *Scleronema* were more closely related to the TSVSG clade than to remaining trichomycterines, a result not corroborated by the present analysis. More recently, Datovo and Bockmann (2010) and Datovo et al. (2016) revised the characters proposed to support these

two alternative hypotheses and concluded that none of them were valid. The authors, however, concluded that the Trichomycterinae exclusive the "*T.*" *hasemani* group could form a monophyletic group on the basis of the sharing of a posterior portion of *levator internus IV* originated from the dorsal face of the posttemporo-supracleithrum. Thus, the present analysis based on representatives of five trichomycterine genera (*Eremophilus*, *Bullockia*, *Ituglanis*, *Scleronema*, and *Trichomycterus*; missing *Hatcheria*, *Rhizosomichthys* and *Silvinichthys*), supported the monophyly of the Trichomycterinae (clade D) as circumscribed by Datovo and Bockmann (2010).

In our topology, the Trichomycterinae is divided into two large clades: one including all species from Atlantic coastal drainages and Upper Paraná (*Scleronema* and part of *Trichomycterus*; clade D1+D2+E+D3) and another primarily including Amazonian and trans-Andean taxa (*Bullockia, Eremophilus, Ituglanis*, and part of *Trichomycterus*; clade D4+D5). Such a scheme contradicts previous hypotheses in which *Scleronema* and *Ituglanis* are in some way closely related (Costa and Bockmann, 1993; de Pinna 1998). The monophyletic status of genus *Ituglanis* (clade D3) is highly supported, in agreement with the morphological studies (Costa and Bockmann, 1993; Datovo and de Pinna, 2014; Datovo et al., 2016; Wosiacki et al., 2012). De Pinna and Keith (2003) tentatively proposed the existence of two monophyletic groups within the genus, one including species from the Amazon and Guiana shield and another primarily formed primarily by species from the La Plata system and Atlantic coastal drainages. This hypothesis has been challenged in light of additional anatomical evidence and newly discovered taxa (Datovo and Landim, 2005; Datovo, 2014; Datovo and de Pinna, 1994). Our analysis refuted de Pinna and Keith's (2003) proposal, with the clustering of species from the Amazon and Paraguay in two occasions: in one clade, including *I. herberti* and *I. parkoi*, and a second lineage that includes *I. amazonicus*, *I. eichhorniarum*. It is worth mentioning, however, that identity and limits of the three last species are poorly understood and some terminals are only tentatively assigned to these taxa (*I. amazonicus*, *I.* cf. *eichhorniarum*, and *I.* cf. *parkoi*). Interestingly, all analyzed species from the Atlantic coastal drainages (*I. boitata*, *I. parahybae*, and *Ituglanis.* sp. n. 1) are herein highly supported as forming a monophyletic lineage. A fourth major clade of *Ituglanis* grouped the epigean *I. goya* and the hypogean *I.* cf. *ramiroi*, both from the lower Tocantins basin.

The sister group of *Ituglanis* is Clade E composed of *Bullockia*, *Trichomycterus areolatus* and an undescribed *Trichomycterus* from Sepotuba River, upper Paraguay River basin. A previous study of DoNascimiento (2015) clustered *Bullockia*, *Hatcheria*, and

*Trichomycterus areolatus*. Datovo and Bockmann (2010) included to this clade another Chilean species, *T. chiltoni* and other two from southern Brazil to this clade, *Trichomycterus immaculatus* and *T. zonatus*, but our molecular results nested those species in different clades, D5 and D4 respectively.

Our molecular results corroborated the non-monophyletic status of *Trichomycterus* as previously hypothesized by several studies (Baskin, 1973; de Pinna, 1989, 1998; Arratia, 1998; Datovo and Bockmann, 2010). Identity of the type species of *Trichomycterus*, *T. nigricans*, is surrounded by uncertainties and conflicting information (A. Datovo, in prep.), but its type locality is certainly an Atlantic coastal drainage in Brazil. In our analysis, species of *Trichomycterus* from this area are restricted to sister clades D4 and D5. Clade D4 also includes two species of *Scleronema*, *S. minutum* and *Scleronema* cf. *operculatum*.

In spite of the present study to be the most comprehensive phylogenetic analysis of the Trichomycterinae published to date, including 70 representative terminals, taxonomic changes at this moment are premature considering the constant increase in the description of new species assigned to the genus *Trichomycterus*, as well as, the incomplete genera representation of some subfamilies. Nevertheless, our results constitute an important evidence for the necessary nomenclatural changes within *Trichomycterus*, which should be ideally associated with the geographical distribution of the clades herein identified. Based on our results, clades containing species of the *Trichomycterus* from trans-Andean basins (D1, D2, E) are more closely related to *Ituglanis* (Clade D3) than to clades containing species of *Trichomycterus* from Atlantic coastal drainages, which probably are more related with the type species of this genus. Finally, we suggest that revisionary studies of key taxa at the generic level, an increase in the molecular information and its integration with morphological data are crucial to confidently to perform accurate nomenclatural decisions in the subfamily.

### *4.3 Timing of diversification*

Molecular-clock methods provide neontological tools for estimating the temporal origins of clades (Hipsley and Müller, 2014) and linking cladogenesis to major events in geohistory. For example, Lundberg et al. (2007) used a fossil-calibrated, relaxed-clock molecular analysis to estimate the divergence of the Neotropical freshwater catfish, *Lacantunia enigmatica*, from its closest relatives which are endemic to Africa. Based on the estimated age of *Lacantunia* (83-86.5 Mya), its occurrence in the New World is more likely attributable to

dispersal via Holarctic land bridges from the Late Cretaceous to Late Miocene than to older vicariant events associated with the breakup of Pangea and subsequently Gondwana. Employing similar methods, Sullivan et al. (2013) estimated that the Neotropical superfamily Pimelodoidea (long-whiskered catfishes) originated between 110 and 95 Mya, and that its five major lineages (family-level clades) split off soon afterward during a period of explosive diversification. Based on the fossil records of pimelodoids and other Neotropical fishes, the freshwater fauna of South America appears to have been essentially modern by the mid-Miocene (Lundberg, 1998; Lundberg et al., 2010; Sullivan et al., 2013).

Trichomycteridae is one of six family-level groups in Loricariodei, a suborder that is endemic to the Neotropic and sister to all other catfishes (Sullivan et al., 2006). Our estimate of the origin of Trichomycteridae (103.2 Mya) is approximated the time of the rifting caused the connection between the Central and South Atlantic (ca. 100 Mya; Torsvik et al. 2008) which severed the last continental connection between Africa and South America. The oldest split within Trichomycteridae is estimated at 84.66 Mya and established the clade Copionodontinae + Trichogeninae. Extant members of this clade are restricted to a few, relatively small Atlantic tributaries in eastern and southeastern Brazil. Ribeiro (2006) used this split to exemplify the initial (Cretaceous) phase of diversification contributing to an ancient fish fauna endemic to Brazilian coastal rivers (his "Pattern A"). He linked Pattern A cladogenesis to the original Atlantic coastal drainages that were probably structurally oriented by megadomes, large faults and grabens. The second major split within the Trichomycteridae appears to have established two large clades, TSVSG and Trichomycterinae, just before the K-T Boundary (ca. 65.5 Mya).

The TSVSG and Trichomycterinae clades provide more examples of cladogenesis involving Atlantic coastal drainages. The first group to diverge in the TSVSG clade is *Listrura* (60.8 Mya), a genus restricted to coastal streams of southern and southeastern Brazil. The remaining members of the TSVSG clade are restricted to larger cis and trans-Andean basins that were variously interconnected throughout the Cenozoic. Within the Trichomycterinae, there are two major clades containing Atlantic coastal taxa, D3 and D4+D5. By our estimates, Clade D4+5 split from the remaining trichomycterines 33.3 Mya (Lower Oligocene). This clade is composed exclusively of taxa from Atlantic coastal drainages and tributaries to the Paraná-Paraguay basin. Clade D3 is younger, established ~24 Mya (Upper Oligocene), and includes species of the genus *Ituglanis* from both Atlantic coastal drainages and the major cis-Andean drainages (Amazonas, Paraná-Paraguay system, Tocantins, and Amazonas River

basin). Our results therefore suggested that separate geological events are responsible for the modern-day fauna of Atlantic coastal trichomycterines.

The distribution and diversification of fishes in Atlantic coastal tributaries are commonly attributed to sea level changes during the Late Pleistocene (Weitzman et al., 1988; Thomaz et al., 2015). Ribeiro (2006) argued that changes in sea level fail to explain the occurrence of closely related taxa in both coastal drainages and upland rivers draining the Brazilian Shield inland towards much larger basins such as the Paraná-Paraguay system. Alternatively, he hypothesized megadome uplifts, rifting, vertical movements between rifted blocks and the erosive retreat of the eastern continental margin of South America to be the main geological forces controlling the biogeography of Atlantic coastal fishes. Those forces gave rise to taphrogenic (rift related) basins along the Brazilian coast that repeatedly captured adjacent upland drainages, providing a one-way conduit for introducing upland taxa to Atlantic coastal basins. Ribeiro (2006) asserted that only significant tectonic deformations could account for dispersal in the opposite direction whereby fishes common to coastal basins are introduced to upland rivers draining inland. Our results point to the Miocene as an active period for such geological events in concert with the diversification of trichomycterins in clades D3 and D4+5.

Of equal interest are dates of origin estimated for trichomycterine clades D1, D2 and E. Together with clade D3 (*Ituglanis*), those groups are one of the first two clades to diverge within Trichomycterinae 33.3 Mya. Clades D1, D2 and E are dominated by taxa from trans-Andean drainages (e.g., Magdalena, Pacific coastal drainages). According to our analysis, clade D1 was the first to split off 30.1 Mya, followed by D2 at 27.2 Mya. The last split between clade E (*Bullockia* and two species of *Trichomycterus*) and clade D3 (*Ituglanis*) is estimated at 24 Mya. Those major cladogenetic events pointed to the Oligocene as an active time for the impact of the Andean uplift on local drainage patterns. Of special interest is the placement of *Trichomycterus* cf *guianensis* within clade D2. *Trichomycterus* cf *guianensis* is a species complex endemic to upland left-bank tributaries of the Essequibo River which drain a portion of the Guiana Shield into the Atlantic Ocean. Our results nested *T.*cf. *guianensis* within clade D2 as sister to a group composed of *Trichomycterus* from the Magdalena and a Pacific versant in Ecuador. As one would expected, the timing of that split, 19.2 Mya (Lower Miocene), predates the estimated isolation of the Magdalena River via the uplift of the Northern Andes ~7-11 Mya (plate 15 in Hoorn and Wesselingh, 2010).

