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Taxonomia integrativa de *Trichomycterus* Valenciennes, 1832 (Siluriformes: Trichomycteridae) das drenagens costeiras do Sul e Sudeste do Brasil

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Dissertação apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Mestre em Biologia Animal.

Área de concentração: Biologia Comparada

Orientador: Dr. Tiago Pinto Carvalho

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Aprovada em ____ de ____________ de _____.

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Dedico este trabalho ao meu querido pai (*in memorium*), Laury Donin.

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Este trabalho é parte integrante dos requerimentos necessários à obtenção do título de mestre em Biologia Animal, e como tal, não deve ser vista como uma publicação no senso do Código Internacional de Nomenclatura Zoológica (artigo 9) (apesar de disponível publicamente sem restrições) e, portanto, quaisquer atos nomenclaturais nela contidos tornam-se sem efeito para os princípios de prioridade e homonímia. Desta forma, quaisquer informações inéditas, opiniões e hipóteses, bem como nomes novos, não estão disponíveis na literatura zoológica. Pessoas interessadas devem estar cientes de que referências públicas ao conteúdo deste estudo, no presente formato, somente devem ser feitas com aprovação prévia do autor.

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SUMÁRIO

CAPÍTULO 1: **A new species of *Trichomycterus* (Siluriformes: Trichomycteridae) from the Ribeira de Iguape River basin, southeastern Brazil, with comments on congeners recorded for the drainage**

CAPÍTULO 2: **Species delimitation in *Trichomycterus* (Siluriformes: Trichomycteridae) from coastal drainages of South and Southeast Brazil**

RESUMO

Trichomycterus é um gênero polifilético que contém espécies com complexas histórias taxonômicas. Esta revisão taxonômica está focada nas espécies de *Trichomycterus* que habitam drenagens costeiras do Sul e Sudeste do Brasil, desde o Sistema do rio Tramandaí (sul) até a bacia do rio Ribeira de Iguape (norte). O objetivo desse estudo é realizar uma revisão taxonômica integrativa do gênero baseado em dados morfológicos e moleculares. Sendo assim, uma nova espécie de *Trichomycterus* foi descrita para a bacia do rio Ribeira de Iguape a o status taxonômico dos seus congêneres presentes na bacia foram discutidos (cap. 1). Adicionalmente, uma extensiva revisão das espécies de *Trichomycterus* das drenagens costeiras do Sul e Sudeste do Brasil foi utilizada para avaliar os limites de espécies em uma análise integrativa e discutimos os processos biogeográficos envolvidos na diversificação das espécies de *Trichomycterus* (cap. 2). Identificamos 12 morfoespécies que habitam as drenagens costeiras do Sul e Sudeste do Brasil baseado em uma combinação de caracteres morfológicos. Confirmamos a presença de seis espécies de *Trichomycterus* anteriormente citadas para a região (*T. davisi*, *T. cubataonis*, *T. guaraquessaba*, *T. jacupiranga*, *T. tupinamba*, and *T. zonatus*), além de *T. alternatus*. Reconhecemos também possíveis novas espécies (*Trichomycterus* sp. ‘ribeira’, *Trichomycterus* sp. ‘malacara’, *Trichomycterus* sp. 'tubarão' and T. aff. *davisi*). Confirmamos a ampla distribuição de *T. balios* tanto do Sistema da Laguna dos Patos (localidade tipo), bacia do rio Uruguay e drenagens costeiras das bacias dos rios Mampituba e Itajaí. Finalmente, sugerimos que a diversificação em *Trichomycterus* do Sul e Sudeste do Brasil parece estar associada a ambos os eventos de troca de fauna, primariamente com trocas de fauna ocorridas entre drenagens costeiras e continentais e secundariamente restritas as paleodrenagens na plataforma continental.

Keywords: Captura de cabeceira, Generalized Mixed Yule-Coalescent (GMYC), Palaeodrenagens, Taxonomia integrativa, Trichomycterinae, Variação de colorido

ABSTRACT

Trichomycterus is a systematic puzzle, highly diverse and contains species with complexes taxonomic histories. This taxonomic review is focused on coastal drainages of South and Southeast Brazil from Tramandaí River System (south) to the Ribeira de Iguape River basin (north). The aim is to provide an integrative taxonomic revision of the genus based on morphological and molecular data. Thus, a new species of *Trichomycterus* was described for Ribeira de Iguape River basin and the taxonomic status of the remaining congeners in the basin is discussed (chapter 1). In addition, an extensive review of species of *Trichomycterus* from coastal drainages of South and Southeastern Brazil was used to evaluate species limit in an integrative analysis, and we discussed the biogeographic processes involved in the recent diversification of *Trichomycterus* species (chapter 2). We identified 12 morphospecies inhabiting coastal drainages of South and Southeast Brazil based on a combination of morphological characters. We confirmed the presence of six species of *Trichomycterus* previously cited for the region (*T. davisi*, *T. cubataonis*, *T. guaraquessaba*, *T. jacupiranga*, *T. tupinamba*, and *T. zonatus*) in addition to *T. alternatus*. We also recognized the putative new species (*Trichomycterus* sp. 'ribeira', *Trichomycterus* sp. 'malacara', *Trichomycterus* sp. 'tubarão' and T. aff. *davisi*. We confirmed the widely distributed of *T. balios* through Laguna dos Patos System (type-locality), Uruguay River basin and two coastal drainages the Mampituba and the Itajaí river basins. Finally, we suggest that the diversification in *Trichomycterus* from South and Southeast Brazil seem to be associated with both events of exchange of fauna, in primary instances with faunal exchanges occurred between coastal and continental drainages and as secondary distributions are contrained by paleodrainage configuration in the atlantic continental shelf.

Palavras chave: Color variation, Generalized Mixed Yule-Coalescent (GMYC), Headwater capture, Integrative taxonomy, Palaeodrainages, Trichomycterinae

INTRODUÇÃO

A diversidade de peixes dulcícolas do Brasil é uma das maiores do mundo, atualmente com mais de 3000 espécies (Reis *et al.*, 2016; Fricke *et al.*, 2019). Isto configura como a maior diversidade de peixes de água doce da região Neotropical e uma das mais ricas do mundo (Reis, 2013; Reis *et al.*, 2016). Conhecer, registrar e entender essa diversidade se faz imprescindível neste momento de crise ambiental e *shortfall* lineana (carência no conhecimento acerca da diversidade de espécies no mundo, incluindo as espécies não descritas e aquelas não catalogadas em bases de dados) e *shortfall* wallaceana (carência no conhecimento sobre a distribuição geográfica das espécies, *i.e.*, conhecimento incompleto sobre a ocorrência das espécies) (*sensu* Hortal *et al.*, 2015) acerca do conhecimento da diversidade biótica da Terra.

Trichomycteridae compreende a família de pequenos bagres neotropicais de água doce, a segunda mais diversa de Loricarioidei, com 307 espécies válidas (Fricke *et al.*, 2019), ocorrendo em quase todas as drenagens neotropicais, desde a Costa Rica até a região da Patagônia (de Pinna & Wosiacki, 2003; Fernandez & Schaefer, 2005; Malabarba & Malabarba, 2014). Grande parte das espécies não ultrapassam 150 mm de comprimento padrão (de Pinna & Wosiacki, 2003); o maior representante, *Trichomycterus rivulatus* Valenciennes, 1846, pode atingir cerca de 380 mm de comprimento padrão (de Pinna & Wosiacki, 2003). Além disso, a família compreende representantes com notáveis estratégias alimentares, desde aqueles que se alimentam de escamas e mucos (Stegophilinae) até os que se alimentam de sangue das brânquias de outros peixes (Vandelliinae). Ocorrem em uma ampla diversidade de habitats, desde águas subterrâneas até riachos e lagos Andinos acima de 4500 m de altitude (Arratia & Menu-Marque, 1984; de Pinna & Wosiacki, 2003; Rizzato *et al.*, 2011).

Trichomycteridae é um grupo monofilética bem corroborada (de Pinna & Wosiacki, 2003; Datovo & Bockmann, 2010; Ochoa *et al.*, 2017a). Atualmente são reconhecidas oito subfamílias, sendo: Copionodontinae (6 espécies), Glanapteryginae (18), Sarcoglanidinae (11), Stegophilinae (29), Trichogeninae (2), Trichomycterinae (221), Tridentinae (11) e Vandelliinae (9). Dentre as sinapomorfias propostas para Trichomycteridae, a estrutura do aparato opercular é, sem dúvida, a mais conspícua, apresentando o opérculo e o interopérculo altamente modificados em uma estrutura compacta suportando odontódeos ligados entre si através de um forte ligamento (de Pinna, 1998). Sua função parece estar ligada à ancoragem e movimentação em substratos duros. Acredita-se que esse sistema permite uma locomoção por impulsos ("elbowing locomotion") na maioria das espécies de Trichomycteridae; auxilia ainda as espécies ectoparasitas de Stegophilinae e Vandellinae a fixarem-se nos hospedeiros e adentrar suas cavidades branquiais (Adriaens *et al.*, 2010) para se alimentarem de sangue.

Trichomycterinae é a maior e mais complexa das subfamílias de Trichomycteridae (de Pinna & Wosiacki, 2003). Encerra 221 espécies (Fricke *et al.*, 2019) compreendidas em oito gêneros: *Rhizosomichthys* Miles, 1943 (1 sp.); *Bullockia* Arratia, Chang, Menu-Marque & Rojas, 1978 (1 sp.); *Eremophilus* Humboldt (1 sp.), 1805; *Hatcheria* Eigenmann, 1909 (1 sp.); *Ituglanis* Costa & Bockmann, 1993 (28 spp.); *Scleronema* Eigenmann, 1917 (3 spp.); *Silvinichthys* Arratia, 1998 (7 spp.); *Trichomycterus* Valenciennes, 1832 (175 spp.) (Eschmeyer *et al.*, 2018). Apenas os representantes de Trichomycterinae estão presentes em toda área de distribuição da família.

As primeiras propostas de relações filogenéticas trazem Trichomycterinae como não monofilética (Baskin, 1973; de Pinna, 1989) em virtude da ausência de sinapomorfias para a subfamília e possível afinidade de algumas espécies dentro de Trichomycterinae com outras subfamílias. No entanto, Arratia (1990), Datovo & Bockmann (2010) e mais recentemente Ochoa *et al.* (2017a) propõem Trichomycterinae como monofilética com base em caracteres morfológicos, miológicos e moleculares.

A maior problemática em torno da sistemática de Trichomycterinae está em *Trichomycterus*. É um gênero merofilético (Fernández & Schaefer, 2009; DoNascimiento, 2015) e o mais rico em espécies da família, com 175 espécies válidas (Fricke *et al.*, 2019). Embora o gênero seja amplamente estudado, com muitas espécies descritas nos últimos anos (Ochoa *et al.*, 2017b; Castellanos-Morales, 2018; Mesa *et al.*, 2018), ainda há muitas incertezas e confusões taxonômicas, principalmente no reconhecimento e delimitação robusta das espécies. De Pinna (1998) enumera três principais motivos a esta problemática: i) condição parafilética do gênero *Trichomycterus*; ii) história taxonômica longa e complexa, com muitos nomes disponíveis e limites diagnósticos tênues e iii) conhecimento incompleto da diversidade real das espécies. Isto, aliado às descrições imprecisas, são fatores que tornam a delimitação precisa dos táxons uma tarefa difícil ou quase impraticável. Nessa perspectiva, há necessidade de integrar dados morfológicos e moleculares no reconhecimento das unidades operacionais, *i.e.*, delimitação precisa das espécies, buscando o entendimento de padrões e processos evolutivos do grupo.

Ochoa *et al.* (2017a) apresentaram, a partir de uma extensa análise baseada em dados moleculares, importante contribuição na elucidação nas relações em Trichomycteridae. Trichomycterinae (*sensu* Datovo & Bockmann, 2010., Ochoa *et al.*, 2017a) é apresentado como grupo monofilético, composto por dois grandes clados: o primeiro inclui as espécies de drenagens atlânticas costeiras e da bacia do alto rio Paraná (*Scleronema* e parte de *Trichomycterus*; clados D4 e D5) e outro com os táxons amazônicos e trans-andinos (*Bullockia*, *Eremophilus*, *Ituglanis* e parte de *Trichomycterus*; clados D1 + D2 + E + D3). Neste mesmo trabalho, *Trichomcyterus* aparece como gênero não monofilético, com espécies do gênero mais próximas a *Bullockia*, *Eremophilus*, *Hatcheria* e *Scleronema* que com os demais *Trichomycterus*, em consonância aos trabalhos anteriores (de Pinna, 1998; Wosiacki, 2002; Datovo & Bockmann, 2010; Ochoa *et al.*, 2017a).

A taxonomia alfa vem sendo amplamente discutida nos últimos anos perante ao desenvolvimento e utilização de novos métodos para o reconhecimento e delimitação de espécies (Wheeler *et al.*, 2004; Dayrat, 2005; Schlick-Steiner *et al.*, 2010; Pereira *et al.*, 2013). No entanto, frente à complexidade da biodiversidade e às limitações que a morfologia como ferramenta de delimitação de espécies apresenta, é necessário abordagens taxonômicas com sinergia de dados. Nesse contexto, diversos trabalhos (Glaw *et al*., 2010; Lumley & Sperling, 2010; Pugedo *et al*., 2016) utilizam o termo taxonomia integrativa (*integrative taxonomy*) para abordar uma taxonomia a partir de perspectivas múltiplas e complementares, normalmente incorporando análises moleculares à hipótese morfológica.

Para as drenagens costeiras do Sul e Sudeste do Brasil (entre as bacias dos rios Tramandaí, ao sul e bacia do rio Ribeira de Iguape, ao norte) delimitadas como área de estudo, estão citadas dez espécies para o gênero *Trichomycterus*: *Trichomycterus balios* Ferrer & Malabarba, 2013; *T. cubataonis* Bizerril, 1994; *T. davisi* Haseman, 1911; *T. guaraquessaba* Wosiachi, 2005; *T. iheringi* (Eigenmann, 1917); *T. jacupiranga* Wosiachi & Oyakawa, 2005; *T. nigricans* Valenciennes, 1832; *T. paolence* (Eigenmann, 1917); *T. tupinamba* Wosiachi & Oyakawa, 2005. Muitas destas espécies foram descritas há muito tempo, com informações insuficientes para diagnosticá-las, impossibilitando reconhecer prontamente seus caracteres diagnósticos, bem como a distribuição geográfica. Além disso, há diversas espécies não descritas para o gênero (*e.g.*, Bertaco *et al*., 2016), evidenciando que a diversidade do gênero está subestimada nesta região.

As drenagens costeiras do Brasil apresentam grande diversidade de peixes de água doce, distribuídas por ecorregiões (Abell *et al*., 2008; Albert *et al*., 2011). Essas drenagens são de grande importância biogeográfica devido a sua ictiofauna altamente endêmica (Ribeiro, 2006). Tal diversidade reflete, provavelmente, aos processos históricos e ecológicos resultantes de milhões de anos de evolução, que moldaram essa fauna (Ribeiro, 2006).