Overall, the tempo of diversification within the Trichomycteridae is consistent with that described by molecular-clock analyses of other Neotropical catfishes (Lundberg et al., 2007; Sullivan et al. 2013). Likewise, trichomycterid diversity appears to have been essentially modern by the mid-Miocene and largely shaped by Paleogene events in the geohistory of South America.

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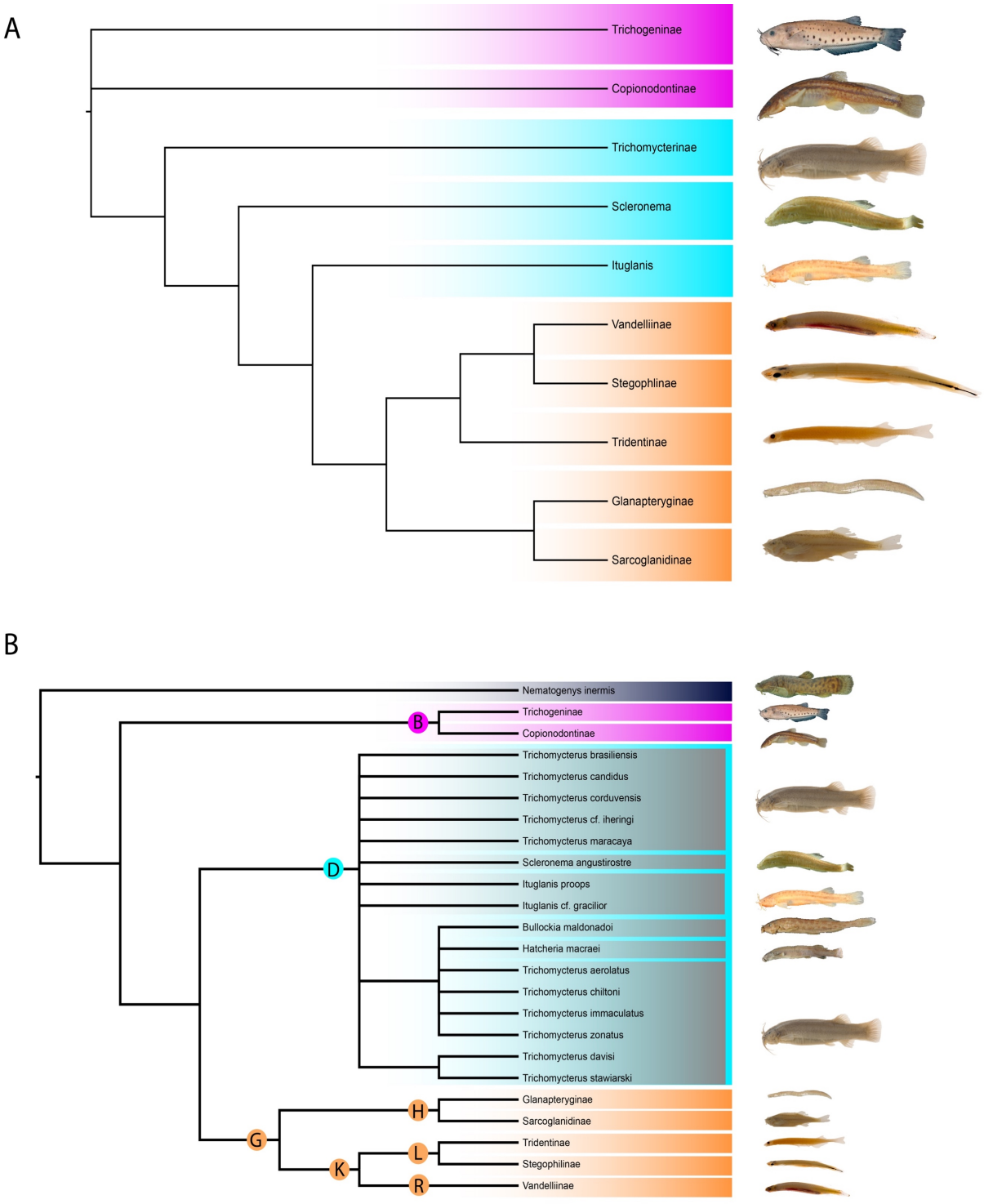


**Figure 1.** Previous phylogenetic hypothesis for the family Trichomycteridae based in **A)** de Pinna (1998); and **B** Datovo and Bockmann (2010).

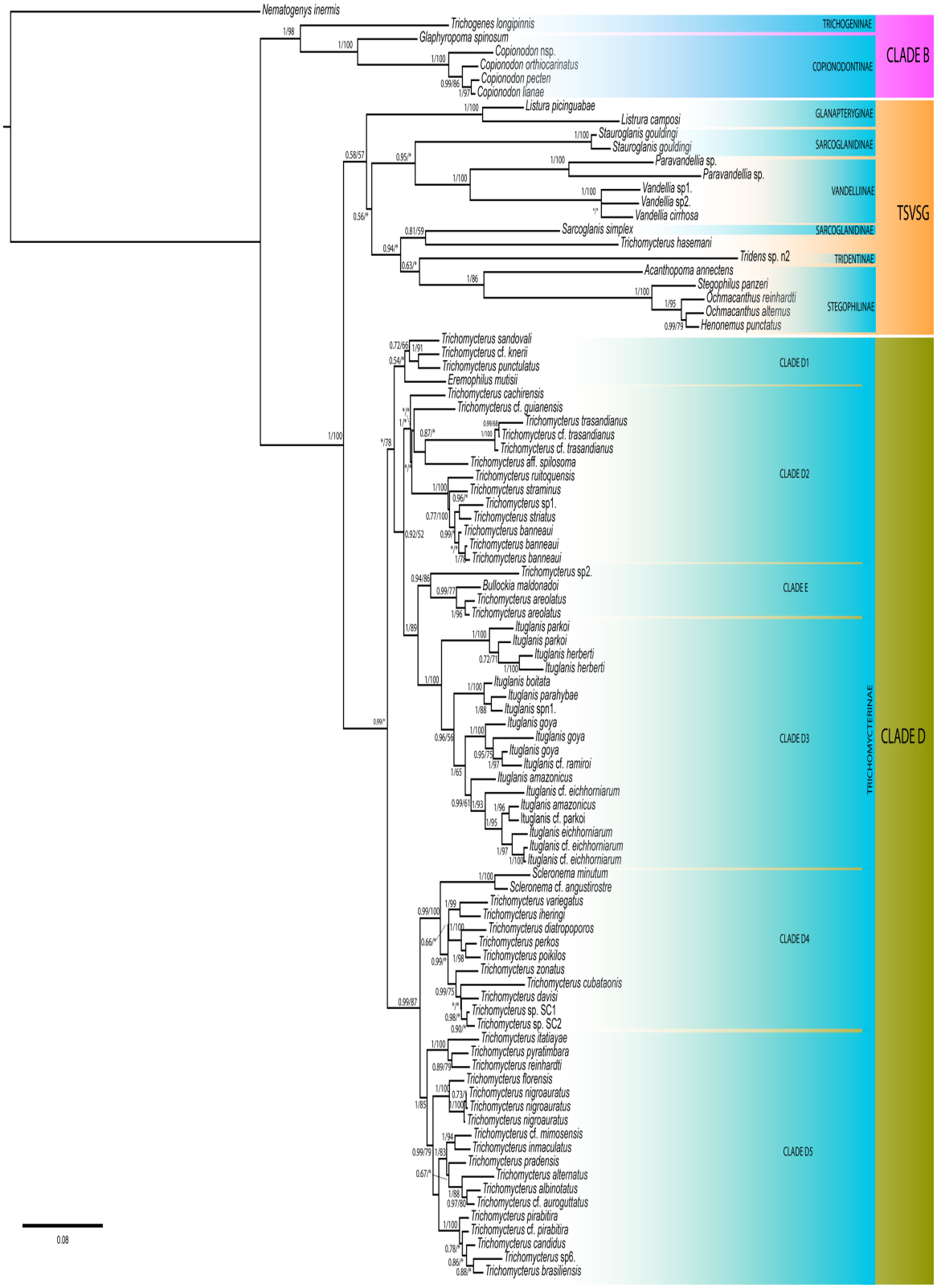


**Figure 2.** Phylogenetic relationships of Trichomycteridae. Node numbers correspond to Bayesian Posterior Probability (P) and Maximum Likelihood (BS) support values. * indicate P and BS values <0.50 and <50 respectively.