Múltiplos processos geológicos como a elevação de megadomes, processos tectônicos e recentes eventos de erosão (Ribeiro, 2006) vem moldando a costa Atlântica do Brasil desde a separação da América do Sul do continente Africano (~112 Ma) (Lundberg *et al*., 1998). Além disso, movimentos eustáticos resultantes das sucessivas glaciações durante o período Pleistoceno (~2.6 Ma) moldaram a extensão das bacias hidrográficas litorâneas expondo a plataforma e conectando bacias atualmente isoladas, promovendo assim, episódios cíclicos de isolamento e contato entre populações de peixes (Weitzman *et al*., 1988; Hewitt, 2000; Dias *et al*., 2014, Tschá, 2016), como foi demonstrado na estruturação dos padrões de diversidade genética em *Hollandichthys* (Thomaz *et al*., 2015). Nessa área, encontra-se o gênero *Trichomycterus*, com espécies tanto amplamente distribuídas, como também espécies endêmicas ou restritas, muitas vezes limitadas pelas porções altas das cabeceiras, podendo assim, ser considerado um bom modelo para estudos biogeográficos nessa região.

Justificativa

Análises revisivas e filogenéticas são pré-requisitos para responder a uma série de importantes questões sobre os peixes de água doce neotropicais, importantes também para os estudos de ecologia e biologia das espécies. Identificações seguras dos táxons nas mais diversas áreas, importantes para a comparação de dados, são praticamente impossíveis sem revisões taxonômicas (Böhlke *et al.*, 1978). Conhecer a diversidade e os limites diagnósticos de cada táxon, através de diagnoses claras, constitui condição *sine qua non* para tomada de decisões acerca das informações biológicas que as espécies congregam. E os estudos filogenéticos, por sua vez, possibilitam agrupar os táxons consoante sua história genealógica, permitindo que os organismos sejam reconhecidos conforme sua história evolutiva. Além disso, revelam apomorfias que possibilitam identificações seguras das espécies e fornecem subsídios para o entendimento dos padrões biogeográficos de distribuição das espécies.

OBJETIVO GERAL

Considerando-se o cenário descrito acima, o presente trabalho tem como objetivo geral realizar uma revisão taxonômica integrativa das espécies do gênero *Trichomycterus* nas drenagens costeiras do Sul e Sudeste do Brasil integrando dados morfológicos e moleculares.

OBJETIVOS ESPECÍFICOS

- Delimitar morfologicamente e geneticamente as espécies na região de estudo;
- Descrever a distribuição geográfica das espécies na região de estudo;
- Descrever possíveis espécies novas encontradas na região;
- Compreender a história evolutiva do gênero *Trichomycterus* nas drenagens de interesse, bem como os processos biogeográficos envolvidos nos padrões de distribuição das espécies.

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A new species of *Trichomycterus* (Siluriformes: Trichomycteridae) from the Ribeira de Iguape River basin, southeastern Brazil, with comments on congeners recorded for the drainage

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A new species of *Trichomycterus* (Siluriformes: Trichomycteridae) from the Ribeira de Iguape River basin, southeastern Brazil, with comments on congeners recorded for the drainage

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Running head. New species of *Trichomycterus* from the Ribeira de Iguape River basin

Abstract

A new species of *Trichomycterus* from Ribeira de Iguape River basin, São Paulo State, southeastern Brazil, is revealed using an integrative taxonomy approach. *Trichomycterus* sp. n. is distinguished from congeners of the Southeast and Northeast Brazil by the outer layer coloration composed of scattered round spots smaller or equivalent in size to the eye circumference (*vs.* round spots absent or, if present, clearly larger than eye circumference or arranged in longitudinal rows). The taxonomic status of the remaining congeners in the Ribeira de Iguape River basin is discussed and an identification key provided.

Keywords. Color variation, Identification key, Incomplete lineage sorting, Integrative taxonomy, *Trichomycterus zonatus*.

Palavras-chave. Chave para identificação, Sorteio de linhagens incompleto, Taxonomia integrativa, *Trichomycterus zonatus*, Variação de colorido.

Introduction

Trichomycteridae is the second richest family of Siluriformes, with about 300 valid species (Fricke *et al.*, 2019), widely distributed in the Neotropical region from Costa Rica to Argentina, with species distributed in both cis- and trans-Andean drainages (de Pinna, Wosiacki, 2003; Fernandez, Schaefer, 2005; Malabarba, Malabarba, 2014). Trichomycterinae is the most species-rich subfamily, with 233 species (Fricke *et al.*, 2019), and the more complex inter-specific relationships (de Pinna, Wosiacki, 2003). This group comprises the problematic genus *Trichomycterus* with a complex taxonomic history and a non-monophyletic status that essentially includes those species lacking the diagnostic characters of other genera of Trichomycterinae (Baskin, 1973; de Pinna, 1989, 1998; Datovo, Bockmann, 2010; Ochoa *et al.*, 2017a; Katz *et al*., 2018).

The Ribeira de Iguape River basin is comprised within the Atlantic Rainforest, a highly impacted environment. Estimates of the remaining forest in this region vary from 1% to 12% (Gentry, 1996; Brown & Brown, 1992; Myers *et al*., 2000; Oyakawa *et al*., 2006; Menezes *et al*., 2007). Knowledge of the biological diversity of this area is eminent to preservation, conservancy, and management. The first ichthyological surveys in the basin date from early XX century with the expeditions of John Haseman and Richard Krone (Haseman, Eigenmann, 1911; Miranda-Ribeiro, 1908a, 1908b). The trichomycterids collected by them were posteriorly studied by Miranda-Ribeiro (1908a, 1908b) and Eigenmann (1917, 1918) who described three new species and identified others as already described taxa (Tab. 1). Along the XXI century, two new records of *Trichomycterus* were pointed out to the Ribeira de Iguape River basin (*cf.* de Pinna, Wosiacki, 2003; Menezes *et al.*, 2007), as well as two new endemic species to this system were described (Wosiacki, Oyakawa, 2005) (Tab. 1). However, some of these records are doubtful and the morphological limits among the species unclear.

Reviewing collections in the Ribeira de Iguape River basin and adjacent drainages we identified a new endemic species of *Trichomycterus* supported by morphological and molecular data, which is described herein. In addition, we confirmed the presence of *T. alternatus* and discuss the taxonomic status of the remaining congeners recorded for the system providing an identification key.

Material and Methods

Samples. A large portion of the samples for this study were recently collected by electric fishing method. After the capture, all specimens were anesthetized and euthanized using a concentrated Eugenol solution (Lucena *et al*., 2013). Tissue samples from fin clips or muscle were preserved in 96% ethanol for molecular analysis and vouchers were fixed in 10% formaldehyde for morphological analysis (preserved in 70% ethanol). All tissue samples are vouchered in fish collections (**S1**). Samples used in this study were examined from the following institutions: DZSJRP, Coleção de Peixes da Universidade Estadual Paulista "Júlio de Mesquita Filho", São José do Rio Preto; FMNH, Division of Fishes, Department of Zoology, Field Museum of Natural History, Chicago; MCN, Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre; MCP, Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre; MNRJ, Museu Nacional, Rio de Janeiro; MPEG, Museu Paraense Emílio Goeldi, Laboratório de Ictiologia, Belém; MZUEL, Museu de Zoologia da Universidade Estadual de Londrina, Londrina; MZUSP, Museu de Zoologia da Universidade de São Paulo, São Paulo; UFRGS, coleção de peixes do Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre; Other abbreviations in the text are: c&s (cleared and stained specimens), HL (head length), SL (standard length), tec (specimens with tissue samples available). Species incorporated in the phylogenetic analysis were identified based on original descriptions, and other papers with informative data (*i.e.*, Eigenmann, 1918; Bizerril, 1994; Wosiacki, Garavello, 2004; Wosiacki, 2005; Wosiacki, Oyakawa, 2005; Ferrer, Malabarba, 2011, 2013; Datovo *et al.*, 2012; Nascimento *et al.*, 2017; Teran *et al.*, 2017) and comparisons with type specimens (see comparative material). In addition, sequences of some species were obtained from GenBank, which we maintained their original identification and provided their respective reference in the cladogram. The nomenclature used for the clades within *Trichomycterus* followed Ochoa *et al*. (2017a).

Morphological procedure. Morphometric data were taken point to point with digital caliper (precision of 0.1 mm) following Costa (1992), except for Tchernavin (1944) for length of barbels; Wosiacki, de Pinna (2008) for length and depth of the caudal peduncle and for supraorbital pore s6 distance; Ferrer, Malabarba (2011) for scapular girdle width; and Ferrer *et al.* (2015) for length of interopercular. Nomenclature of bones and cartilage followed Bockmann *et al.* (2004), except for the use of parurohyal that followed Arratia, Schultze (1990) and sesamoid supraorbital according to Adriaens *et al*. (2010). Laterosensory canal system and associated pores followed Rizzato, Bichuette (2016). Osteological information of the new taxon was taken from two c&s specimens according to the method of Taylor, van Dyke (1985) and posteriorly dissected. Vertebral counts excludes those in the Weberian complex and the compound caudal centrum, (PU1+U1 = urostyle), is counted as one element. Distribution map was made using software Quantum GIS version 1.8.0 (Scherman *et al.*, 2016). Counts of unbranched and branched rays, as well as the analysis of the laterosensory canal system were done in all type specimens. Principal Component Analysis (PCA) was used to check overall morphometric variation among samples and species. Measurements were treated using Aitchinson (1986) log-ratio transformation, thus the first principal component that mostly accounts for size variation (Strauss, 1985) was maintained. Principal component analysis (PCA) of the Aitchinson-transformed data were done using Past (Hammer *et al.*, 2001) and multiple linear regressions of selected characters were applied to describe morphometric differences among species using Sigma Plot and the raw data (Brannan *et al*., 2000). In the diagnosis, the new species was compared with species that are included in clade D5 (Ochoa *et al.*, 2017a) and the *Trichomycterus sensu stricto* (Katz *et al*., 2018) in addition to other species of *Trichomycterus* from Southeast and Northeast Brazil that are putatively related with these clades. Description of coloration in life was based on photographs taken from freshly collected and anesthetized specimens using the phototank-immersion method following Sabaj Pérez (2009).

Molecular procedure. DNA extractions from ethanol preserved samples followed a modified CTAB protocol (Doyle, Doyle, 1987). Partial sequences of the two mitochondrial genes Cytochrome Oxidase I (COI) (Ivanova *et al*., 2007; Melo *et al*., 2011) and Cytochrome B (CYTB) (Barros *et al*., 2015; Unmack *et al*., 2009) were amplified by polymerase chain reaction (PCR) using the primers cited in the supplementary file 2 (S2). Amplifications were performed in a solution with a total volume of 20 µL with 13.8 µL of ultra pure water; 2.0 µL of 10x PCR buffer; 0,2 µL each 5 mM primer; 0.2 µL of Taq DNA Polymerase Ludwig Biotec; 1.0 µL of DNA template (10-50 ng). The PCR reactions consisted of initial denaturation of 94°C for 5 min, 35 cycles with a chain denaturation of 94°C for 45s, primer hybridization of 48°C to 54°C for 1,5 min and final extension of 72°C for 10 min. PCR products were checked by electrophoresis in agarose gel, purified using ExoSap Ilustra ® Exonuclease I and Shrimp Alkaline Phosphatase- GE Healthcare) following manufacturer instructions. The purified PCR products were sequenced in both directions at Macrogen Korea. Sequences were edited in Geneious v8.0 (Biomatters Ltd., Auckland, New Zealand)

and the consensus sequences were obtained. DNA sequences from samples used in molecular analysis were deposited in GenBank (respective vouchers in **S1**). Additional sequences added to our analysis were taken from GenBank from the works of Nascimento *et al*. (2017), Ochoa *et al*. (2017a), Ochoa *et al*. (2017b) and Pereira *et al*. (2013). Sequences were aligned in MUSCLE (Edgar, 2004) algorithm within Geneious v8.0 under default parameters. The genes COI and CYTB were concatenated into a single matrix for phylogenetic analysis. The single matrix was partitioned by gene and codon positions to determine codon-specific models and best partition scheme of molecular evolution in PartitionFinder v1.1.1 (Lanfear *et al.*, 2012) under the Bayesian Information Criterion (BIC) (Tab. 2). Phylogenetic relationships were inferred by Bayesian Inference using the uncorrelated relaxed molecular clock (lognormal) in BEAST v.1.7 (Drummond *et al*., 2012), programmed to run for 10 million generations of MCMC chains (saving one best tree every 10 thousand generations), under a Birth-Death tree prior. The first one million generations (10%) were discarded as burn-in and the remaining trees were used to summarize the results of the Bayesian analysis using the Maximum Clade Credibility Tree in TreeAnnotator. The concatenated data set was analyzed assuming an evolutionary rate of 0.01/site/Myr following rates previously proposed to mitochondrial markers in fishes (Bermingham *et al.*, 1997; Reeves & Bermingham, 2006; Ornelas-Garcia *et al.*, 2008) including studies in the Atlantic Rainforest region (Hirschmann *et al.*, 2015; Thomaz *et al.*, 2015).

Results

We confirmed the presence of four species of *Trichomycterus* previously cited for the Ribeira do Iguape River basin (*T. davisi*, *T. jacupiranga*, *T. tupinamba*, and *T. zonatus*) in addition to *T. alternatus* and the new taxon described below.

Trichomycterus sp. n. is endemic to the Ribeira do Iguape River basin being largely distributed in its headwaters (Fig. 1). *Trichomycterus jacupiranga* is so far known for its type locality, córrego do Queimado, a stream located in the right bank of the Ribeira de Iguape River. The distribution of *Trichomycterus tupinamba* is extended to north of the basin (including the Juquiá River) in addition to Betari and Iporanga rivers, two neighbor tributaries of the left bank of the Ribeira de Iguape River mentioned in its description. As so far, *Trichomycterus zonatus* has a restrict distribution in the basin, found only in the Betari River and its small tributaries (see below for more details about paratype from Santos, SP). *Trichomycterus davisi* is restricted to few records in the headwaters of Ribeira de Iguape River basin, near to its watershed limits with its neighboring Paranapanema and Iguaçu river basins. *Trichomycterus alternatus,* in contrast to these congeners, is abundant and has the widest distribution in the Ribeira de Iguape River basin, covering practically all its headwaters, and also occurring in the adjacent tributaries of the Paranaguá Bay.

Another putative new species morphologically not aligned with none of these species listed above is also recorded from this region. Since just two specimens (MZUSP 51971) are known for this morphospecies, additional investigation and collections are needed to evaluate its taxonomical status.