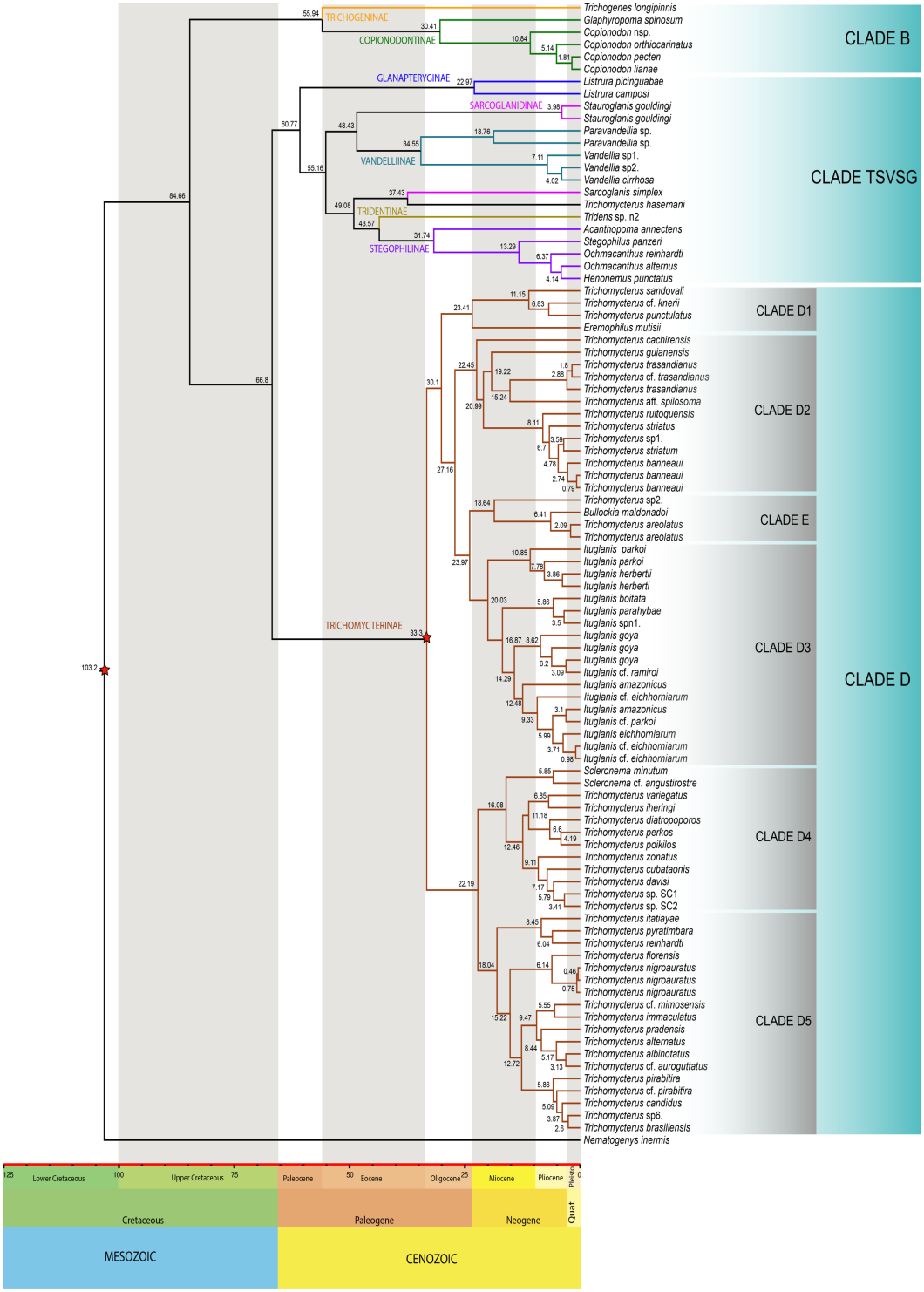


**Figure 3.** Maximum clade credibility (MCC) tree of Trichomycteridae obtained from BEAST analysis.

**Table 1.** Characteristics of the matrix for each gene and total matrix.

| | **Mitochondrial** | | | **Nuclear** | | |
|---|---|---|---|---|---|---|
| | **16S** | **COI** | **Cytb** | **Myh6** | **Rag2** | **Concatenated matrix** |
| Total number of sequences | 92 | 89 | 66 | 86 | 62 | 93 |
| Base-pairs (bp) after alignment | 466 | 524 | 859 | 544 | 891 | 3284 |
| Number of variable sites | 139 | 216 | 403 | 193 | 329 | 1278 |
| Number of invariants (I) sites | 327 | 308 | 456 | 351 | 566 | 2006 |
| Number of informative characters under parsimony | 95 | 205 | 360 | 140 | 214 | 1010 |
| Nucleotide frequency | | | | | | |
| A | 22.0 | 25.0 | 28.0 | 25.4 | 26.4 | 25.5 |
| C | 24.2 | 28.2 | 15.2 | 22.3 | 24.2 | 22.6 |
| G | 23.7 | 17.4 | 29.0 | 22.2 | 25.2 | 23.7 |
| T | 30.1 | 29.4 | 27.7 | 30.1 | 24.2 | 28.1 |
| Overall mean genetic distance (S.E.) | 0.042±0.004 | 0.147±0.009 | 0.151±0.007 | 0.046±0.004 | 0.050±0.003 | 0.090±0.003 |

**Table 2.** Evolutionary models by gene and codon position found in PartitionFinder.

| Subset | Scheme of partition | Partitions | Best-fit model |
|---|---|---|---|
| 1 | 16S | 1-466 | GTR+I+G |
| 2 | COI 1st position | 467-990\3 | K81+I+G |
| | Cytb 1st position | 991-1849\3 | |
| 3 | COI 2nd position | 468-990\3 | F81+I |
| 4 | COI 3rd position | 469-990\3 | GTR+I+G |
| 5 | Cytb 2nd position | 992-1849\3 | GTR+G |
| 6 | Cytb 3rd position | 993-1849\3 | HKY+I+G |
| | Myh6 2nd position | 1851-2393\3 | |
| 7 | Myh6 1st position | 1850-2393\3 | TVMEF+G |
| | Rag2 1st position | 2394-3284\3 | |
| 8 | Myh6 3rd position | 1852-2393\3 | TRNEF+I+G |
| | Rag2 2nd position | 2395-3284\3 | |
| | Rag2 3rd position | 2396-3284\3 | |
| lnL: -36058.22 | | Number of parameters: 250 | |

**Table 3.** Results from topology test in Consel, assessing the significance between the unconstrained RAxML best tree and a tree constrained to the subfamilial relationships proposed by Datovo and Bockmann (2010).

| Analysis | lnL score | Rank | Delta lnL | AU- P value | SH- P value | KH - P value |
|---|---|---|---|---|---|---|
| Unconstrained tree | -36106.04 | 1 | -26.6 | 0.976 | 0.964 | 0.964 |
| Constrained tree | -36133.43 | 2 | 26.6 | 0.024 | 0.036 | 0.036 |

## Supplementary material

**S1**. Taxon sampling, voucher catalogue number, country, drainage and GenBank accession number for every gene sequenced in the present study.

| Subfamily | Species | Voucher Cat # | Tissue | Country | Drainage | 16S | COI | Cytb | Myh6 | Rag2 |
|---|---|---|---|---|---|---|---|---|---|---|
| Trichogeninae | *Trichogenes longipinnis* | LBP 3862 | 22411 | Brazil | Camburi (Atlantic) | KY807238 | KY857961 | KY858035 | KY858113 | KY858185 |
| Copionodontinae | *Glaphyropoma spinosum* | LBP 17359 | 52577 | Brazil | Paraguaçu (Atlantic) | KY807208 | KY857932 | | KY858087 | KY858172 |
| | *Copionodon* n. sp. | LBP 17361 | 59311 | Brazil | Paraguaçu (Atlantic) | KY807206 | KY857930 | | KY858085 | KY858170 |
| | *Copionodon orthiocarinatus* | LBP 1964 | 13699 | Brazil | Paraguaçu (Atlantic) | KY807204 | KY857928 | | KY858083 | KY858168 |
| | *Copionodon pecten* | LBP 17357 | 38993 | Brazil | Paraguaçu (Atlantic) | KY807205 | KY857929 | | KY858084 | KY858169 |
| | *Copionodon lianae* | LBP 17358 | 38978 | Brazil | Paraguaçu (Atlantic) | KY807203 | KY857927 | | KY858082 | KY858167 |
| Glanapteryginae | *Listrura picinguabae* | LBP 3864 | 22414 | Brazil | Picinguaba (Atlantic) | KY807228 | KY857951 | KY858027 | | |
| | *Listrura camposi* | LBP 7438 | 35362 | Brazil | Ribeira de Iguape (Atlantic) | KY807227 | KY857950 | | KY858106 | |
| Sarcoglanidinae | *Stauroglanis gouldingi* | LBP 3159 | 19300 | Brazil | Negro (Amazonas) | KY807235 | KY857958 | KY858032 | KY858110 | |
| | *Stauroglanis gouldingi* | LBP 3159 | 19301 | Brazil | Negro (Amazonas) | KY807236 | KY857959 | KY858033 | KY858111 | |
| | *Sarcoglanis simplex* | LBP 3172 | 19335 | Brazil | Negro (Amazonas) | KY807233 | KY857956 | KY858030 | KY858108 | |
| Vandelliinae | *Paravandellia* sp. | LBP 1666 | 12749 | Brazil | Negro (Amazonas) | KY807232 | KY857955 | KY858029 | | KY858183 |
| | *Paravandellia* sp. | LBP 3176 | 19345 | Brazil | Negro (Amazonas) | KY807231 | KY857954 | | | |
| | *Vandellia* sp. 1 | LBP 15976 | 66164 | Brazil | Xingu (Amazonas) | KY807292 | KY858013 | KY858079 | KY858165 | |
| | *Vandellia* sp. 2 | LBP 10155 | 47557 | Venezuela | Apure (Orinoco) | KY807291 | KY858012 | KY858078 | KY858164 | |
| | *Vandellia cirrhosa* | LBP 3178 | 19329 | Venezuela | Apure (Orinoco) | KY807290 | KY858011 | KY858077 | KY858163 | KY858226 |

| Subfamily | Species | Voucher Cat # | Tissue | Country | Drainage | 16S | COI | Cytb | Myh6 | Rag2 |
|---|---|---|---|---|---|---|---|---|---|---|
| Tridentinae | *Tridens* sp. n 2 | LBP 12070 | 51642 | Brazil | Madeira (Amazonas) | KY807289 | KY858010 | | KY858162 | |
| Stegophilinae | *Acanthopoma annectens* | ANSP 181146 | 891 | Peru | Amazonas | KY807201 | KY857925 | | KY858080 | |
| | *Stegophilus panzeri.* | LBP 1858 | 13281 | Brazil | Araguaia (Tocantins) | KY807237 | KY857960 | KY858034 | KY858112 | |
| | *Ochmacanthus reinhardti* | LBP 10987 | 50481 | Brazil | Madeira (Amazonas) | KY807230 | KY857953 | KY858028 | | |
| | *Ochmacanthus alternus* | LBP 4351 | 24089 | Brazil | Branco (Negro-Amazonas) | KY807210 | KY857934 | KY858015 | KY858089 | KY858174 |
| | *Henonemus punctatus* | LBP 15974 | 66160 | Brazil | Xingu (Amazonas) | KY807209 | KY857933 | KY858014 | KY858088 | KY858173 |
| Trichomycterinae | | | | | | | | | | |
| Clade D1 | *Eremophilus mutisii* | no voucher | 11306 | Colombia | Magdalena | KY807207 | KY857931 | | KY858086 | KY858171 |
| | *Trichomycterus sandovali* | LBP 19833 | 77947 | Colombia | Magdalena | KY807261 | KY857985 | KY858052 | KY858136 | KY858205 |
| | *Trichomycterus* cf. *knerii* | LBP 18717 | 18717 | Colombia | Meta (Orinoco) | KY807263 | KY857987 | KY858054 | KY858138 | KY858206 |
| | *Trichomycterus punctulatus* | ANSP 180733 | 903 | Peru | Pisco (Pacific) | KY807259 | KY857983 | | KY858134 | KY858203 |
| Clade D2 | *Trichomycterus cachiraensis* | LBP 19832 | 77943 | Colombia | Magdalena | KY807248 | KY857971 | | KY858122 | KY858195 |
| | *Trichomycterus* cf. *guianensis* | LBP 17444 | 69015 | Guyana | Potaro (Essequibo) | KY807251 | KY857974 | KY858043 | KY858125 | |
| | *Trichomycterus* aff. *spilosoma* | LBP 19339 | 77975 | Ecuador | Pacific | KY807218 | KY857942 | | KY858097 | |
| | *Trichomycterus* cf. *transandianus* | LBP 19844 | 77965 | Colombia | Magdalena | KY807277 | KY857999 | KY858067 | KY858150 | KY858216 |
| | *Trichomycterus* cf. *transandianus* | LBP 19844 | 77964 | Colombia | Magdalena | KY807282 | KY858004 | KY858070 | KY858155 | KY858220 |
| | *Trichomycterus transandianus* | LBP 19845 | 77967 | Colombia | Magdalena | KY807285 | KY858007 | KY858073 | KY858158 | KY858222 |
| | *Trichomycterus ruitoquensis* | LBP 19838 | 77955 | Colombia | Magdalena | KY807260 | KY857984 | | KY858135 | KY858204 |
| | *Trichomycterus straminius* | LBP 19834 | 77958 | Colombia | Magdalena | KY807284 | KY858006 | KY858072 | KY858157 | KY858221 |