Molecular approach. We investigated the phylogenetic position and genetic distinctiveness of the new species, sympatric congeners and other *Trichomycterus* species using two molecular markers (Fig. 2). A total concatenated matrix of 1,866 bp. was obtained for the mitochondrial genes COI (789 bp.) and CYTB (1,077 bp.). In the concatenated dataset, 567 sites were variable, 445 were parsimony informative and 1,299 were invariant. The nucleotide composition was of 28.8% (T), 28.4% (C), 25.4% (A) and 17.5% (G). The best-fit model of nucleotide evolution estimated by PartitionFinder by gene and codon positions are showed in

Tab. 2. *Trichomycterus* sp. n., *T. alternatus*, and *T. jacupiranga* are strongly supported in the clade D5 of Ochoa *et al*. (2017a) (Fig. 2) whereas *T. davisi*, *T. tupinamba*, and *T. zonatus* are included in the clade D4 of Ochoa *et al*. (2017a) (S3). *Trichomycterus* sp. n. is sister group to a large assemblage of *Trichomycterus* species from Atlantic coastal drainages of eastern and southern Brazil including *Trichomycterus albinotatus*, *T. alternatus*, *T.* aff. *alternatus*, *T. brasiliensis*, *T. candidus*, *T.* cf. *auroguttatus*, *T.* cf. *mimosensis*, *T.* cf. *pradensi*s, *T. giganteus*, *T. immaculatus sensu* Pereira *et al*. (2013), *T. immaculatus sensu* Ochoa *et al*. (2017a), *T. jacupiranga*, *T. nigroauratus*, and *T. pirabitira*. The two specimens available of *T. jacupiranga* are nested within samples of *T. alternatus* from the Ribeira de Iguape River basin with high support (Posterior Probability; PP = 1.0). Sequences from *T. jacupiranga* of both COI and CYTB are highly similar or identical (between 99.9-100%; S3) to the sympatric specimens of *T. alternatus* (S4). Sequences included herein of *T. alternatus* from tributaries of the Paranaguá Bay represent the southern limit of the species included in clade D5 of Ochoa *et al*. (2017a) within the coastal region. The other species of *Trichomycterus* from Ribeira do Iguape River basin are included in clade D5 of Ochoa *et al*. (2017a) (see also Katz *et al*., 2018). *Trichomycterus davisi* was recovered as a polyphyletic assemblage suggesting it as a species complex (S2; see also chapter II for more details). *Trichomycterus zonatus,* which is restricted to Betari River basin is also included in clade D5 and is sister group to one lineage of *T. davisi* from Iguaçu River basin being both sister group of *T. pascuali*, a species from upper Paraná River basin (S3; see chapter II). *Trichomycterus tupinamba*, which is endemic to Ribeira de Iguape River basin, is closely related to *T. guaraquessaba* from tributaries of the Paranaguá Bay (S3; see chapter II).

Morphological approach. Due to their genetic similarity, we investigated morphological differences between *T. jacupiranga* and populations *T. alternatus* from the Ribeira de Iguape River basin and another neighboring drainage (Paranaguá Bay) (Tab. 3). Principal component analyses (PCA) on the Aitchinson-transformed data showed overlap of the populations of *T. alternatus* and specimens examined of *T. jacupiranga*. PC1 explains 22.65 and PC2 17.58% of the variation for analyzed specimens (Fig. 3). Although having some overlap, the length of the interopercular and its shape in *T. jacupiranga* is emphasized (Tab. 3; Figs. 4-5). The examination of color pattern (*in vivo* and in alcohol preserved specimens) evidence two very distinct color patterns (Fig. 6). *Trichomycterus alternatus* has dorsal black bars variable in width extending laterally (saddle-like blotches) (Fig. 6a), contrasting with the homogeneous grayish coloration in *T. jacupiranga* (Fig. 6b).

We also investigated morphological differences between the endemic *Trichomycterus zonatus* and the widely distributed *T. alternatus*. These two species can be easily distinguished by external characters as the pectoral fin with eight rays being the first, usually, prolonged as a filament in *T. alternatus* (Fig. 7a; *vs.* the pectoral fin with seven rays with the first never prolonged as a filament: Fig. 7b), the laterosensoy system with the antorbital segment of infraorbital canal present with the pores “i1” and “i3” and a single median pore “s6” in the supraorbital canal in *T. alternatus* (Fig. 8; *vs.* the antorbital segment of infraorbital absent and two pores “s6” in the supraorbital canal), a broad interspace between the insertions of the pelvic fins in *T. alternatus* (Fig. 9; *vs.* interspace reduced with the pelvic-fin insertions almost in contact). In addition, the pattern color is distinct between the two species: *T. alternatus* has dorsal black bars extending laterally resembling saddles (Fig. 6a) whereas the black blotches of *T. zonatus* are variable in shape but not forming marked saddles (Fig. 10c).

Specimens of *T. davisi* from Ribeira de Iguape River basin (Fig. 10a) were identified based on absence of morphological differences with the type material and populations analyzed by Nascimento *et al*. (2017) from Iguaçu and Tibagi river basins. According to these authors, *T. davisi* has an intraspecific color variation, impossible to evaluate in the Ribeira de Iguape River basin in view of few samples available for now. In addition, these three allopatric populations were found as a non-monophyletic unit in the molecular analysis and further studies are needed to resolve their species identities. *Trichomycterus davisi* and *T. zonatus* are very similar externally, however, some osteological aspects are distinct, such as the number of branchiostegal rays (9-10 *vs.* 8) and the first pterygiophore of the anal fin position (anterior to haemal spine of 22-23th vertebra *vs.* anterior to haemal spine of 21th vertebra). Additionally, it is possible to diagnose the two species analyzing the color pattern of adults: *T. davisi* has the lateral surface with smaller and more coalescent black blotches forming a mottled pattern (Fig. 10a) whereas *T. zonatus* has well defined and larger black blotches, if coalescent, only in the midline of the caudal peduncle (Fig. 10c). Additional comments are provided in the "Discussion", and see also chapter II for comments on *T. davisi*, *T. zonatus*, and *T. tupinamba*.

Identification key for species of *Trichomycterus* from Ribeira de Iguape River basin

1a. First pectoral-fin ray not prolonged as a filament……………………………………… 2

1b. First pectoral-fin ray prolonged as a filament………………………………………….. 4

2a. Antorbital segment of infraorbital canal with pores i1 and i3 present; lateral surface of body with a longitudinal row of black spots forming a stripe in at the anterior region……….. *Trichomycterus tupinamba* (Fig. 10b)

2b. Antorbital segment of infraorbital canal absent; lateral surface of body with black blotches variable in shape and size not forming a row of tiny black spots medially…………………...... 3

3a. Body with black blotches forming a mottled pattern along the lateral surface……………………………………………………….. *Trichomycterus davisi* (Fig. 10a)

3b. Body with black blotches variable in shape and size not forming a mottled pattern along the lateral surface…………..……………………………… *Trichomycterus zonatus* (Fig. 10c)

4a. Body with scattered black spots smaller or equivalent in size to the eye circumference; pectoral fin usually with I+8-9 rays, rarely I+7……........ *Trichomycterus* sp. n. (Fig. 11-12)

4b. Body homogeneously gray or with dorsal black bars variable in width extending laterally; pectoral fin with I+7 rays…………………………………………………………………... 5

5a. Body homogeneously gray……………………….. *Trichomycterus jacupiranga* (Figs. 6b)

5b. Body with dorsal black bars variable in width extending laterally ……………………….……………………………… *Trichomycterus alternatus* (Fig. 6a)

***Trichomycterus* sp. n.**

Figs. 11-12, Tab. 4

Pygidium iheringi non Eigenmann, 1917. –Eigenmann (1918: 330), listed in the synonym of *Pygidium iheringi* (=*Trichomycterus iheringi*) as *Trichomycterus punctulatus* and *T. dispar*.

Trichomycterus dispar (*non* Tschudy, 1846). –Miranda Ribeiro (1908a), listed and with commented on its occurrence in the Ribeira de Iguape River basin. –Miranda Ribeiro (1908b: 5, fig. 1), listed and corrected of *Trichomycterus dispar* to *T. punctulatus* from Ribeira de Iguape River basin. –Miranda Ribeiro (1911:222), briefly described with figure (Est. 35 - fig. 2).

Trichomycterus punctulatus non Valenciennes, 1846. –Miranda Ribeiro (1908b: 5, fig.1), listed and compared with the previously listed *Trichomycterus dispar* from Ribeira de Iguape River basin.

Holotype. UFRGS 24536, 114.7 mm SL, Brazil, São Paulo State, Cajati, córrego do Queimado, stream tributary of rio Jacupiranguinha, Ribeira de Iguape River basin, 24°48’07’’S 48°13’47’’W, 14 Dec 2017, L. Donin, N. Pio, P. M. Ito & T. P. Carvalho.

Paratypes. All from Brazil. São Paulo State, Ribeira de Iguape River basin. MNRJ 17719, 5, 89.5-46.0 mm SL, Iporanga, stream tributary of rio Ribeira de Iguape, 24°35’06’’S 48°50’04’’W, 12 Nov 1991, C. R. S. F. Bizerril. MZUSP 60130, 9, 87.14-73.44 mm SL, Iporanga, rio dos Pilões, 24°29’09’’S 48°29’01’’W, 29 Jan 2000, P. Gerhard & R. Moraes. UFRGS 24536, 18 (4 tec, 1 c&s), 124.5-60.8 mm SL, collected with holotype. UFRGS 24537, 19 (4 tec, 1 c&s), 81.9–54.9 mm SL, Iporanga, rio Betari, 24°36’13’’S 48°36’40’’W, 13 Dec 2017, L. Donin, N. Pio, P. M. Ito & T. P. Carvalho. UFRGS 24539, 3 (2 tec), 94.4-80.6 mm SL, Itariri, rio das Pedras, tributary of rio do Azeite, 24°19’51’’S 47°11’06’’W, 14 Dec 2017, L. Donin, N. Pio, P. M. Ito & T. P. Carvalho. UFRGS 24548, 6 (2 tec), 90.7-56.9 mm SL, Juquiá, tributary to rio Juquiá, 24°08’20’’S 47°38’35’’W, Dec 2017, L. Donin, N. Pio, P. M. Ito & T. P. Carvalho.

Diagnosis. *Trichomycterus* sp. n. is distinguished from more closely related congeners of the Southeast and Northeast Brazil according to Ochoa *et al.* (2017a; D5 clade) and Katz *et al.* (2018: *Trichomycterus sensu stricto* clade) by having the outer layer coloration composed of scattered round dark brown or black spots smaller or equivalent in size to the eye circumference (Figs. 11-12; *vs.* round spots absent or, if present, clearly larger than eye circumference or arranged in longitudinal rows). The high number of pectoral-fin rays (modally I+8 *vs*. I+7 or less) also distinguishes *Trichomycterus* sp. n. from most of these more closely related congeners with the exception of *T. caipora*, *T. giganteus*, *T. immaculatus*, *T. nigricans* and *T. pradensis*.

Description. Morphometric data in Tab. 4. Maximum body length observed 124.5 mm SL. Body robust, elongate, trunk roughly cylindrical and gradually compressed towards caudal fin. Dorsal profile of trunk convex along anterior half of body to insertion of dorsal fin. Ventral profile of trunk straight to slightly convex. Dorsal and ventral profiles of caudal peduncle straight to slightly concave depressed on mid-length.

Head depressed, trapezoidal from dorsal view, wider posteriorly. Dorsal and ventral profile of head straight to slightly convex. Snout straight to slightly round from dorsal view. Eyes, round to elliptical, located dorsolaterally on anterior half of head at same horizontal line of nasal barbel. Eyes covered with thin and transparent skin, without free orbital rim.

Anterior nostril approximately same size as posterior nostril, surrounded by tubular flap of integument posterolaterally and continuous with nasal barbel base. Posterior nostril surrounded anterolaterally by thin flap of integument. Gill openings not constricted, united with isthmus anteriorly forming a free fold reaching pectoral-fin insertion. Mouth subterminal and slightly curved with corners posteriorly oriented. Upper lip with thicker distally. Lower lip with fleshy lobes laterally, internally united to origin of rictal barbels. Lips with numerous rounded papillae of approximately same size.

Barbels with large bases, tapering gradually towards tips. Nasal barbel emerging from lateral region of anterior nostril, its posterior tip when adpressed to body, reaching between pores infraorbital 10 and infraorbital 11. Maxillary and rictal barbels about same size, its posterior tip when adpressed to body, reaching anterior portion of interopercular patch of odontodes.

Mesethmoid with anterior margin straight to slightly concave and cornua rounded at distal margins, width of their bases similar to their length. Anterior cranial fontanel restricted to drop-shaped opening situated between frontals and epiphyseal bar. Posterior cranial fontanel long and wide extending from posterior portion of frontals to parieto-supraoccipital. Epiphyseal bar longer than wide.

Antorbital slightly elongated extending over at least one third of autopalatine.

Sesamoid supraorbital elongated, straight rod shape, without lateral processes. Sphenotic, prootic, and pterosphenoid totally fused. Vomer arrow-shaped with long posterior process extending to parasphenoid. Parasphenoid with long and pointed process extending to basioccipital. Weberian capsule with small lateral opening and with anterior margin fused to basioccipital.

Premaxilla rectangular with conical teeth similar in size and distributed approximately in three irregular rows. Maxilla boomerang-shaped with approximately same size of premaxilla. Autopalatine with lateral margin straight; anterior margin slightly concave followed by convex medial margin and posterior elongated process extending over posterior portion of metapterygoid. Metapterygoid large and laminar, connected with quadrate through cartilage. Quadrate L-shaped with concavity in anterior portion followed by process in posterior portion. Hyomandibula well-developed with prominent notch in dorsal margin. Opercular patch of odontodes oval to rounded, with anterior process directed upward, with 28-29 conical odontodes arranged in six irregular rows (counted in two c&s specimens). Interopercular patch of odontodes elongate with at least 54-62 conical odontodes posteriorly concentrated, arranged in three irregular rows (counted in two c&s specimens). Odontodes of both opercular and interopercular patches gradually curving medially and increasing in size posteriorly.

Hyoid arch with ventral hypohyal trapezoid-shaped. Anterior ceratohyal elongate and widening at anterior and posterior limits. Posterior ceratohyal short with posterior rounded process. Seven branchiostegal rays: three in contact with anterior ceratohyal, two with interceratohyal cartilage, one with posterior ceratohyal and one lateral free to posterior ceratohyal. Last four branchiostegal rays widening distally. Parurohyal with expanded anterior head, two elongate lateral processes with wide bases and decreasing in width distally with rounded tips and laminar, elongate, narrow posterior process located between rounded tips;

medial foramen of hypobranchial artery; posterior process smaller than lateral processes.

Gill arches with basibranchials 2 and 3 elongated, connected to each other by cartilage; basibranchial 2 slightly wider and longer than basibranchial 3. Basibranchial 4 hexagonal and entirely cartilaginous. Hypobranchial 1 elongated, slightly larger than basibranchial 2 with cartilage at tips. Hypobranchials 2 and 3 approximately with same size and with narrow anterolateral ossified processes with large area of cartilage distally. Hypobranchial 4 absent. Five elongate ceratobranchials with cartilage at tips. Ceratobranchial 1 and 4 with straight margin. Ceratobranchial 2 and 3 with distinct concavity in posterior margin, more proeminent on ceratobranchial 3. Ceratobranchial 5 with approximately 31 conical, elongated, and pointed teeth. Four epibranchials; first three elongated and narrow with cartilage at tips. First epibranchial with elongated and pointed process at mid-length; epibranchial 2 with small process at mid-length; epibranchial 3 with distinct curved process in posterior margin. Epibranchial 4 rectangular, wider posteriorly. Pharyngobranchial 3 elongated, shorter than hypobranchial 1, with cartilage at tips. Pharyngobranchial 4 ossified and connected to curved plate with approximately 22 conical, elongated, and pointed teeth arranged in up to two irregular rows; teeth increasing in length posteriorly.

Pectoral fin with distal margin rounded, one unbranched ray prolonged as short filament (two of 55 examined specimens with unbranched rays not prolonged on both sides), and seven (2), eight (45), nine (8), branched rays. Axillary pore present near opercular opening. Pelvic fin inserted anterior to origin of dorsal fin with distal margin convex reaching to anterior margin of urogenital papilla. Inner margin of pelvic fins distant from each other. Pelvic fin with one unbranched ray and four branched rays (two of 55 with one unbranched ray and three branched rays). Dorsal fin with distal margin truncate to slightly convex with three procurrent rays, two unbranched rays (one of 55 specimens with one unbranched ray) and seven branched rays (one of 55 specimens with six and one with eight rays). Origin of dorsal fin located anteriorly to anal fin with posterior tip when adpressed to body reaching at vertical through near of posterior portion of pelvic fin. Dorsal fin with eight and nine pterygiophores, first inserted anterior to neural spine of 17th vertebrae.

Anal fin elongate with distal margin convex and slightly smaller than dorsal fin, with three procurrent rays, two unbranched rays and five branched rays (55). Origin of anal fin located posterior to base of dorsal fin. Anal fin with six pterygiophores, first inserted anterior to haemal spine of 22th vertebrae.

Caudal fin with distal margin truncated or slightly forked. Procurrent caudal-fin rays 15 and 12 dorsally and 11 and ten ventrally. Upper plate with one unbranched ray and five branched rays with separate uroneural; hypural 3 autogenous and hypurals 4 and 5 fused. Single lower caudal plate with two unbranched rays and six branched rays with parhypural and hypurals 1 and 2 co-ossified and fused to compound caudal centrum. Branched rays splitting two times. Free vertebrae 36 and 12 ribs.

Laterosensory canals with simple (non-dendritic) tubes ending in single pores. Nasal and frontal canals of supraorbital line fused to each other, with three pores ("s1", "s3" and "s6"); single pore "s6" present in 47 specimens of 55; eight of 55 with pore "s6" paired and very close. Pore supraorbital "s1" located in medial portion of anterior nostrils; pore supraorbital "s3" located in longitudinal line in posterior portion of posterior nostrils; singles pore "s6" located in longitudinal line in posterior portion of orbital and equidistant in intraorbital space. Antorbital segment of infraorbital canal present with two pores "i1" and "i3" located laterally to anterior and posterior nostrils. Sphenotic canal present with two pores "i10" and "i11". Pore "i10" located located laterally to posterior margin of eye; pore "i11" located at same horizontal line of single pore supraorbital "s6". Otic, postotic, and scapular canals present with preoperclemandibular and pterotic branches short and with one associated pore each. Postotic canal with preopercularmandibular and pterotic located anterodorsal to opercular patch of odontodes; pore preoperculo-mandibular located specifically at same vertical line to pore “i11”; pore pterotic located in the posterior margin of the opercular patch of odontodes internally to pore preoperculo-mandibular. Trunk canal short with two pores (one of 55 specimens with three pores on right side) located between pectoral-fin base and opercular patch.

Color in alcohol. Dorsal and lateral surfaces of body with scattered round dark brown or black spots, smaller or equivalent in size to eye circumference, over lighter background (Figs. 11-12). Belly light brown surrounded by scattered dark brown round spots which extending to caudal peduncle. Smaller specimens with scattered round black spots covering all ventral surface. Head with dorsal surface totally blackened with round black spots, its lateral and ventral surfaces light brown. Opercular and interopercular patch of odontodes light brown. Barbels dark brown dorsally near base and light brown ventrally and at tips. Pectoral, dorsal, anal, and caudal fins with scattered round dark brown or black spots, smaller or equivalent in size to eye circumference, over lighter background fading towards distal margins. Pelvic fin unpigmented basally and with scattered dark brown spots distally. General color pattern *in vivo* similar to preserved specimens but with background reddish brown or dark yellow to dark brown or grayish in few specimens.

Geographical distribution. *Trichomycterus* sp. n. is known from several tributaries of the Ribeira de Iguape River basin (Fig. 1) including (or tributaries of them) Betari, Iporanga, do Pilões, Juquiá, and Jacupiranga rivers.

Ecological notes. *Trichomycterus* sp. n. is found in fast flowing clear waters running over rocky bottom (Fig. 13) together with the riffle-associated fauna of species such as *Ancistrus* *multispinnis*, *Astyanax laticeps*, *Characidium pterostictum*, *Chasmocranus lopezae, Kronichthys lacerta*, *Lampiella gibbosa*, *Rineloricaria kronei* and *Schizolecis guentheri* (at type locality). *Trichomycterus* sp. n. was collected at its type locality, córrego Queimado, with two congeners, *T. alternatus* and *T. jacupiranga*. This stream is also the type locality of *T. jacupiranga.* In its original description (Wosiacki, Oyakawa, 2005), authors provided information about the specific stretches where *T. jacupiranga* was found. According to them, *T. jacupiranga* inhabit clear water sand beds with strong to moderate current flowing where was possible to observe the fishes buried, mentioning its syntopical occurrence with *T. alternatus* (identification of *T. zonatus* by authors likely corresponds to *T. alternatus,* see discussion). Although these general microhabitat conditions were observed at the type locality of *Trichomycterus* sp. n., this species was collected in the rocky portions, composed by boulders of medium to large sizes and usually covered by periphyton, while the only two specimens collected of *T. jacupiranga* were found in the sand beds. *Trichomycterus alternatus*, however, was observed occupying a large variety of microhabitats in the córrego do Queimado stream and are found in high abundance. *Trichomycterus* sp. n. was also collected in two localities with *T. tupinamba* in the Betari and Iporanga rivers in similar microhabitats.

Conservation status. *Trichomycterus* sp. n. is apparently endemic to Ribeira de Iguape River basin where it can be found in, at least, six localities distributed in different tributaries within the drainage. Most of these streams and rivers are surrounded by riparian vegetation of relative well-preserved forest, but in some locations were replaced by banana plantations, one of the main economic activities in the Ribeira de Iguape River valley. This situation was highlighted in the description of other *Trichomycterus* species for the region and concern freshwater fish conservation (Wosiacki, Oyakawa, 2005). The Extent of Occurrence (EOO) for *Trichomycterus* sp. n. estimated as 5,239,878 km^2 therefore this species may considered

Near Threatened (NT: B1b(iii)) according to IUCN criteria (IUCN, 2016).

Discussion

A recent and comprehensive multilocus phylogeny of trichomycterids supported the monophyly of Trichomycterinae (Ochoa *et al*., 2017), previously accessed by Datovo & Bockmann (2010) based on myological evidence. In this molecular phylogeny, the clade that incorporates the trichomycterines was partitioned into six groups designated as clade *D1*, *D2*, *E*, *D3*, *D4*, and *D5* (Ochoa *et al.*, 2017). Two of these monophyletic assemblages, D4 and D5 are sister groups, and includes the species of *Trichomycterus* from southeastern South America and *Scleronema*. The clade D5 is formed by species from coastal drainages of northeastern and southeastern Brazil and continental drainages such as the upper Paraná and São Francisco and were posteriorly designated as *Trichomycterus sensu stricto* clade by Katz *et al.* (2018). The clade D4 is formed by species from coastal and continental drainages of southern Brazil that were recently proposed as the new genus *Cambeva* by Katz *et al.* (2018) plus also the genus *Scleronema*. Our analysis supported the new species, *T. alternatus* and *T. jacupiranga* in the clade D5 of Ochoa *et al.* (2017a) and the species *Trichomycterus davisi*, *T. tupinamba*, and *T. zonatus* in their clade D4. Thus, even with the inclusion of additional samples the groups suggested by Ochoa *et al*. (2017a) and reviewed by Katz *et al*. (2018) are supported as divergent monophyletic lineages in the present study.

The Ribeira de Iguape River basin is a large coastal drainage comprised within the Atlantic Rainforest, a highly impacted environment, which estimates of the remaining forest in this region vary from 1% to 12% (Gentry, 1996; Brown & Brown, 1992; Myers *et al*., 2000; Oyakawa *et al*., 2006; Menezes *et al*., 2007). Knowledge of the biological diversity of this area, including fishes, is eminent to preservation, conservancy, and management of this region.

Nine species of *Trichomycterus* were previously cited in the literature from Ribeira de

Iguape River basin (Tab. 1): *Trichomycterus brasiliensis* Lütken, 1874; *T. davisi* (Haseman, 1911); *T. dispar* (Tschudi, 1846), *T. iheringi* (Eigenmann, 1917), *T. immaculatus* (Eigenmann & Eigenmann 1889), *T. jacupiranga* Wosiacki & Oyakawa, 2005; *T. punctulatus* Valenciennes, 1846; *T. tupinamba* Wosiacki & Oyakawa, 2005, and *T. zonatus* (Eigenmann, 1918) (*cf*. Miranda-Ribeiro, 1908a; de Pinna & Wosiacki, 2003; Miranda-Ribeiro, 1908a; Eigenmann, 1917; Menezes *et al*., 2007; Wosiacki & Oyakawa, 2005; Miranda Ribeiro, 1908b; Wosiacki, Oyakawa, 2005; Eigenmann, 1918). Our results contrasts in part with these previous records and recognize only six species to the Ribeira de Iguape River basin: *T. alternatus*, *T. davisi*, *T. jacupiranga*, *T. tupinamba*, *T. zonatus* and the new species described herein. Most species of this genus are endemic to single basins and have restricted distributions (Nogueira *et al.*, 2009; Ferrer, Malabarba, 2011), as appears to be also the case of *T. tupinamba*, *T. jacupiranga*, *T. zonatus* and *Trichomycterus* sp. n. On the other hand, other species present in the basin have large distributions in drainages of South and Southeast Brazil: *T. alternatus* (discussed below) and *T. davisi*. The latter is restricted to the headwaters of the Ribeira de Iguape River basin that neighbor other continental basins (*e.g.* Iguaçu and Paranapanema drainages). *Trichomycterus davisi* status as a possible species complex and its polyphyly was already discussed in other studies (Morais-Silva *et al.*, 2018) and this species needs further revision that involves other nominal species (see also chapter 2). Additionally, another putative new species (that would be the 7th in the Ribeira de Iguape), which can be recognized by a low number of pectoral-fin rays and color pattern distinct from congeners was recorded from the Ribeira de Iguape River basin deserving further investigation.

Causes of the high species diversity and endemism of *Trichomycterus* in the Ribeira de Iguape River basin compared to most coastal basins in South and Southeast Brazil may be associated with their specificity to habitat of fast flowing waters running over rocky bottoms a feature found typically in headwaters. This headwaters in relative large drainage basins, as the

Ribeira de Iguape River basins, are tipically isolated by main lowland portions of rivers and allopatric species may play an important role in the (as observed in loricariids, *e.g.*, Roxo *et al.*, 2017). Another reason that may contribute for the high diversity of the Ribeira de Iguape River basin may be associated with the polyphyletic origin of its *Trichomycterus* fauna, an aspect that is not observed in most northern or southern coastal river basins. Within *Trichomycterus* clades recognized as monophyletic (clades D1, D2, D3, D4, and D5) show mostly allopatric distributions, as it is the case of clades D4 and D5 in the coastal basins of Southeast Brazil. Southern limit, of the more northern distributed D5 clade, is observed by populations of *T. alternatus* occurring in northern tributaries of the Paranaguá Bay and the northern limit of clade D4 is represented by records of *T. iheringi* in small coastal drainages of São Paulo State (Wosiacki & Oyakawa, 2005; Silva *et al.*, 2010). Therefore most of the distributional overlap of these two clades occurs within the Ribeira de Iguape River basin. Other species historically recorded to Ribeira de Iguape River basin (*e.g.*, *T. brasiliensis*, *T. dispar*, *T. iheringi*, *T. immaculatus*, and *T. punctulatus*) are likely misidentifications as discussed above.

Interestingly, the new species described here was collected by Krone in early XX century and posteriorly cited, commented and illustrated by Miranda Ribeiro (1908a, 1908b; 1911). Firstly, the taxon was identified as *T. dispar* by Miranda Ribeiro (1908a) and later cited as *T. dispar* var. *punctulata* and *T. punctulatus* (Miranda Ribeiro, 1908b). However, Miranda Ribeiro (1911) again cited the taxon as *T. dispar* providing a brief description, which fits in several aspects with the new species described herein. All these species are from Peru and Eigenmann (1918) based on the description of *T. iheringi* suggested the records of Miranda Ribeiro (1908a, 1908b) as synonym. Unfortunately, this specimen could not be found and analyzed – as well as the taxon cited as *T. brasiliensis* by Miranda Ribeiro (1908a) – but our results indicated that the fish illustrated by Miranda Ribeiro (1908b, fig. 1) (Fig. 14) and posteriorly briefly described as *T. dispar* (Miranda Ribeiro, 1911) is the new species described herein. This hypothesis is suggested based on features of *Trichomycterus* sp. n. observed in the Fig. 134 as the spotted coloration, the filament of the first pectoral-fin ray and the truncate margin of the caudal fin. Wosiacki (2005) and Silva *et al*. (2010) already confirmed that the type locality of *T. iheringi* is Santos, São Paulo. Thus, we can suggest here that the new species described here from Ribeira de Iguape River basin was erroneously included in the synonymy list of *T. iheringi* by Eigenmann (1917), as well cited by Miranda Ribeiro (1908a, 1908b, 1911), supposedly as *T. dispar* and *T. punctulatus*.

Taxonomic status of *Trichomycterus alternatus* and *T. zonatus*. *T. alternatus* has a broad distribution occurring from rio Doce River basin (its type locality) to northern tributaries of the Paranaguá Bay (Fig. 1). As mentioned above to *T. davisi*, *T. alternatus* also was hypothesized as part of a species complex that may include other nominal species considering the high genetic divergence between allopatric populations (S3; and *cf*. Lima, 2009; Reis, 2018) and deserve further studies. Our results confirm the presence of several lots of *T. alternatus* to Ribeira de Iguape River basin from that were equivocally identified as *T. zonatus* in collections as well as in the literature (Menezes *et al.*, 2007; Oyakawa *et al.*, 2007;). Consequently, some putative occurring localities of *T. zonatus* cited in the literature (*e.g.* Lima *et al*., 2009; Wosiacki, Oyakawa, 2005) were presently refuted and correspond to *T. alternatus*. The difficulty in establishing clear limits between these two species may be associated with the mixed type series of *T. zonatus* examined by one of us (JF). The paratype of *T. zonatus* from “rio Cubatão, seven miles west of Santos, São Paulo State” (Figs. 8-9; FMNH 58572) does not match in several aspects with its holotype (Fig. 7b; FMNH 58573) and the other two paratypes (FMNH 58574) collected in the type locality, “Água Quente, Iporanga”. Therefore, this specimen erroneously assumed as a paratype of *T. zonatus* (FMNH

58572) seems to be *T. alternatus* based on analysis of its holoype (Fig. 7a), some paratypes and a large amount of nontypes. Eigenmann (1918) noted differences in the color pattern between these two species describing the coloration of the specimen from west of Santos (FMNH 58572; herein identified as *T. alternatus*) composed by "obscure bars across the back" (probably the saddles-like blotches observed in the Fig. 6a) separated from those specimens from "Agua Quente" (*T. zonatus*), which were defined as possessing the "back and lower part of the sides with conspicuous spots".