| Subfamily | Species | Voucher Cat # | Tissue | Country | Drainage | 16S | COI | Cytb | Myh6 | Rag2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Trichomycterus* sp. 1 | LBP 19842 | 77963 | Colombia | Magdalena | KY807280 | KY858002 | KY858068 | KY858153 | |
| | *Trichomycterus striatus* | LBP 19846 | 77968 | Colombia | Magdalena | KY807281 | KY858003 | KY858069 | KY858154 | KY858219 |
| | *Trichomycterus banneaui* | LBP 19847 | 77973 | Colombia | Magdalena | KY807245 | KY857968 | | KY858119 | KY858192 |
| | *Trichomycterus banneaui* | LBP 19847 | 77974 | Colombia | Magdalena | KY807246 | KY857969 | | KY858120 | KY858193 |
| | *Trichomycterus banneaui* | LBP 19537 | 77971 | Colombia | Magdalena | KY807279 | KY858001 | | KY858152 | KY858218 |
| Clade E | *Trichomycterus* sp. 2 | LBP 8563 | 43332 | Brazil | Tapajós (Amazonas) | KY807283 | KY858005 | KY858071 | KY858156 | |
| | *Bullockia maldonadoi* | LBP 3112 | 19795 | Chile | Biobio (Pacific) | KY807202 | KY857926 | | KY858081 | KY858166 |
| | *Trichomycterus areolatus* | LBP 997 | 10320 | Chile | Paicavi (Pacific) | KY807240 | KY857963 | | | KY858187 |
| | *Trichomycterus areolatus* | LBP 3118 | 19819 | Chile | Toltén (Pacific) | KY807241 | KY857964 | KY858036 | KY858115 | KY858188 |
| Clade D3 | *Ituglanis parkoi* | LBP 14153 | 59188 | Brazil | Tapajós (Amazonas) | KY807213 | KY857937 | KY858018 | KY858092 | |
| | *Ituglanis parkoi* | LBP 7995 | 37376 | Brazil | Arinos (Tapajós) | KY807226 | KY857949 | KY858026 | KY858105 | |
| | *Ituglanis herberti* | LBP 676 | 8028 | Brazil | Pirai (Paraná) | KY807224 | KY857947 | KY858025 | KY858103 | |
| | *Ituglanis herberti* | LBP 2442 | 16211 | Brazil | Araguaia (Tocantins) | KY807211 | KY857935 | KY858016 | KY858090 | |
| | *Ituglanis boitata* | LBP 14546 | 60865 | Brazil | Jacuí (Laguna dos Patos) | KY807272 | KY857994 | KY858063 | KY858145 | |
| | *Ituglanis parahybae* | LBP 10730 | 49719 | Brazil | Macabú (Atlantic) | KY807219 | | KY858023 | KY858098 | KY858178 |
| | *Ituglanis* sp. n. 1 | LBP 7416 | 35678 | Brazil | Ribeira de Iguape (Atlantic) | KY807217 | KY857941 | KY858022 | KY858096 | |
| | *Ituglanis goya* | LBP 19465 | 77969 | Brazil | das Brancas (Tocantins) | KY807278 | KY858000 | | KY858151 | KY858217 |
| | *Ituglanis goya* | LBP 17131 | 68592 | Brazil | das Almas (Tocantins) | KY807222 | KY857945 | | KY858101 | |
| | *Ituglanis goya* | LBP 17137 | 68599 | Brazil | Dos Couros (Tocantins) | KY807223 | KY857946 | | KY858102 | KY858180 |
| | *Ituglanis* cf. *ramiroi* | LBP 15293 | 63262 | Brazil | Paranã (Tocantins) | KY807276 | KY857998 | | KY858149 | KY858215 |

| Subfamily | Species | Voucher Cat # | Tissue | Country | Drainage | 16S | COI | Cytb | Myh6 | Rag2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Ituglanis amazonicus* | LBP 16129 | 66849 | Brazil | Tapajós (Amazonas) | KY807225 | KY857948 | | KY858104 | KY858181 |
| | *Ituglanis* cf. *eichhorniarum* | LBP 1916 | 14038 | Brazil | Paraguay (Paraná) | KY807220 | KY857943 | | KY858099 | KY858179 |
| | *Ituglanis amazonicus* | LBP 11003 | 50532 | Brazil | Mamoré (Madeira) | KY807212 | KY857936 | KY858017 | KY858091 | KY858175 |
| | *Ituglanis* cf. *parkoi* | LBP 5407 | 27103 | Brazil | Jari (Amazonas) | KY807221 | KY857944 | KY858024 | KY858100 | |
| | *Ituglanis eichhorniarum* | LBP 4686 | 24825 | Brazil | Paraguay (Paraná) | KY807215 | KY857939 | KY858020 | KY858094 | KY858176 |
| | *Ituglanis* cf. *eichhorniarum* | LBP 7667 | 36473 | Brazil | Aquidauana (Paraguay) | KY807216 | KY857940 | KY858021 | KY858095 | KY858177 |
| | *Ituglanis* cf. *eichhorniarum* | LBP 10777 | 49859 | Brazil | Taquari (Paraguay) | KY807214 | KY857938 | KY858019 | KY858093 | |
| Clade D4 | *Scleronema minutum* | LBP 3310 | 19841 | Brazil | Laguna dos Patos (Atlantic) | KY807234 | KY857957 | KY858031 | KY858109 | KY858184 |
| | *Scleronema* cf. *angustirostre* | LBP 13185 | 55146 | Brazil | Laguna dos Patos (Atlantic) | KY807239 | KY857962 | | KY858114 | KY858186 |
| | *Trichomycterus variegatus* | LBP 10289 | 47222 | Brazil | Santo Antônio (São Francisco) | KY807269 | KY857991 | KY858060 | | KY858211 |
| | *Trichomycterus iheringi* | LBP 4512 | 24563 | Brazil | Tietê (Paraná) | KY807286 | KY858008 | KY858074 | KY858159 | KY858223 |
| | *Trichomycterus diatropoporos* | LBP 14694 | 61155 | Brazil | Jacuí (Laguna dos Patos) | KY807274 | KY857996 | KY858065 | KY858147 | KY858213 |
| | *Trichomycterus perkos* | LBP 17033 | 66403 | Brazil | Passo Fundo (Uruguay) | KY807257 | KY857981 | KY858050 | KY858132 | KY858202 |
| | *Trichomycterus poikilos* | LBP 14693 | 61154 | Brazil | Jacuí (Laguna dos Patos) | KY807273 | KY857995 | KY858064 | KY858146 | |
| | *Trichomycterus zonatus* | LBP 2653 | 17409 | Brazil | Ribeira de Iguape (Atlantic) | KY807262 | KY857986 | KY858053 | KY858137 | |
| | *Trichomycterus cubataonis* | LBP 3123 | 19690 | Brazil | Itapocu (Atlantic) | KY807288 | KY858009 | KY858076 | KY858161 | KY858225 |
| | *Trichomycterus davisi* | LBP 7130 | 34219 | Brazil | Paranapanema (Paraná) | KY807264 | KY857988 | KY858055 | KY858139 | |
| | *Trichomycterus* sp. SC1 | LBP 727 | 8276 | Brazil | Itapocu (Atlantic) | KY807256 | KY857979 | KY858048 | KY858130 | KY858200 |
| | *Trichomycterus* sp. SC2 | LBP 3121 | 19686 | Brazil | Itapocu (Atlantic) | KY807255 | KY857978 | KY858047 | KY858129 | KY858199 |

| Subfamily | Species | Voucher Cat # | Tissue | Country | Drainage | 16S | COI | Cytb | Myh6 | Rag2 |
|---|---|---|---|---|---|---|---|---|---|---|
| Clade D5 | *Trichomycterus itatiayae* | LBP 16356 | 62282 | Brazil | Paraiba do Sul (Atlantic) | KY807254 | KY857977 | KY858046 | KY858128 | KY858198 |
| | *Trichomycterus pyratimbara* | LBP 9004 | 42138 | Brazil | Grande (Paraná) | KY807247 | KY857970 | KY858040 | KY858121 | KY858194 |
| | *Trichomycterus reinhardti* | LBP 16302 | 61942 | Brazil | Paraopeba (São Francisco) | KY807275 | KY857997 | KY858066 | KY858148 | KY858214 |
| | *Trichomycterus florensis* | LBP 1021 | 10264 | Brazil | Paraiba do Sul (Atlantic) | KY807250 | KY857973 | KY858042 | KY858124 | KY858196 |
| | *Trichomycterus nigroauratus* | LBP 6301 | 29341 | Brazil | Paraiba do Sul (Atlantic) | | KY857980 | KY858049 | KY858131 | KY858201 |
| | *Trichomycterus nigroauratus* | LBP 8042 | 37769 | Brazil | Paraiba do Sul (Atlantic) | KY807265 | | KY858056 | | KY858207 |
| | *Trichomycterus nigroauratus* | LBP 6347 | 29864 | Brazil | Grande (Paraná) | KY807249 | KY857972 | KY858041 | KY858123 | |
| | *Trichomycterus* cf. *mimosensis* | LBP 8290 | 38358 | Brazil | Jequitinhonha (Atlantic) | KY807266 | KY857989 | KY858057 | KY858140 | KY858208 |
| | *Trichomycterus immaculatus* | LBP 8351 | 40419 | Brazil | Doce (Atlantic) | KY807253 | KY857976 | KY858045 | KY858127 | KY858197 |
| | *Trichomycterus pradensis* | LBP 8291 | 38359 | Brazil | Jequitinhonha (Atlantic) | KY807267 | | KY858058 | KY858141 | KY858209 |
| | *Trichomycterus alternatus* | LBP 1014 | 10261 | Brazil | Doce (Atlantic) | KY807244 | KY857967 | KY858039 | KY858118 | KY858191 |
| | *Trichomycterus albinotatus* | LBP 6326 | 29802 | Brazil | Paraiba do Sul (Atlantic) | KY807243 | KY857966 | KY858038 | KY858117 | KY858190 |
| | *Trichomycterus* cf. *auroguttatus* | LBP 8374 | 40446 | Brazil | Paraiba do Sul (Atlantic) | KY807268 | KY857990 | KY858059 | KY858142 | KY858210 |
| | *Trichomycterus pirabitira* | LBP 18381 | 72641 | Brazil | Grande (Paraná) | KY807258 | KY857982 | KY858051 | KY858133 | |
| | *Trichomycterus* cf. *pirabitira* | LBP 2714 | 17464 | Brazil | Grande (Paraná) | KY807287 | | KY858075 | KY858160 | KY858224 |
| | *Trichomycterus candidus* | LBP 11630 | 58011 | Brazil | Uberaba (Grande) | KY807242 | KY857965 | KY858037 | KY858116 | KY858189 |
| | *Trichomycterus* sp. 6 | LBP 10282 | 47992 | Brazil | Grande (Paraná) | KY807270 | KY857992 | KY858061 | KY858143 | KY858212 |
| | *Trichomycterus brasiliensis* | LBP 11778 | 58144 | Brazil | Upper São Francisco | KY807271 | KY857993 | KY858062 | KY858144 | |
| | *Trichomycterus hasemani* | LBP4198 | 23792 | Brazil | Rio Negro (Amazonas) | KY807252 | KY857975 | KY858044 | KY858126 | |
| Nematogenyidae | *Nematogenys inermis* | LBP3105 | 19783 | Chile | Concepción | KY807229 | KY857952 | | KY858107 | KY858182 |

**S2**. Primers used to amplify and sequence every gene used in this study.

| Gene | *Primer* | Sequences (5'-3') | Reference |
|---|---|---|---|
| COI | FishF1 | TCAACCAACCACAAAGACATTGGCAC | Ward et al. (2005) |
| | FishR1 | TAGACTTCTGGGTGGCCAAAGAATCA | |
| 16S | 16Sa-L | ACGCCTGTTTATCAAAAACAT | Palumbi (1996) |
| | 16Sb-H | CCGGTCTGAACTCAGATCACGT | Palumbi (1996) |
| Cyt B | L14841 | AAATCAAAGCATAACACTGAAGATG | Kocher et al. (1989) |
| | H15915 | CCAATTTGCATGGATGTCTTCTCGG | Irwing et al. (1991) |
| Myh6 | F459 | CATMTTYTCCATCTCAGATAATGC | Li et al. (2007) |
| 1º PCR | R1325 | ATTCTCACCACCATCCAGTTGAA | Li et al. (2007) |
| Myh6 | F507 | GGAGAATCARTCKGTGCTCATCA | Li et al. (2007) |
| 2º PCR | R1322 | CTCACCACCATCCAGTTGAACAT | Li et al. (2007) |
| RAG2 | 164F | AGCTCAAGCTGCGYGCCAT | Oliveira et al. (2011) |
| 1° PCR | RAG2-R6 | TGRTCCARGCAGAAGTACTTG | Lovejoy e Collette (2001) |
| RAG2 | 176R | GYGCCATCTCATTCTCCAACA | Oliveira et al. (2011) |
| 2º PCR | Rag2Ri | AGAACAAAAGATCATTGCTGGTCGGG | Oliveira et al. (2011) |

# Supplement 2

**New species of *Trichomycterus* (Siluriformes: Trichomycteridae) lacking pelvic fins from Paranapanema basin, southeastern Brazil.**

# New species of *Trichomycterus* (Siluriformes: Trichomycteridae) lacking pelvic fins from Paranapanema basin, southeastern Brazil

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## Abstract

A new species of trichomycterid catfish, *Trichomycterus pascuali*, is described from Paranapanema basin and is distinguished from all congeners by the possession of five pectoral-fin rays and the absence of pelvic fin, girdle, and muscles. Additional features further differentiate the new species from the other congeners lacking pelvic fins, *T. candidus*, *T. catamarcensis*, and *T. tropeiro*. The identification of *T. pascuali* is additionally corroborated by genetic divergence based on DNA-barcode analysis. Osteological and myological data unequivocally support the inclusion of the new species in the Trichomycterinae and molecular analyses justify its allocation to the genus *Trichomycterus* rather than *Eremophilus*, a trichomycterine taxon traditionally diagnosed by the lack of pelvic fins. Our genetic analysis further indicates that pelvic fins were independently lost in *E. mutisii*, *T. candidus*, and *T. pascuali*.

**Keywords:** Upper Paraná Basin; Freshwater catfish; pelvic-fin loss; taxonomy.

## Introduction

Trichomycteridae is one of the most species-rich groups of freshwater fishes (de Pinna 1998) in the Neotropics with approximately 290 valid species (Eschmeyer *et al.* 2017) distributed into 41 genera and eight subfamilies. The highest species diversity of the family is concentrated in the Trichomycterinae, especially in the genus *Trichomycterus*, which currently

includes about 190 valid species (Eschmeyer *et al.* 2017), most of which are characterized by restricted geographic distributions and high levels of endemism (de Pinna 1992; Malabarba *et al*. 2009).

In the last decades, studies on the diversity of genus *Trichomycterus* increased with the description of several new species and the identification of smaller putative monophyletic subunits (de Pinna 1989; Costa 1992; Wosiacki 2002; Barbosa & Costa 2003a; Lima & Costa 2004; Wosiacki & de Pinna 2008, Dutra *et al*. 2012; Barbosa & Costa 2010; Fernández & Osinaga 2006; Datovo *et al.* 2012). Nevertheless, the non-monophyletic status of the genus and the often poorly informative characters used to describe species are considered some of the main obstacles to the understanding of the phylogenetic relationships of the Trichomycteridae (de Pinna 1998; Datovo & Bockmann 2010). New and old species descriptions of *Trichomycterus* are frequently characterized by ambiguous diagnoses, vague descriptions and limited taxonomic comparison with little concern about similar species previously described (Garcia-Melo *et al*. 2016). Additionally, the intra- and interspecific phenotypic variability are commonly overlapping among possibly related species, which demand innovative approximations such as the use of molecular tools for the identification of biological species (Hebert *et al*. 2003).

In a collection performed in a small tributary of the Paranapanema basin, Upper Paraná drainage, we collected a trichomycterid species lacking pelvic fins. Morphological survey and genetic analysis based on DNA barcode support the allocation of these specimens in the genus *Trichomycterus*, which is herein described.

**Material and methods**

Sample collection and preservation. All specimens belonging to the new species (Table S1) were collected at the same location. After capture, the individuals were anesthetized and euthanized using a solution of 1% benzocaine. Specimens were preserved in 95% ethanol for molecular studies, and vouchers were fixed in 10% formaldehyde for morphological studies. All samples used in morphological and molecular analysis were cataloged in the the Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista, Botucatu, Brazil (LBP) and remaining types were deposited in the Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP).

Morphological analysis. Measurements and counts were taken from the left side of the specimens. Measurements were made from point to point, taken with a digital caliper to the nearest 0.1 mm and following Tchernavin (1944) for the barbel lengths, Wosiacki and de Pinna (2008) for the peduncle-caudal length and depth and Costa (1992) for the remaining measurements. Morphometrics are given as percentages of standard length (SL), except for subunits of the head region that are expressed as percentages of head length (HL). Specimens were cleared and double stained (c&s) according to anatomical preparations in Datovo and Bockmann (2010), which follow the protocol of Taylor and Van Dyke (1985) with some modifications. Vertebral counts do not include those in the Weberian apparatus and the compound caudal centrum (PU1+U1; = urostyle) is counted as a single element. Nomenclature for laterosensory system and associated pores followed Bockmann *et al.* (2004).

DNA Extraction and Sequencing. Total DNA was extracted from ethanol preserved muscle samples with the DNeasy Tissue Kit (Qiagen), following manufacturer's instructions. Partial sequences of the gene cytochrome c oxidase subunit I (COI) and the 16S genes were amplified using polymerase chain reaction (PCR) using the primers FishF1 (5'-TCAACCAACCACAAA GACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') to COI and 16Sa-L 5'-ACGCCTGTTTATCAAAAACAT-3' and 16Sb-H 5'-CCGGTCTGAACTCAGATCACGT-3' (Palumbi 1996). Amplifications were performed in a total volume of 12.5 µl with 1.25 µl of 10X buffer (10 mM Tris-HCl+15 mM MgCl2), 0.5 µl dNTPs (200 nM of each), 0.5 µl each 5 mM primer, 0.05 µl Platinum® *Taq* Polymerase (Invitrogen), 1 µl template DNA (12 ng), and 8.7 µl ddH2O. The PCR reactions consisted of intial denaturation (4 min at 95ºC) followed by 30 cycles with a chain denaturation (30 s at 95°C), primer hybridization (30-60 s at 54°C) and nucleotide extension 30-60 s at 72°C. All PCR products were first visually identified on a 1% agarose gel and then purified using ExoSap-IT® (USB Corporation) following instructions of the manufacturer. The purified PCR products were sequenced using the "Big DyeTM Terminator v 3.1 Cycle Sequencing Ready Reaction Kit" (Applied Biosystems), purified again by ethanol precipitation and loaded on an automatic sequencer 3130-Genetic Analyzer (Applied Biosystems) in the Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil.