Taxonomic status of *Trichomycterus jacupiranga*. *T. jacupiranga* was described based on six specimens from córrego do Queimado, a tributary of the Ribeira de Iguape River basin, and known, at moment, only from the type locality, with apparently low abundance (Wosiacki, Oyakawa, 2005). The species was diagnosed by a combination of characters: "first pectoral-fin ray prolonged as short filament (*vs.* not prolonged), one supraorbital pore s6 fused (*vs.* two supraorbital pores s6 paired), eight [total] pectoral fin rays (*vs.* six or seven), pelvic fin covering anal and urogenital opening (*vs.* not covering), anal and urogenital opening closer to anal-fin origin than to pelvic-fin base (*vs.* midway between anal-fin origin and pelvic-fin base), caudal fin truncated with attenuated edges (*vs.* rounded or truncated without attenuated edges), and the following morphometric characteristics: head length 20.7-22.4% SL, and head width 90.6-104.6% HL".

Among the co-occurring species, *T. jacupiranga* is more similar to *T. alternatus* and share several morphological characters that are often used in *Trichomycterus* taxonomy: pectoral-fin with one unbranched ray and seven branched rays, first ray prolonged with a short filamentous, single supraorbital pore "s6" and antorbital segment of infraorbital canal present with two pores "i1" and "i3". A feature that readily distinguishes both species is the color pattern that in *T. alternatus* is characterized by a series of large dark spots and saddles (Fig. 6a) with the absence of these in *T. jacupiranga* (Fig. 6b; *cf.* Wosiacki, Oyakawa, 2005).

Our results suggest two hypotheses regarding similarity between *T. jacupiranga* and *T. alternatus*: the first hypothesis, supported by molecular data, predict that the haplotype sharing among *T. alternatus* and *T. jacupiranga* is result of an intraspecific variation in the color pattern of *T. alternatus*. The low abundance of *T. jacupiranga* specimens compared to the morphologically similar *T. alternatus* could suggest that the former is a color morphotype occurring in a low representativity and limited to that drainage basin (córrego do Queimado). The second, and contrasting hypothesis is that both species are distinct and the morphological distinctiveness and genetic similarity (mitochondrial data; see S3 for genetic distance) could be result of a recent speciation event (*e.g.*, incomplete lineage sorting) or alternatively an older event with posterior introgression (*e.g.*, mitochondrial introgression); both hypotheses are discussed below.

High intraspecific color variation in Trichomycterinae was already discussed by several studies (Arratia *et al.*, 1978; Arratia, Menu-Marque, 1981; Arratia, 1983), which reported variation of body pigmentation associated with ontogeny and camouflage with the type of substrata. Recent works have recognized intrapopulational variation in color pattern associated with ontogeny as observed in populations of *Trichomycterus caipora* Lima, Lazzarotto & Costa , 2008; *T. davisi* (Haseman, 1911); *T. poikilos* Ferrer & Malabarba, 2013; and *T. santanderensis* Castellanos-Morales, 2007 (Lima *et al.*, 2008; Nascimento *et al*., 2017, Ferrer, Malabarba, 2013; Castellanos- Morales, 2007), microhabitat preference (*e.g.*, Arratia *et al.*, 1978; Arratia & Menu-Marque, 1981; Arratia, 1983) or both (Silva *et al*., 2010). Color pattern differences observed between *T. jacupiranga* and *T. alternatus* could not be associated with ontogeny since we evaluate individuals of different size classes of both species (Fig. 6) and association with microhabitat preference deserve further investigation. Other than color pattern, one additional morphological feature (interopercular length) can putatively distinguish *T. alternatus* from *T. jacupiranga* (Tab 2; Fig. 5) suggesting that differences between these color morphs are in fact interspecific differences and arguing against a possible conspecificity suggested by mitochondrial data.

The low genetic divergence between *Trichomycterus alternatus* and *T. jacupiranga* can be explained due relative recent speciation process and incomplete lineage sorting. Thus, these species are not distinguished by genetic approach due to lack of reciprocal monophyly between them, as previously reported for other groups with a complex taxonomic history (Pereira *et al*., 2010; Henriques *et al.*, 2015, Costa-Silva 2015). In a similar case, despite low genetic divergences Costa-Silva *et al.* (2015), consider that the morphological differences between species of *Rineloricaria* are adequate to discriminate between them.

Genetic similarity on mitochondrial marker between *Trichomycterus jacupiranga* and *T. alternatus* may also be associated with mitochondrial introgression due to hybridization. Interspecific introgression and replacement of mtDNA is relatively common in fishes (*e.g.*, Bernatchez *et al.*, 1995; Chow, Kishino, 1995; Glemet *et al*., 1998; Gerber *et al.*, 2001; Sullivan *et al*., 2007) as well as in other organisms (*cf.* Avise, 2000). To test this hypothesis, further investigation is needed using other molecular markers such as nuclear genes.

For this moment, we agree that inferences regarding species boundaries based on genetic data alone are likely inadequate, and robust species delimitation should be conducted with consideration of other sources of data, such as morphology, in an integrative approach (Pereira *et al*., 2013, Roxo *et al*., 2017, Camelier *et al*., 2018; Serrano *et. al*., 2018). Finally, we opted for a conservative decision to maintain the principle of taxonomy stability, assuming that the morphological differences between *T. alternatus* and *T. jacupiranga* discriminate between, at the moment is also adequate to maintain as distinct species.

COMPARATIVE MATERIAL EXAMINED

All from Brazil.

Cambeva cubataonis. MNRJ 12490, holotype, 46.2 mm L_S, rio Cubatão, Joinville, Santa Catarina. MNRJ 12491, 9, paratypes, 38.3-49.5 mm L_S, rio Cubatão, Joinville, Santa Catarina.

Cambeva davisi. FMNH 60309, holotype (xr), 43.8 mm L_S; rio Iguaçu, Serrinha, Paraná. FMNH 52242, 4, paratypes, 20.9-44.5 mm L_S, rio Iguaçu, Serrinha, Paraná.

Cambeva guaraquessaba. **Paraná State:** MPEG 7916, holotype, 88.5 mm L_S; rio Bracinho, Fazenda Salto Dourado, Guaraqueçaba. MHNCI 7916, 3, paratypes, 67.7-80.9 mm L_S, rio Bracinho, Fazenda Salto Dourado, Guaraqueçaba. MZUSP 85531, 2, paratypes, 83.1-85.5 mm L_S, rio Bracinho, Fazenda Salto Dourado, Guaraqueçaba.

Cambeva iheringi**.** **São Paulo State:** MNRJ 24008, 3, 30.2-75.0 mm L_S, rio Piagui, Guaratinguetá. MNRJ 23931, 5, 51.5-74.3 mm L_S, tributary of ribeirão Piquete, Piquete.

Cambeva tupinamba. **São Paulo State, Ribeira de Iguape River basin:** MZUSP 62362, 1, paratype, 41.7 mm L_S, rio Iporanga, Iporanga. MZUSP 62382, holotype, 83.6 mm L_S, rio Betari, tributary of rio Ribeira de Iguape, in front of the camping area of Núcleo Santana, Parque Estadual Turístico do Alto Ribeira (PETAR), Iporanga. MZUSP 63495, 1, paratype, 37.2 mm L_S, rio Betari, Iporanga. MZUSP 84094, 4, 27.8-70.9 mm L_S, rio Betari, tributary of rio Ribeira de Iguape, in front of the camping area of Núcleo Santana, Parque Estadual Turístico do Alto Ribeira (PETAR), Iporanga, collected with holotype. MPEG 7895, 5, paratypes, 30.9-61.6 mm L_S, rio Betari, PETAR, Iporanga.

Cambeva zonata. **São Paulo State, Ribeira de Iguape River basin:** FMNH 58573, holotype (xr), 53.6 mm L_S, Agua Quente, Iporanga. FMNH 58574, 2, paratypes (2xr), 42.2-48.0 mm L_S, Agua Quente, Iporanga. The following lots are from rio Betari, Iporanga, Ribeira de Iguape River basin: DZSJRP 13630, 2, 26.6-46.6 mm L_S; MCP 20621, 1, 50.5 mm L_S, MZUSP 28415, 2, 64.5-70.3 mm L_S; MZUSP 38784, 1, 26.4 mm L_S; MZUSP 61696, 1, 68.4

mm L_S; MZUSP 61715, 2, 30.1-30.9 mm L_S; MZUSP 62380, 3, 56.5-57.8 mm L_S; MZUSP 82685, 2, 65.0-67.0 mm L_S; MZUSP 82771, 2, 57.7-63.7 mm L_S; MZUSP 82932, 3, 43.0-56.7 mm L_S; MZUSP 83139, 1, 51.9 mm L_S ;MZUSP 83714, 11, 28.4-67.3 mm L_S; MZUSP 83730, 2, 21.9-27.3 mm L_S; MZUSP 83724, 1, 67.5 83714 mm L_S; MZUSP 83751, 1, 48.5 mm L_S; UFRGS 24538, 15 (1c&s), 47.1-61.6 mm L_S.

Trichomycterus alternatus. **Minas Gerais State:** FMNH 58082, holotype (xr), 66.2 mm L_S, rio Doce. FMNH 58083, 8, paratypes (4xr), 47.5-58.1 mm L_S, rio Doce. MZUSP 52542, 1, 45.9 mm L_S, ribeirão Pitangas at the confluence with rio Santo Antônio, Braúnas, Doce River basin. MZUSP 107892, 5, 28.1-33.8 mm L_S, rio do Peixe, Juiz de Fora. MZUSP 110928, 1, 59.4 mm L_S, rio Gualaxo do Sul, Mariana, Doce River basin. **Rio de Janeiro State:** MNRJ 45333, 3, 58.2-72.1 mm L_S, unnamed stream at REBIO do Tinguá, Duque de Caxias. **São Paulo State:** FMNH 58572, 1, paratype of *Cambeva zonata*, 51.0 mm L_S, rio Cubatão, Santos. MNRJ 19629, 5, 51.4-52.3 mm L_S, rio Formoso, tributary of rio Paraíba do Sul, Paraíba do Sul River basin. MZUSP 54166, 4, 39.7-60.5 mm L_S, rio Boiçucanga at Vila de Boiçucanga, São Sebastião. MZUSP 62933, 6, 52.0-42.7 mm L_S, rio Boiçucanga, São Sebastião. MZUSP 107318, 2, 30.2-65.5 mm L_S, córrego das Tocas, Cachoeira Água Branca, Parque Estadual de Ilha Bela. MZUSP 115423, 10, 22.0-33.6 mm L_S, rio Cubatão under the bridge of the rodovia dos Imigrantes. UFRGS 10169, 1, 63.0 mm L_S, stream on the Km 208 of Rio-Santos highway, Bertioga. UFRGS 10240, 5, 32.8-60.3 mm L_S, stream near to Rio-Santos road, Ubatuba River basin. **The following lots are from Ribeira de Iguape River basin:** DZSJRP 13668, 8, 39.6-57.4 mm L_S, ribeirão das Areias, Tapirai; MCP 32472, 1, 43.1 mm L_S, stream tributary of rio Pardo, Iporanga; MZUSP 36551, 10, 56.9-65.1 mm L_S, stream tributary of rio Bananal, Miracatu; MZUSP 55022, 4, 39.7-55.5 mm L_S, stream tributary of rio Juquiá; MZUSP, 60268, 10, 37.2-58.7 mm L_S, Córrego da Onça, stream tributary of rio Pardo; MZUSP 68179, 10, 42.3-26.1 mm L_S, Córrego do Queimado, stream tributary of

Jacupiranguinha, Cajati; MZUSP 68188, 2, 25.7-37.6 mm L_S, Córrego do Queimado, stream tributary of rio Jacupiranguinha, Cajati; MZUSP 68189, 6, 39.2-55.6 mm L_S, Córrego do Queimado, stream tributary of Jacupiranguinha, Cajati; MZUSP 78354, 4, 42.8-63.5 mm L_S, Córrego Vargedo, stream tributary of rio Juquiá at Cachoeira da Fumaça, Ibiúna; MZUSP 78649, 10, 39.7-55.2 mm L_S, stream tributary of rio Areado, Itariri; MZUSP 78652, 3, 53.3-57.3 mm L_S, rio areado, stream tributary of rio Guanhanhã, Itariri; MZUSP 78922, 1, 32.4 mm L_S, ribeirão do Chiqueiro at Aldeinha, São Lourenço; MZUSP 78923, 2, 61.5-68.4 mm L_S, stream tributary of Ribeirão do Chiqueiro near to Pesqueiro Araponga, São Lourenço da Serra; MZUSP 79955, 5, 40.9-49.2 mm L_S, rio Espraiado at Estação Ecológica Juréia-Itatins, Iguape; MZUSP 79989, 2, 46.3-52.4 mm L_S, rio Espraiado at Estação Ecológica Juréia-Itatins, Iguape; MZUSP 100603, 6, 37.9-57.2 mm L_S, rio Catas Altas, Barra do Chapéu; UFRGS 24542, 7 (1 c&s), 44.5-55.1 mm L_S, rio Iporanga, Iporanga; UFRGS 24544, 40, 64.8-22.6 mm L_S, córrego do Queimado, stream tributary of rio Jacupiranguinha, Cajati; UFRGS 24545, 5, 53.4-46.4 mm L_S, rio Juquiá at Manoel Gama, Juquiá; UFRGS 24557, 14, 37.2-49.6 mm L_S rio Turvo at córrego dos Micos, Barra do Turvo; UFRGS 24559, 3, 50.9-53.7 mm L_S, rio Pardinho, Barra do Turvo; UFRGS 24560, 3, 25.9-40.9 mm L_S, tributary of rio Turvo, Barra do Turvo; UFRGS 24561, 9, 18.7-35.0 mm L_S, rio Guanhanha, stream tributary of rio do Azeite, Itariri. **Paraná State**: MZUSP 70646, 1, 38.8 mm L_S, rio João Surra, tributary of rio Pardo near to Parque das Lauraceas, Adrianópolis, Ribeira de Iguape River basin. MZUSP 70652, 1, 41.3 mm L_S, rio João Surra, tributary of rio Pardo near to Parque das Lauraceas, Adrianópolis, Ribeira de Iguape River basin. MZUEL 14730, 2, 39.8-39.9 mm L_S, ribeirão tributary of rio Ribeira, Cerro Azul, Ribeira de Iguape River basin. The following lots are from Paranaguá Bay, Guaraqueçaba: MNRJ 38983, 4, 21.4-42.2 mm L_S, rio do Cedro, stream tributary of rio Guaraqueçaba; UFRGS 24540, 37, 36.0-56.0 mm L_S, unnamed stream tributary of rio Guaraqueçaba; UFRGS 24546, 28, 29.1-38.5 mm L_S, stream tributary of rio

Guaraqueçaba; UFRGS 24551, 3, 48.0-57.4 mm L_S, stream tributary of rio Serra Nova.

***Trichomycterus jacupiranga*. São Paulo State, Ribeira de Iguape River basin.** UFRGS 24555, 2, 35.1-44.8 mm L_S, córrego do Queimado, stream tributary of rio Jacupiranguinha, Cajati. **The following lots are from córrego do Queimado, Parque Estadual de Jacupiranga, Cajati, São Paulo:** MZUSP 67818, holotype, 56.5 mm L_S; MZUSP 67819, 1, paratype, 48.1 mm L_S; MZUSP 67820, 1, paratype, 49.3 mm L_S; MZUSP 67821, 1, paratype, 49.7 mm L_S; MZUSP 67822, 1, paratype, 48.2 mm L_S; MZUSP 84095, 1, paratype, 32.8 mm L_S. See too material examined in chapter 2.