All individual sequences for each species were initially analyzed using the program Geneious v7.1.7 (Biomatters Ltd., Auckland, New Zealand) and the consensus sequences were obtained. All sequences were aligned using MUSCLE (Edgar, 2004) under default parameters and the alignment was inspected by eye for any obvious misalignments such as sequencing errors due to contamination, paralogy or pseudogenes.

Nucleotide variation, substitution patterns, and genetic distances were examined using MEGA 6.0 (Tamura *et al*. 2013). To evaluate the occurrence of substitution saturation in the sequences, we estimated the index of substitution saturation (Iss) described by Xia *et al*. (2003) and the rate of transitions/transversions (Xia and Lemey 2009) in DAMBE 5.2.31 (Xia 2013). The genetic divergence among specimens was estimated using COI matrix and Kimura two parameter (K2P) distance model (Kimura 1980). To infer the congeneric relationships of the new species, we used a concatenated matrix including two loci (cytochrome oxidase I and 16S, 1222 pb) to estimate a maximum likelihood tree using RAxML (Stamatakis 2006) via CIPRES web portal (Miller *et al*. 2010). The number of alternative runs was 100 and the analyses were performed under the model GTR+G.

***Trichomycterus pascuali,* new species**

(Figs. 1–3, Table 1)

**Holotype.** MZUSP 121681, 48.8 mm SL; Brazil: São Paulo State, Municipality of Itatinga; unnamed tributary of Tamanduá river, Paranapanema Basin, 23°13’27.06”S 48°31’45.34”W, G.S.C. Silva, G.J. Costa e Silva, L.E. Ochoa, 14 Feb 2017.

**Paratypes**. LBP 23323, 8 (20.76-46.53 mm SL), MZUSP 121682 (2 ex): 2 CS (20.76, 22.30 mm SL), 1 MS (46.53), 1 genetype (20.76 mm, Genbank accession number: MF034463-COI, MF034462-16S); same data as holotype.

**Diagnosis**. *Trichomycterus pascuali* is readily distinguished from its congeners by two remarkable features: possession of five pectoral-fin rays (*vs.* six or more) and absence of pelvic fin, girdle, and muscles (*vs.* presence). Within *Trichomycterus*, only *T. candidus* (Rio Grande basin, Brazil), *T. catamarcensis* (Laguna Blanca basin, Argentina), and *T. tropeiro* (laguna dos Patos basin, Brazil) also lack pelvic structures. *Trichomycterus pascuali* differs from these three species in having the aforementioned five pectoral-fin rays (*v*s. six, eight, and seven,

respectively) and seven branchiostegal rays (*vs.* eight, five or six, and nine, respectively). It addition, it differs from *T. candidus* by the possession of anterior and posterior cranial fontanels completely separated from each other by a broad epiphyseal bar (*vs.* fontanels partially or completely fused, with epiphyseal bar absent or minute and incomplete) and 37 post-Weberian vertebrae (*vs.* 38–39 in *T. candidus*); from *T. catamarcensis* by the first pectoral fin prolonged as a small filament (*vs.* pectoral filament absent in most specimens), 14 ribs (*vs.* 18–20 in *T. catamarcensis*), 17–18 dorsal procurrent caudal-fin rays (*vs.* 12–13), and 15–16 ventral procurrent caudal-fin rays (*vs.* 11–12); and from *T. tropeiro* by the presence of pectoral filament (*vs.* absence), 17–18 dorsal procurrent caudal-fin rays (*vs.* 13–14), 15–16 ventral procurrent caudal fin rays (*vs.* 9–10), and absence of pores $i_1$ and $i_3$ (*vs.* presence).

Members of the so-called *"Trichomycterus" hasemani* group, a clade of highly derived Amazonian miniature trichomycterids, also exhibit a low count of pectoral-fin rays and one of its species, *"T." anhanga*, further lacks pelvic fins. However, the *"T." hasemani* group is demonstrably a new subfamily that is more closely related to the Vandelliinae-group than to trichomycterines (de Pinna 1989; Dutra *et al.* 2012; Datovo 2014, Wosiacki 2002). The nominal assignment of these species to the genus *Trichomycterus* is merely due to taxonomic inertia, as the formal description of the new subfamily is still underway (by W. Wosiacki and M.C.C. de Pinna, pers. comm.). In any event, *T. pascuali* differs from all species of the *T. hasemani* group by the relatively large body size, with up to 49.4 mm SL (*vs.* miniature body, with less than 18.6 mm SL); presence of an ossified neurocranial roof with two small fontanels (*vs.* roof of neurocranium mostly unossified and forming a single wide fontanel); and 14 ribs (*vs.* 1–3).

**Description.** Morphometric data are given in Table 1. Body elongate, maximum total length 52.2 mm SL, cross section of trunk roughly cylindrical at pectoral girdle and progressively more compressed towards caudal-fin. In lateral view, dorsal profile of body straight from snout tip to interorbital region, slightly convex to dorsal-fin origin, and slightly convex to end of caudal peduncle. Ventral surface of body convex from tip of snout to anal-fin insertion. Caudal peduncle smoothly continues profile of trunk region, with dorsal and ventral profiles slightly convex. Greatest body depth at dorsal-fin origin. Head wide, longer than wide, nearly trapezoidal in dorsal view. Eyes elliptical, covered by thin and translucent skin separate from surface of eyeball. Anterior nostril larger than posterior one, surrounded by fleshy flap of integument continuous with base of nasal barbel. Posterior nostril partially surrounded anteriorly by thin flap of skin. Seven branchiostegal rays visible in c&s individuals. Mouth

subterminal, its corners laterally or slightly posteriorly oriented. Lower lip with conspicuous fleshy lobes along lateral limits, lobes situated internal to base of rictal barbels. Lower lip with anterior and, to lesser degree, anteroventral surfaces covered by small papillae. Anterior margin of upper lip rounded with numerous papillae. Barbels relatively short and with larger bases. Nasal barbel thick always surpassing posterior margin of eye but not reaching opercular patch of odontodes. Maxillary barbel reaching posterior tip of interopercular patch of odontodes. Rictal barbel reaching interopercular patch of odontodes. Interopercular patch of odontodes posteriorly elongate with 11 or 12 conic odontodes imbedded in flesh. Opercular patch of odontodes very small, with 10–12 thin odontodes with thick tips.

Cephalic laterosensory system including incomplete supraorbital and infraorbital canals. Infraorbital canal restricted to its posterior fragment corresponding to pores $i_{10}$ and $i_{11}$. Supraorbital pores $s_1$, $s_3$ and $s_6$. Two bilaterally paired pores $s_6$. Lateral line of trunk reduced, comprising two pores dorsal to middle of pectoral fin. Vertebra 37, ribs 14.

Pectoral fin narrow, with rounded margin. Pectoral-fin rays i,4, first one longest, unbranched, prolonged as small filament. Dorsal fin with distal margin rounded, ii,5-7 rays (modally ii,6,i). Anal fin slightly smaller than dorsal fin, with distal margin rounded, ii,4-5,i rays, origin at vertical through posterior portion of dorsal fin. Pelvic fin, girdle and muscles absent. Caudal fin with distal margin truncate with rounded corners and dorsal and ventral edges slightly convex, i,5+6,i principal rays, branched rays splitting tree times. Procurrent caudal-fin rays 17 or 18 dorsally and 15 or 16 ventrally.

**Color in alcohol.** Two main body-coloration patterns. First pattern (Fig. 2A) observed in specimens 20.8–48.8 mm SL. Ground color of body yellowish. Head covered by irregular brown spots and two regular longitudinal stripes along lateral region of body, one dorsolateral and another midlateral. Ventrolateral row of spots observed at abdominal region and caudal peduncle. Dorsal, anal and caudal fins slightly pigmented.

Second color pattern (Fig. 2B) found in specimens 49.4–52.2 mm SL. Head with some regular brown spots in the dorsal region, gradually lighter laterally; barbels slightly pigmented with the same coloration as head spots. Trunk yellowish with dorsal and abdominal lateral portion of the body covered by irregular spots from the lateral head region to caudal peduncle without defined pattern. Pectoral fin unpigmented, dorsal, anal, and caudal fins rays pigmented with the same coloration as the body spots.

**Etymology**. The name "pascuali" was given in honor to José Pascual Ochoa, L. Ochoa's father.

**Distribution.** Known only from the type locality, a small stream tributary of Tamanduá river, Paranapanema river, Upper Paraná, close to the town of Itatinga, São Paulo state, southeastern Brazil (Fig. 4).

**Habitat, ecological notes, and conservation status**. The new species was discovered in a small stream with clear water and moderate water flow at the altitude of approximately 600 meters above sea level. The specimens were collected under rocks and aquatic vegetation in a muddy bottom area bordered by riparian vegetation. The region is highly impacted by agricultural activity, more specifically eucalyptus monoculture. Rivers in the region are poor in both alpha diversity and specimen abundance. *Trichomycterus pascuali* was the only species of fish found in the type locality and the number of individuals sampled was low. This species has a restricted area of occupancy and its vulnerability to the threats from anthropic impacts could drive the taxon to critically endangered in a short time. According to the IUCN (2001) criteria, *T. pascuali* should be classified in the category vulnerable VU D2.

**Genetic identification.** Aligned sequences were obtained for 573 bp of COI from the reference set of 11 tissue samples of trichomycterines, the translation of sequences did not result in stop codons, indicating the amplification of functional domains. The nucleotide composition of the COI matrix was 29.8% (T), 26.8% (C), 25.1% (A), 18.3% (G), with a proportion of 400 invariables sites, 173 variables sites and 113 parsimony informative sites. The overall mean genetic distance based in the COI gene was 0.138±0.011. The genetic divergence between species was superior to 3% and the results show concordance with the morphological identification. The pairwise species comparison ranged from 0.5 to 24.1% (Table 2) and the divergence genetic within the *Trichomycterus* species was 0.097±0.009. *Trichomycterus pascuali* presented a genetic divergence with its congeneric species from 9.3 to 11.7%, with *T. candidus* and *T. brasiliensis* respectively. the genetic divergence between *E. mutisii* and *T. pascuali* was (10.8%) similar with other species of the *Trichomycterus*, the maximum likelihood analysis using a concatenated matrix with COI and 16S genes (1122 pb, 30.2%(T), 25.0 (C), 23.7%(A), 21.1%(G), with 112 conserved sites, 254 variables sites and 145 parsimony informative sites) shows that *T. pascuali* is more related with *T. inhering* and *T. variegatus* than other genera included in this analysis with a bootstrap support of 77% (Fig. 5).