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ANEXOS

Tab. 1. Nominal *Trichomycterus* species listed to Ribeira de Iguape River (RI) basin.

Species (as originally cited)	Author(s)	Distribution according to original description	Present study
Trichomycterus dispar Tschudi	Miranda-Ribeiro A (1908a; 1911)	Peruvian Andes	absent
Trichomycterus brasiliensis Lütken	Miranda-Ribeiro A (1908a)	rio São Francisco basin	absent
Trichomycterus dispar Tschudi var. *punctulata*	Miranda-Ribeiro A (1908b)	rio Remac near Lima	absent
Trichomycterus punctulatus (Cuv. & Val.)	Miranda-Ribeiro A (1908b)	Western Peru	absent
Pygidium iheringii spec. nov.	Eigenmann (1917) when included the records of T. dispar and T. punctulatus of Miranda-Ribeiro A (1908a) in the synonym list	rio Paraná basin and São Paulo coastal streams	absent
Pydigium zonatum sp. nov.	Eigenmann (1918)	rio Ribeira de Iguape basin and coastal stream near Santos (SP)	endemic to RI
Trichomycterus davisi (Haseman, 1911)	de Pinna & Wosiacki (2003)	rio Iguaçu basin	present
Trichomycterus immaculatus (Eigenmann & Eigenmann, 1889)	Menezes *et al.* (2007)	rio Paraíbuna and Paraíba basins	absent
Trichomycterus tupinamba, new species	Wosiacki & Oyakawa (2005)	rio Ribeira de Iguape basin	endemic to RI
Trichomycterus jacupiranga, new species	Wosiacki & Oyakawa (2005)	rio Ribeira de Iguape basin	endemic to RI
Trichomycterus sp. n.	present paper	rio Ribeira de Iguape basin	endemic to RI
Trichomycterus alternatus	present paper	rio Doce basin	present
Trichomycterus sp.	present paper	rio Ribeira de Iguape basin	endemic to RI

Tab. 2. Evolutionary models by gene and codon position selected using PartitionFinder.

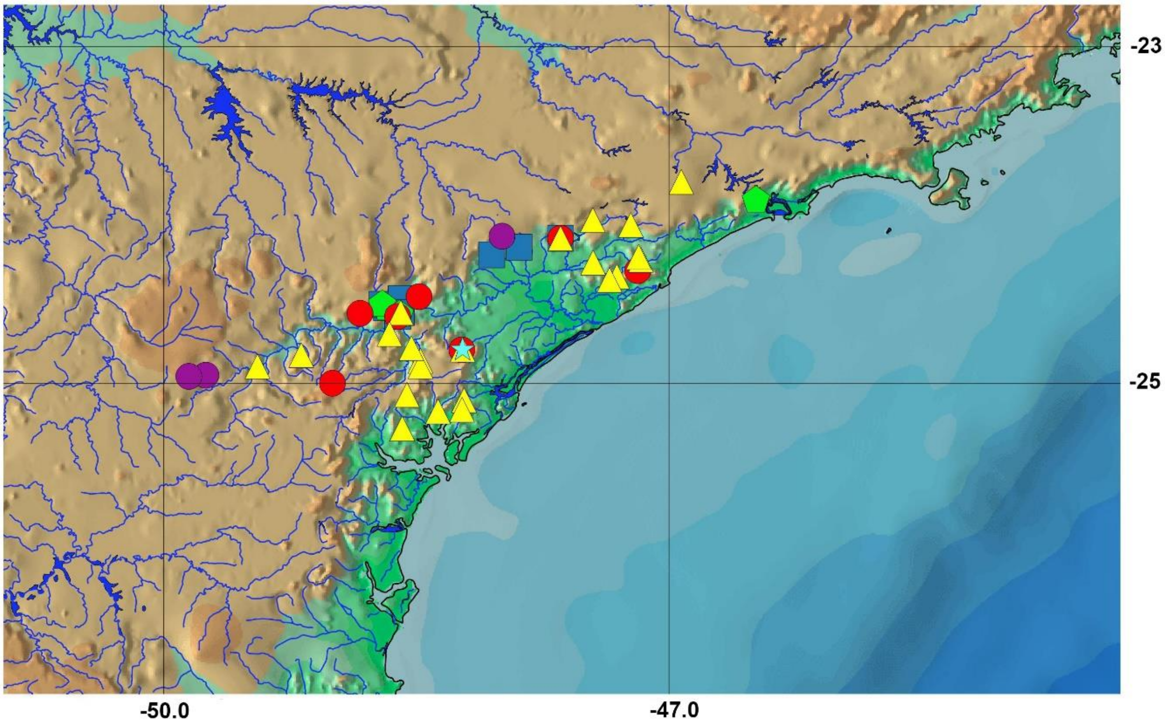
Subset	Subset Partitions	Subset Sites	Best-fit model
1	COI 1st, CYTB 1st	1-789\3, 790-1866\3	K80+I+G
2	COI 2nd, CYTB 2nd	2-789\3, 791-1866\3	KHKY+I
3	COI 3rd	3-789\3	TrN+G
4	CYTB 3rd	792-1866\3	TrN+G

Tab. 3. Comparative morphometric data between *T. alternatus* (N =30) and *T. jacupiranga* (N = 7). SD = standard deviation.

	Range	**Mean**	**SD**	**Range**	**Mean**	**SD**
	Trichomycterus alternatus			*Trichomycterus jacupiranga*		
Standard length (mm)	38.7-65.5	50.2	-	35.1-56.5	47.4	-
Percents of standard length						
Head length	20.1-23.6	22.1	0.88	21.9-22.7	22.3	0.31
Predorsal length	57.4-64.8	61.4	1.49	59.4-62.9	61.5	1.27
Prepelvic length	54.6-59.3	56.5	1.03	53.7-56.4	55.2	0.97
Preanal length	69.6-77.4	73.2	1.56	70.1-73.1	71.5	1.11
Scapular girdle width	16.4-19.3	17.9	0.87	17.2-19.8	18.4	0.95
Trunk length	35.6-40.4	37.7	1.26	33.5-39.1	36.6	1.67
Pectoral-fin length	13.7-18.0	15.9	0.89	14.7-16.5	15.6	0.59
Pelvic-fin length	9.8-12.3	10.9	0.64	9.9-11.4	10.6	0.49
Distance between pelvic-fin base and anus	8.3-11.5	9.9	0.88	8.8-10.8	9.9	0.71
Caudal peduncule length	16.7-22.5	19.4	1.17	19.3-20.9	20.3	0.57
Caudal peduncule depth	9.0-13.1	11.2	1.15	10.6-12.8	11.5	0.83
Body depth	10.9-16.0	13.7	1.44	11.4-15.0	13.6	1.28
Length of dorsal-fin base	11.8-15.3	13.4	0.91	12.0-16.1	13.8	1.26
Length of anal-fin base	7.7-10.8	9.0	0.79	8.7-9.7	9.2	0.37
Percents of head length						
Head width	79.8-92.4	85.4	3.73	76.6-86.7	81.4	4.41
Nasal barbel length	41.7-69.8	52.9	5.83	49.7-59.4	54.9	5.02
Maxillary barbel length	42.3-76.5	58.6	8.79	58.2-67.5	64.5	0.89
Rictal barbel length	34.8-53.0	44.6	4.84	46.6-67.3	51.5	0.44
Snout length	38.0-45.5	41.8	1.71	38.7-48.4	41.5	0.97
Interorbital	15.3-22.0	18.7	1.69	17.9-22.5	19.3	1.84
Mouth width	22.0-29.9	25.8	1.74	22.3-25.4	23.9	0.01
Eye diameter	10.4-16.5	13.9	1.50	12.9-14.1	13.3	0.36
Interopercular length	21.4-32.8	28.9	2.57	30.8-35.3	32.7	1.15

Tab. 4. Morphometric data for *Trichomycterus* sp. n., new species. Ranges include holotype and paratypes. N = 12; SD = standard deviation.

	Holotype	**Range**	**Mean**	**SD**
Standard length (mm)	114.7	60.7-124.5	83.2	-
Percent of standard length				
Head length	21.8	20.8-24.7	22.2	0.96
Predorsal length	62.0	57.9-63.8	61.6	1.71
Prepelvic length	57.7	55.4-58.6	56.7	1.03
Preanal length	74.6	71.8-74.7	73.5	1.04
Scapular girdle width	18.8	17.4-19.3	18.5	0.51
Trunk length	38.2	36.7-40.2	38.5	1.09
Pectoral-fin length	13.5	13.6-16.5	15.2	0.92
Pelvic-fin length	10.3	10.1-11.8	10.8	0.49
Distance between pelvic-fin base and anus	11.1	10.1-13.0	11.2	0.91
Caudal peduncule length	16.9	16.9-21.2	18.2	1.21
Caudal peduncule depth	11.7	10.4-12.5	11.4	0.60
Body depth	16.8	14.9-19.2	16.4	1.52
Length of dorsal-fin base	14.1	11.8-14.2	13	0.66
Length of anal-fin base	8.9	8.6-10.3	9.5	0.49
Percent of head length				
Head width	86.8	75.7-86.8	82.9	3.17
Nasal barbel length	27.3	27.3-51.0	44.5	6.51
Maxillary barbel length	40.1	31.1-51.4	43.6	6.00
Rictal barbel length	28.8	28.8-47.6	39.6	4.98
Snout length	42.2	37.8-45.5	41.7	1.87
Interorbital	21.2	17.9-23.1	20.9	1.60
Mouth width	26.1	21.4-26.4	24.6	1.34
Eye diameter	11.1	11.1-15.2	12.5	1.10
Interopercular length	31.2	31.9-37.3	34.0	1.41



1. Distribution of *Trichomycterus* species from Ribeira de Iguape River basin. Yellow triangles: *T. alternatus*; red circles: *T.* sp. n. “ribeira”; blue star: *T. jacupiranga* and type locality of *T.* sp. n.; purple circles: *T. davisi*; blue squares: *T. tupinamba*; green pentagon: *T. zonatus* – northernmost record in Santos is a misidentification of *T. zonatus* and represents *T. alternatus* (see text). Distribution of *T. alternatus* is also shown in Paranaguá Bay tributaries, which represents its southernmost limit.

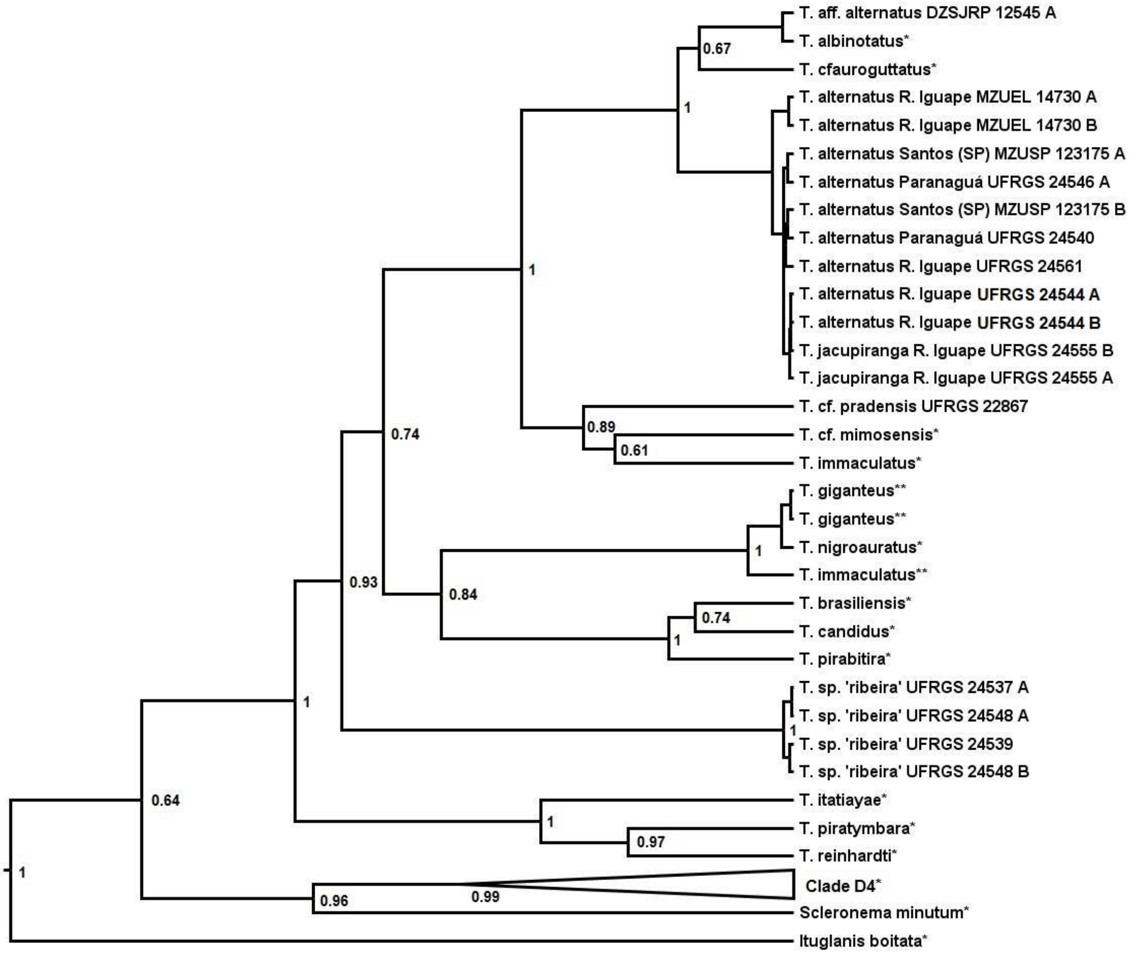


Fig. 2. Phylogenetic relationships of *Trichomycterus* species (Clade D5) and related lineages based on mitochondrial data (COI, CYTB) using Bayesian inference. Node numbers correspond to Bayesian Posterior Probability. * represent specimens from Ochoa *et al*., 2017; ** represent specimens from Nascimento *et al*., 2017.

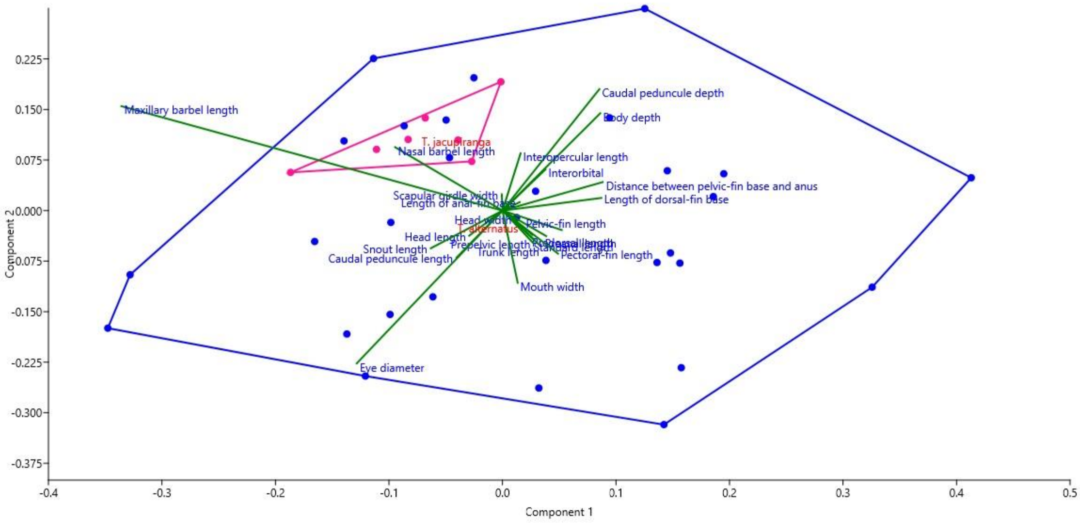


Fig. 3. Principal component analysis of morphometric data of *Trichomycterus alternatus* (blue) and *T. jacupiranga* (pink).