**Discussion**

Osteological, myological and meristic characters have been used to classify species of the Trichomycteridae (e.g., Baskin 1973; de Pinna 1989; Datovo & Bockmann 2010). The subfamily Trichomycterinae belongs to a well-supported clade that includes all trichomycterid subfamilies except Copionodontinae and Trichogeninae (de Pinna 1992, 1998; Wosiacki 2002; Datovo & Bockmann 2010). In traditional classifications, the Trichomycterinae basically includes taxa lacking the synapomorphies for the TSVSG clade, a lineage formed by the most specialized trichomycterid subfamilies (Sarcoglanidinae, Glanapteryginae, Tridentinae, Stegophilinae, and Vandelliinae; de Pinna 1998). According to the most recent phylogenetic hypothesis based on myological data and proposed by Datovo & Bockmann (2010), the only character that may potentially support the monophyly of the Trichomycterinae is the posterior part of the *levator internus IV* originating from the dorsal face of the posttemporo-supracleithrum. *Trichomycterus pascuali* simultaneously shows this synapomorphic condition and lacks the synapomorphies for the TSVSG clade, so that its allocation in the Trichomycterinae is unequivocal.

Whereas the genus *Trichomycterus* is a non-monophyletic group that includes those trichomycterines lacking the diagnostic features of the remaining genera, *Eremophilus* has been traditionally distinguished by its lack of pelvic fins (Eigenmann 1918; Myers 1944). However, several studies indicate that pelvic fins were lost independently many times during the trichomycterid radiation and that species with such a feature are often more closely related to taxa with pelvic fins (de Pinna 1988, 1989; Barbosa & Costa 2003b; de Pinna & Wosiacki 2003; Datovo 2014). As a result, the genus *Eremophilus* has been eroded by the removal of some species originally assigned to this genus, namely *E. candidus* and *E. camposi* (Miranda-Ribeiro 1949; 1957) that were transferred to *Trichomycterus* (*T. candidus*, de Pinna & Wosiacki 2003) and *Listrura* (*L. camposae*, Glanapteryginae; de Pinna 1988), respectively. In the last decade, two new trichomycterines lacking pelvic fins and girdles were described and allocated to the genus *Trichomycterus* following similar reasoning: *T. catamarcensis* (Fernandéz & Vari 2000) from Catamarca, Argentina, and *T. tropeiro* (Ferrer & Malabarba 2011) from rio das Antas, laguna dos Patos system. As a result, *Eremophilus* is currently a monotypic genus that includes only its type species, *E. mutisii*, from the Bogotá River, Colombia.

The allocation of *Trichomycterus pascuali* to the genus *Trichomycterus* is supported by DNA-barcode and phylogenetic analyses that indicate the new species as being more closely related to other species of this genus than to *Eremophilus mutisii* (Fig. 4, Table 2). These analyses also include *T. candidus*, which is the congener lacking pelvic fins occurring in closest geographical proximity (Rio Grande basin, Upper Paraná) to *T. pascuali* (Rio Paranapanema basin, Upper Paraná). Interestingly, the two species are not resolved as sister taxa, indicating that their pelvic structures were lost independently, thus reinforcing the view that such a feature has an erratic distribution across the Trichomycteridae.

**Comparative material**

*Bullockia maldonadoi* (Eigenmann 1920): LBP 3112 (8), municipality of Monte Aguila, rio Bio Bio, La laja basin, Chile. *Eremophilus mutisii* Humboldt 1805: ANSP 11306, municipality of Ubaté, Laguna de Fuquene, Colombia; MZUSP 35409 (1 c&s), no locality data. *Ituglanis parahybae* (Eigenmann 1918): LBP 10730 (3) municipality of Conceição do Macabu, rio Macabu, Paraíba do Sul basin, Brazil. *Scleronema minutum* (Boulenger 1891): LBP 3310 (11) municipality of Pelotas, arroio dos Corrientes, Atlántico, Brazil. *Trichomycterus areolatus* Valenciennes, 1846: LBP 3118 (29) municipality of Cararrehue, Lago Villarica, rio Maichin, Chile. *Trichomycterus brasiliensis* Lütken, 1874: LBP 11778 (2) municipality of São Roque de Minas, Córrego da Cachoeira, São Francisco basin, Brazil. *Trichomycterus candidus* (Miranda Ribeiro, 1949): LBP 11630 (18) municipality of Uberaba, riacho Grotão, Paraná basin, Brazil; MZUSP 55641 (4, 3 c&s), municipality of Delfinópolis, tributary of rio Grande, Paraná basin, Brazil; MZUSP 55642 (3), municipality of Delfinópolis, tributary of rio Grande, Paraná basin, Brazil; MZUSP 109234 (5), municipality of Carmo do Rio Claro, tributary of rio Grande, Paraná basin, Brazil. *Trichomycterus iheringi* (Eigenmann, 1917): LBP 4512 (7), municipality of Santo André, rio Paranapiacaba, Paraná basin, São Paulo, Brazil. *Trichomycterus tropeiro* Ferrer & Malabarba 2011: MZUSP 108296 (1 paratype), MZUSP 108297 (1 paratype), MZUSP 108298 (1 paratype), municipality of São José dos Ausentes, rio das Antas, Brazil. *Trichomycterus variegatus* Costa 1992: LBP 10289 (3), municipality of São Roque de Minas, Córrego do Lavapés, tributary rio São Francisco, Brazil.

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**Figure 1**. *Trichomycterus pascuali*, MZUSP 121681, holotype 48.8 mm SL; Brazil: São Paulo State, Municipality of Itatinga; unnamed river tributary of Tamandua river, Paranapanema basin, in ventral, lateral, and dorsal views.


**Figure 2**. Body coloration patterns observed in *Trichomycterus pascuali,* LBP 23323, (A) 45.4 mm SL, (B) 52.2 mm SL.

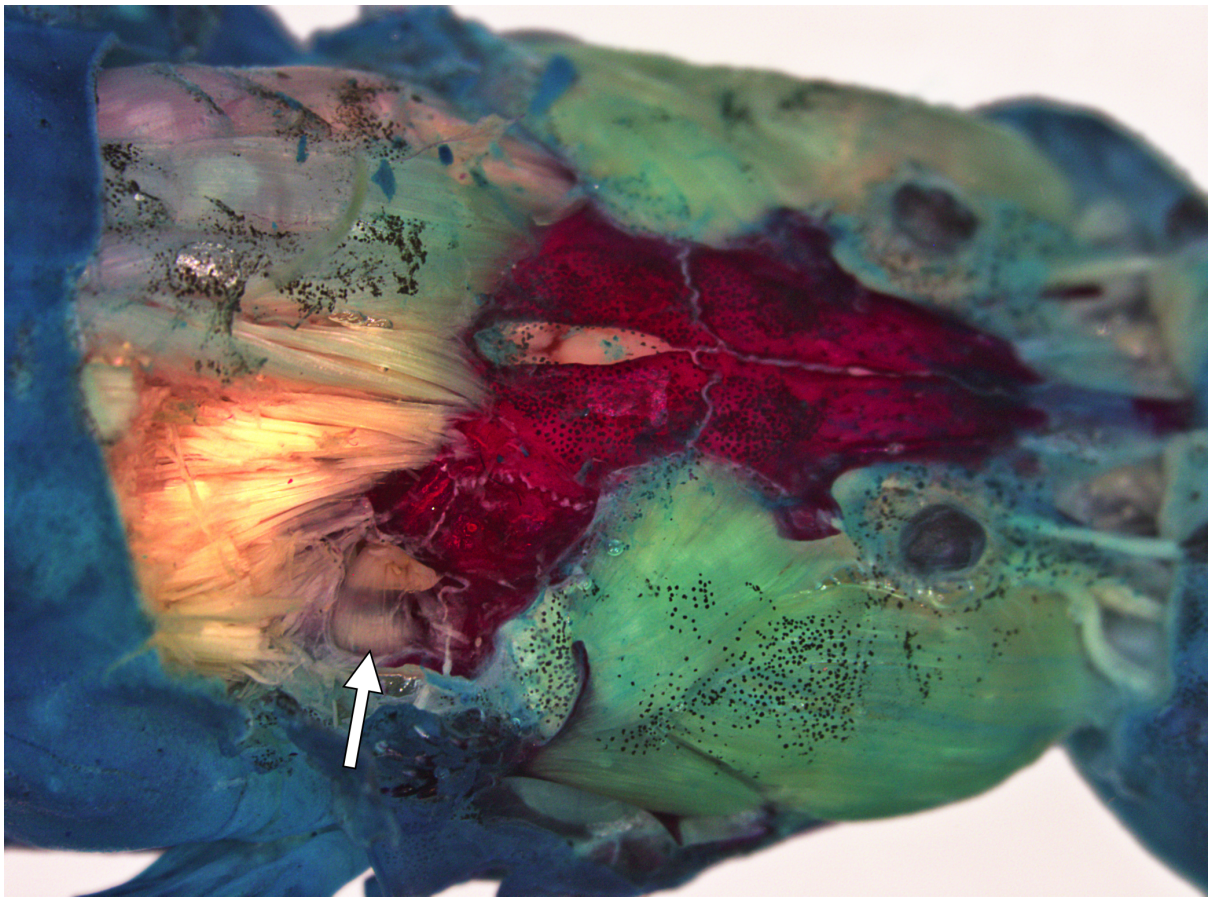

**Figure 3**. Dorsolateral view of head of *Trichomycterus pascuali*, LBP 23323 (51.2 mm SL); arrow indicates *levator internus IV* originating from the dorsal face of posttemporo-supracleithrum.