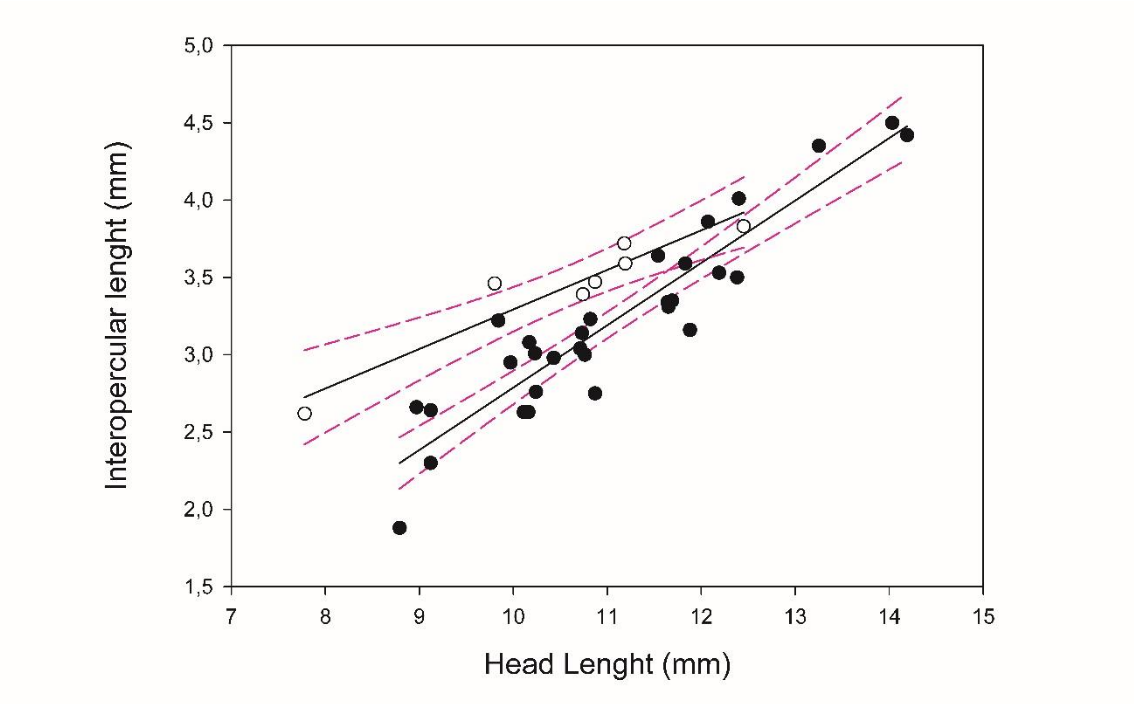


Fig. 4. Linear regression of selected measurements (interopercular length vs. head length) between *Trichomycterus alternatus* (black circles) and *Trichomycterus jacupiranga* (white circles).

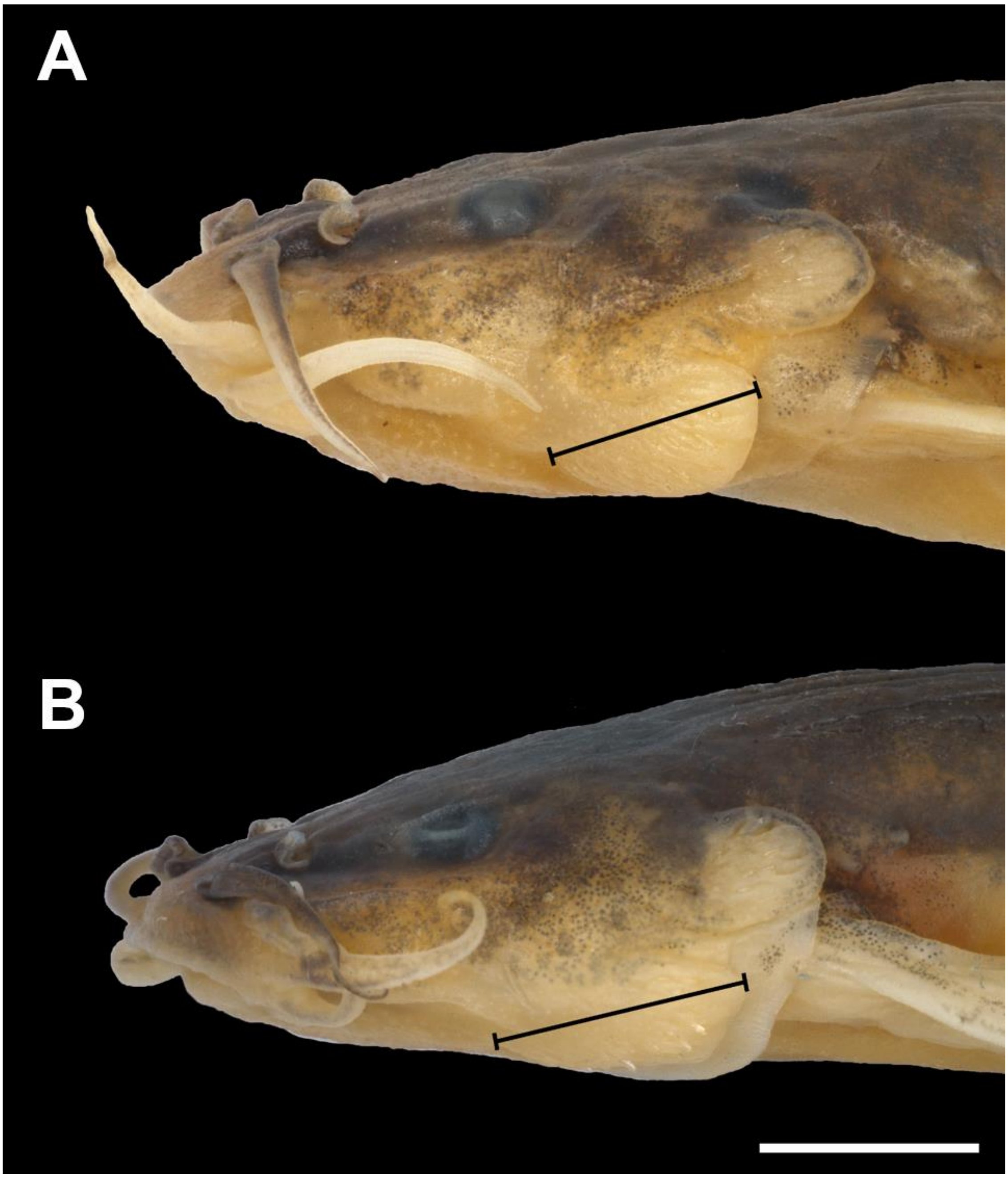


Fig. 5. Lateral view of the head of selected *Trichomycterus* species showing the interopercular patch of odontodes. A. *Trichomycterus alternatus* UFRGS 24544, 45.5 mm SL. **B.** *T. jacupiranga*, UFRGS 24555, 44.8 mm SL. Black bar indicates interopercle length. Scale bar: 3 mm.

Fig. 6. Coloration *in vivo* and ontogenetic series of selected *Trichomycterus* species. **A.** *Trichomycterus alternatus*, UFRGS 24540, 57.2 and 40.9 mm SL, tributary of Guaraqueçaba River, Paranaguá River basin. **B.** *T. jacupiranga*, UFRGS 24555, 44.8 mm SL and 35.1 mm SL, córrego do Queimado, Ribeira de Iguape River basin.

Fig. 7. A. *Trichomycterus alternatus*, holotype, FMNH 58082; **B.** *T. zonatus*, holotype, FMNH 58573, ventral view. Images obtained from All Catfish Species Inventory website (ACSI, credit to Mike W. Littmann) and posteriorly edited.

Fig. 8. Detail of head of specimen of *Trichomycterus alternatus* included in the type series of *T. zonatus*, FMNH 58572, paratype. White arrow = pores i3, black arrow = pore s6, red arrow = filament of the first pectoral-fin ray. Images obtained from All Catfish Species Inventory website (ACSI, credit to Mike W. Littmann) and posteriorly edited.

Fig. 9. Specimen of *Trichomycterus alternatus* included in the type series of *T. zonatus*, FMNH 58572, paratype. **A.** dorsal view. **B.** Ventral view. Images obtained from All Catfish Species Inventory website (ACSI, credit to Mike W. Littmann) and posteriorly edited.

Fig. 10. *Trichomycterus* species from Ribeira de Iguape River basin. **A.** *Trichomycterus davisi*, MZUEL 17202, 72.8 mm SL, fixed in alcohol. **B.** *Trichomycterus tupinamba*, UFRGS 24550, 63.0 mm SL, *in vivo*. **C.** *Trichomycterus zonatus*, UFRGS 24538, 48.4 mm SL, *in vivo.*

Fig 11. *Trichomycterus* sp. n., UFRGS 24536, holotype, 114.7 mm SL, córrego do Queimado, stream tributary of rio Jacupiranguinha, Ribeira de Iguape River basin.

Fig. 12. Variation on the coloration *in vivo* of *Trichomycterus* sp. n. **A.** UFRGS 24536, 60.7 mm SL, córrego do Queimado. **B.** UFRGS 24536, 68.6 mm SL, córrego do Queimado. **C.** UFRGS 24537, 77.0 mm SL, rio Betari. **D.** UFRGS 24537, 68.9 mm SL, rio Betari.

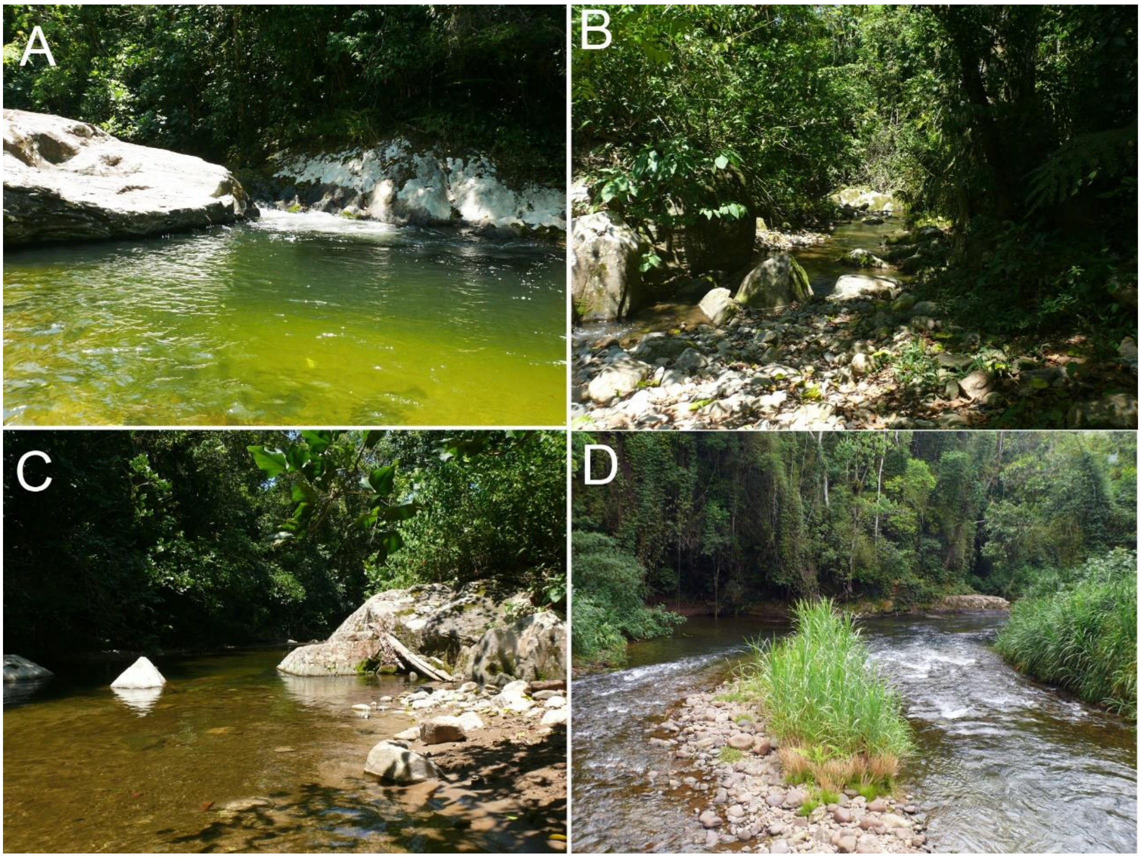


Fig. 13. Type locality of *Trichomycterus* sp., new species, at córrego do Queimado tributary of rio Jacupiranguinha, Cajati (**A-C**) and locality of collection of paratypes (UFRGS 24537) at rio Betari, Iporanga (**D**), both from Ribeira de Iguape River basin.

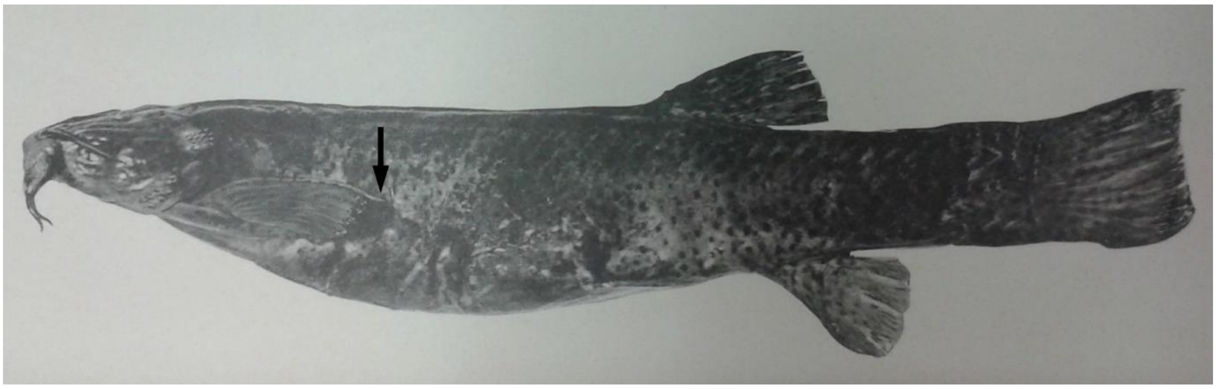


Fig. 14. Illustration of the specimen identified by Miranda Ribeiro (1908b) as *Trichomycterus dispar*, Tschudi var. *punctulata*, Cuvier & Vallencienes, which represents an erroneous identification of the new species. Arrow indicates the filament of the first pectoral-fin ray.