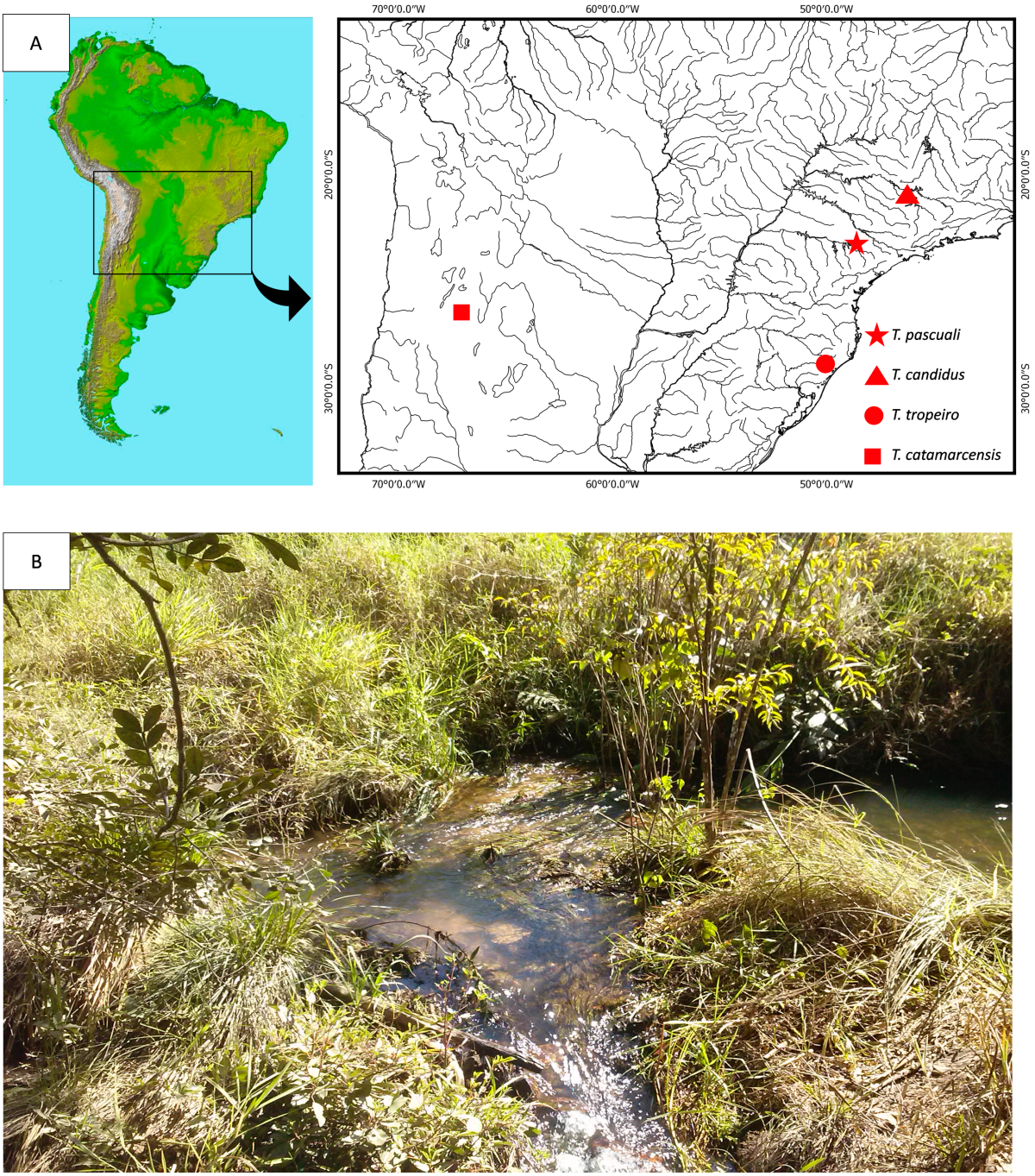


**Figure 4. A.** Map showing the type localities of *Trichomycterus pascuali*, 23°13'27.06"S 48°31'45.34"W, and congeners lacking pelvic fins and girdle. **B**. Type locality of *T. pascuali*, unnamed tributary of Tamanduá river, Paranapanema basin.

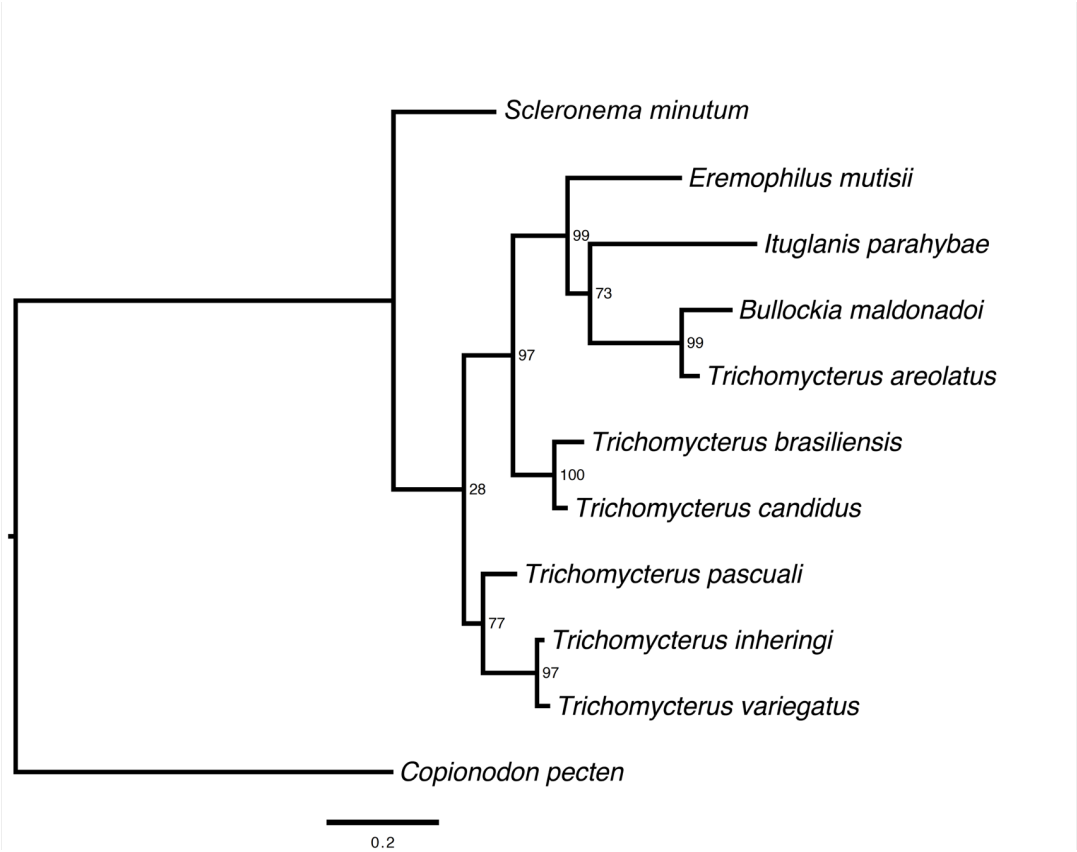


**Figure 5**. Maximum likelihood tree built with concatenated matrix using cytochrome oxidase sub-united I (COI) and 16S genes, showing the relationships of *Trichomycterus pascuali* within Trichomycterinae. Numbers on branches of tree denote bootstrap (B) values.

**Table 1**. Morphometric data for holotype and paratype of *Trichomycterus pascuali* (n=8 including the holotype). SD= Standard deviation.

| | **Holotype** | **Range** | **Mean** | **SD** |
|---|---|---|---|---|
| Standard length (mm) | 48.8 | 31.37-49.36 | 40.4 | 6.1 |
| **Percents of Standard Length** | | | | |
| Head length | 15.5 | 14.30-16.77 | 15.8 | 0.9 |
| Predorsal length | 67.3 | 65.24-68.31 | 67.2 | 1.1 |
| Preanal length | 73.3 | 68.27-73.81 | 71.8 | 1.9 |
| Scapular girdle width | 10.8 | 10.68-12.08 | 11.4 | 0.5 |
| Pectoral-fin length | 8.2 | 8.21- 12.56 | 10.4 | 1.5 |

| | Holotype | Range | Mean | SD |
|---|---|---|---|---|
| Caudal peduncle length | 17.5 | 14.49-21.33 | 17.8 | 1.9 |
| Caudal peduncle depth | 11.5 | 9.66-11.53 | 10.7 | 0.7 |
| Body depth | 13.7 | 8.19-14.87 | 12.5 | 2.0 |
| Length of dorsal-fin base | 10.9 | 6.57-13.13 | 10.3 | 1.8 |
| Length of anal-fin base | 8.4 | 5.77-9.54 | 8.1 | 1.1 |
| **Percents of Head Length** | | | | |
| Head width | 96.0 | 80.66-96.03 | 91.2 | 6.4 |
| Nasal barbel length | 66.1 | 64.20-79.92 | 68.9 | 4.9 |
| Maxillary barbel length | 55.4 | 55.36-76.31 | 62.7 | 7.1 |
| Rictal barbel length | 66.0 | 53.30-71.43 | 63.2 | 6.9 |
| Snout length | 12.9 | 7.14-17.35 | 12.8 | 3.4 |
| Interorbital | 27.7 | 25.27-31.86 | 29.0 | 2.2 |
| Mouth width | 33.0 | 26.72-47.08 | 35.3 | 5.9 |
| Supra-orbital pore distance | 11.7 | 10.52-16.07 | 12.9 | 1.9 |

**Table 2.** Pairwise comparison of nucleotide divergence (K2P distances) at COI from 10 species representatives of four Trichomycterinae genera

| | *I. parahybae* | *T. areolatus* | *B. maldonadoi* | *S. minutum* | *E. mutisii* | *T. brasiliensis* | *T. candidus* | *T. pascuali* | *T. variegatus* |
|---|---|---|---|---|---|---|---|---|---|
| *Ituglanis parahybae* | | | | | | | | | |
| *Trichomycterus areolatus* | 0.145 | | | | | | | | |
| *Bullockia maldonadoi* | 0.144 | 0.047 | | | | | | | |
| *Scleronema minutum* | 0.142 | 0.149 | 0.150 | | | | | | |
| *Eremophilus mutisii* | 0.130 | 0.125 | 0.130 | 0.135 | | | | | |
| *Trichomycterus brasiliensis* | 0.132 | 0.134 | 0.135 | 0.125 | 0.100 | | | | |
| *Trichomycterus candidus* | 0.125 | 0.129 | 0.133 | 0.132 | 0.101 | 0.032 | | | |
| *Trichomycterus pascuali* | 0.162 | 0.150 | 0.150 | 0.108 | 0.117 | 0.095 | 0.093 | | |
| *Trichomycterus variegatus* | 0.183 | 0.138 | 0.132 | 0.112 | 0.127 | 0.100 | 0.106 | 0.057 | |
| *Trichomycterus inheringi* | 0.182 | 0.138 | 0.132 | 0.114 | 0.131 | 0.104 | 0.111 | 0.062 | 0.005 |

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