Supplementary S1. Primers used in this study to amplify sequences of COI and CYTB genes.

Gene	***Primer***	**Sequences (5'-3')**	**Reference**
COI	FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCA	Ivanova *et al.*, 2007
	FishR2_t1	CAGGAAACAGCTATGACACTTCAGGGTG	
	FR1d_t1	CAGGAAACAGCTATGACACCTCAGGGTG	
	VF2_t1	TGTAAAACGACGGCCAGTCAACCAACCA	
	L6252-Asn	AAGGCGGGGAAAGCCCCGGCA	Melo *et al*., 2011
	H7271-COXI	TCCTATGTAGCCGAATGGTTCTTTT	
CYTB	Cat.Thr29	ACCTTCGATCTCCTGATTACAAGAC	Unmack *et al*., 2009
	Glu31	GTGACTTGAAAAACCACCGTT	
	CB23	CCCTCAGAATGATATTTGTCCTCA	Barros *et al*., 2015
	GLU5	TGACTTGAACCACCGTTG	

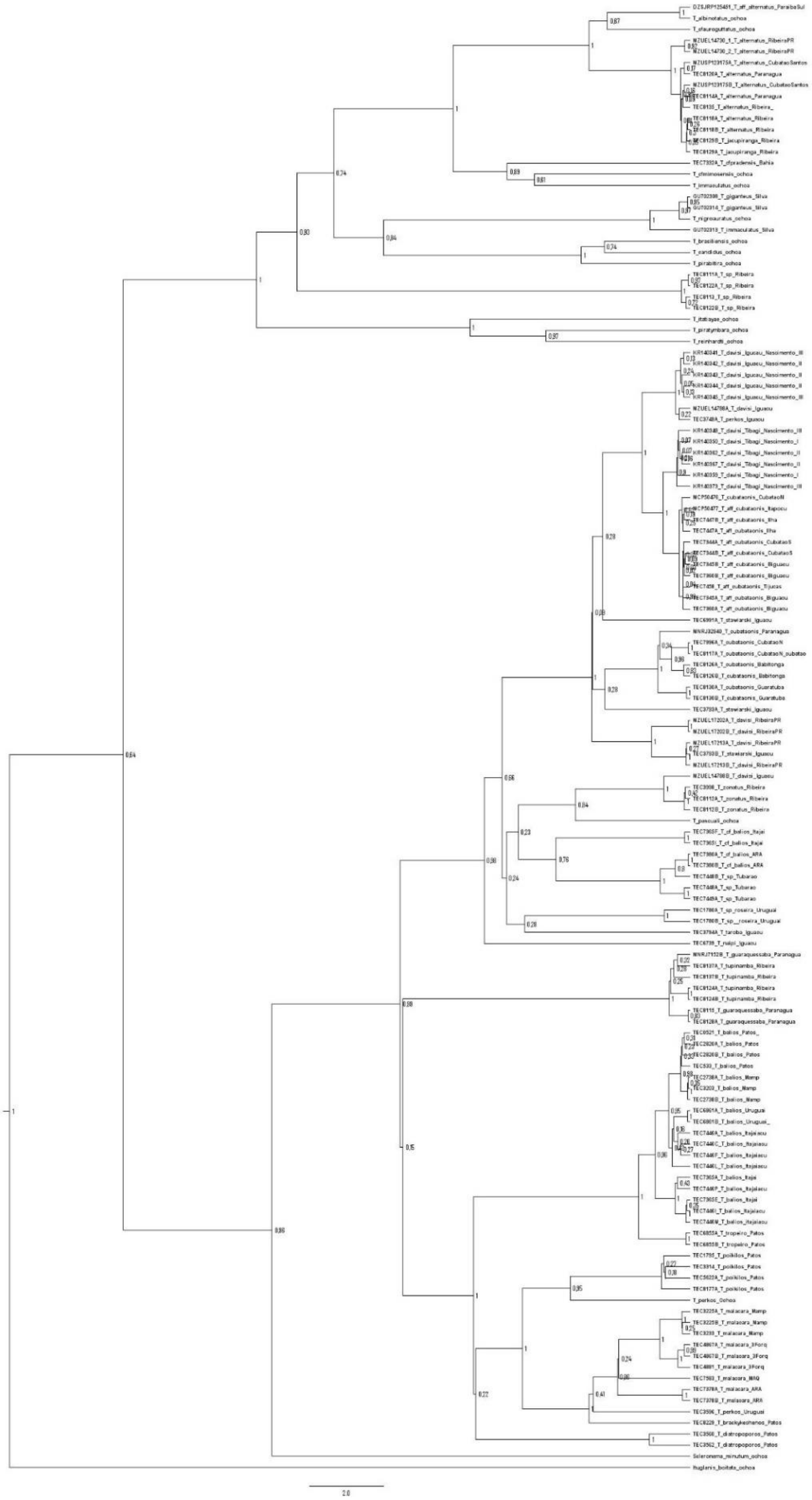
Supplementary S2. Taxon sampling, voucher catalogue number, tissue, drainage, and gene sequenced in the present study.

Species	Voucher	Tissue	Drainage	COI	CYTB
Trichomycterus alternatus	UFRGS 24540	8114A	Paranaguá River basin	X	X
Trichomycterus alternatus	UFRGS 24546	8120A	Paranaguá River basin	X	
Trichomycterus alternatus	UFRGS 24544	8118A	Ribeira de Iguape River	X	X
Trichomycterus alternatus	UFRGS 24544	8118B	Ribeira de Iguape River	X	
Trichomycterus alternatus	UFRGS 24561	8135	Ribeira de Iguape River	X	
Trichomycterus alternatus	MZUEL 14730	A	Ribeira de Iguape River	X	
Trichomycterus alternatus	MZUEL 14730	B	Ribeira de Iguape River	X	
Trichomycterus alternatus	MZUSP 123175	A	Ribeira de Iguape River	X	X
Trichomycterus alternatus	MZUSP 123175	B	Ribeira de Iguape River	X	
Trichomycterus aff. *davisi*	UFRGS 22906	7344A	Cubatão Sul River basin	X	X
Trichomycterus aff. *davisi*	UFRGS 22906	7344B	Cubatão Sul River basin	X	X
Trichomycterus aff. *davisi*	UFRGS 22932	7360A	Biguaçu River basin	X	X
Trichomycterus aff. *davisi*	UFRGS 22932	7360B	Biguaçu River basin	X	
Trichomycterus aff. *davisi*	UFRGS 22907	7345A	Biguaçu River basin	X	X
Trichomycterus aff. *davisi*	UFRGS 22907	7345B	Biguaçu River basin	X	
Trichomycterus aff. *davisi*	UFRGS 23235	7458	Tijucas River basin	X	X
Trichomycterus aff. *davisi*	UFRGS 23183	7447A	Ilha de Florianópolis	X	X
Trichomycterus aff. *davisi*	UFRGS 23183	7447B	Ilha de Florianópolis	X	X
Trichomycterus aff. *davisi*	MCP 50477		Itapocu River basin	X	
Trichomycterus aff. *davisi*	MCP 50476		Cubatão Norte River basin	X	X
Trichomycterus brachykechenos	UFRGS 24666	8229	Laguna dos Patos	X	X
Trichomycterus	UFRGS 17887	3560	Laguna dos Patos	X	
Trichomycterus	UFRGS 17889	3562	Laguna dos Patos	X	X
Trichomycterus balios	UFRGS 16435	2820B	Laguna dos Patos	X	
Trichomycterus balios	UFRGS 6844	521	Laguna dos Patos	X	
Trichomycterus balios	UFRGS 6842	533	Laguna dos Patos	X	X
Trichomycterus balios	UFRGS 16284	2738A	Mampituba River basin	X	X
Trichomycterus balios	UFRGS 16284	2738B	Mampituba River basin	X	
Trichomycterus balios	UFRGS 17198	3203	Mampituba River basin	X	
Trichomycterus balios	UFRGS 23182	7446A	Itajaí Açu	X	
Trichomycterus balios	UFRGS 23182	7446C	Itajaí Açu	X	
Trichomycterus balios	UFRGS 23182	7446F	Itajaí Açu	X	
Trichomycterus balios	UFRGS 23182	7446I	Itajaí Açu	X	
Trichomycterus balios	UFRGS 23182	7446L	Itajaí Açu	X	
Trichomycterus balios	UFRGS 23182	7446M	Itajaí Açu	X	
Trichomycterus balios	UFRGS 23182	7446P	Itajaí Açu	X	
Trichomycterus balios	UFRGS 22939	7365A	Itajaí	X	X
Trichomycterus balios	UFRGS 22939	7365E	Itajaí	X	
Trichomycterus balios	UFRGS 21886	6861A	Upper Uruguay	X	X
Trichomycterus balios	UFRGS 21886	6861B	Upper Uruguay	X	
Trichomycterus cf. *pradensis*	UFRGS 22867	7332A	Jequitinhonha River basin	X	
Trichomycterus "tubarão"	UFRGS 22964	7380A	Ararangua River basin	X	X

Species	Voucher	Tissue	Drainage	COI	CYTB
Trichomycterus "tubarão"	UFRGS 22964	7380B	Ararangua River basin	X	X
Trichomycterus cubataonis	UFRGS 24543	8117A	Cubatão Norte River basin	X	X
Trichomycterus cubataonis	UFRGS 24311	7996A	Cubatão Norte River basin	X	X
Trichomycterus cubataonis	UFRGS 24552	8126A	Babitonga River basin	X	X
Trichomycterus cubataonis	UFRGS 24552	8126B	Babitonga River basin	X	
Trichomycterus cubataonis	UFRGS 24556	8130A	Guaratuba River basin	X	X
Trichomycterus cubataonis	UFRGS 24556	8130B	Guaratuba River basin	X	X
Trichomycterus cubataonis	MNRJ32940	MNHM464	Paranaguá River basin	X	X
Trichomycterus davisi	MZUEL 17202	A	Ribeira de Iguape River	X	X
Trichomycterus davisi	MZUEL 17202	B	Ribeira de Iguape River	X	X
Trichomycterus davisi	MZUEL 17213	A	Ribeira de Iguape River	X	X
Trichomycterus davisi	MZUEL 17213	B	Ribeira de Iguape River	X	X
Trichomycterus	UFRGS 24541	8115	Paranaguá basin	X	X
Trichomycterus	UFRGS 24554	8128A	Paranaguá basin	X	X
Trichomycterus	MNRJ 40846	7152B	Paranaguá basin	X	
Trichomycterus naipi	UFRGS 11405	6739	Iguaçu River basin	X	X
Trichomycterus perkos	UFRGS 17923	3596	Uruguai River Basin	X	X
Trichomycterus poikilos	UFRGS 15014	1795	Laguna dos Patos	X	X
Trichomycterus poikilos	UFRGS 17419	3314	Laguna dos Patos	X	
Trichomycterus poikilos	UFRGS 20352	5622A	Laguna dos Patos	X	
Trichomycterus poikilos	UFRGS 24604	8177A	Laguna dos Patos	X	
Trichomycterus stawiarski	UFRGS 18307	3793A	Iguaçu River basin	X	X
Trichomycterus stawiarski	UFRGS 18307	3793B	Iguaçu River basin	X	
Trichomycterus taroba	UFRGS 18308	3794A	Iguaçu River basin	X	X
Trichomycterus tupinamba	UFRGS 24563	8137A	Ribeira de Iguape River	X	X
Trichomycterus tupinamba	UFRGS 24563	8137B	Ribeira de Iguape River	X	
Trichomycterus tupinamba	UFRGS 24550	8124A	Ribeira de Iguape River	X	X
Trichomycterus tupinamba	UFRGS 24550	8124B	Ribeira de Iguape River	X	
Trichomycterus tropeiro	UFRGS 21878	6855A	Laguna dos Patos	X	X
Trichomycterus tropeiro	UFRGS 21878	6855B	Laguna dos Patos	X	
Trichomycterus zonatus	UFRGS 24538	8112A	Ribeira de Iguape River	X	X
Trichomycterus zonatus	UFRGS 24538	8112B	Ribeira de Iguape River	X	X
Trichomycterus zonatus	UFRGS 18675	3998	Ribeira de Iguape River	X	X
Trichomycterus sp.	UFRGS 23494	7583	Tramandaí River basin	X	X
Trichomycterus sp.	UFRGS 19149	4867A	Tramandaí River basin	X	
Trichomycterus sp.	UFRGS 19178	4881	Tramandaí River basin	X	
Trichomycterus sp.	UFRGS 17220	3225A	Mampituba River basin	X	X
Trichomycterus sp.	UFRGS 17220	3225B	Mampituba River basin	X	
Trichomycterus sp.	UFRGS 17228	3233	Mampituba River basin	X	
Trichomycterus sp.	UFRGS 22962	7378A	Araranguá River basin	X	X
Trichomycterus sp. "roseira"	UFRGS 14998	1780A	Uruguay River basin	X	X
Trichomycterus sp. "roseira"	UFRGS 14998	1780B	Uruguay River basin	X	
Trichomycterus sp "itajaí"	UFRGS 22939	7365F	Itajaí River basin	X	X
Trichomycterus sp "itajaí"	UFRGS 22939	7365I	Itajaí River basin	X	X
Trichomycterus sp "Tubarão"	UFRGS 23184	7448A	Tubarão River basin	X	X
Trichomycterus sp "Tubarão"	UFRGS 23184	7448B	Tubarão River basin	X	
Trichomycterus sp "Tubarão"	UFRGS 23185	7449A	Tubarão River basin	X	X

Species	Voucher	Tissue	Drainage	COI	CYTB
Trichomycterus sp "Tubarão"	UFRGS 23185	7449B	Tubarão River basin	X	
Trichomycterus sp "ribeirae"	UFRGS 24548	8122A	Ribeira de Iguape River	X	X
Trichomycterus sp "ribeirae"	UFRGS 24548	8122B	Ribeira de Iguape River	X	
Trichomycterus sp "ribeirae"	UFRGS 24539	8113	Ribeira de Iguape River	X	X
Trichomycterus sp "ribeirae"	UFRGS 24537	8111A	Ribeira de Iguape River	X	X

Supplementary S3. Phylogenetic relationships of *Trichomycterus* species based on Bayesian inference. Node numbers correspond to Bayesian Posterior Probability (P).



Supplementary S4. Pairwise K2P genetic distance values for two mitochondrial genes Cytochrome Oxidase I (COI) and cytochrome B (CYTB) concatenated sequences among *Trichomycterus alternatus* and *T. jacupiranga*.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1. *Ituglanis boitata*												
2. MZUEL14730.2. *T. alternatus*	0.151											
3. MZUEL14730.1. *T. alternatus*	0.151	0.000										
4. TEC8129A. *T. jacupiranga*	0.149	0.004	0.006									
5. TEC8135. *T. alternatus*	0.151	0.001	0.003	0.003								
6. TEC8120A. *T. alternatus*	0.151	0.001	0.003	0.000	0.000							
7. TEC8114A. *T. alternatus*	0.155	0.001	0.003	0.003	0.000	0.000						
8. MZUSP123175B. *T. alternatus*	0.151	0.001	0.003	0.003	0.000	0.000	0.000					
9. MZUSP123175A. *T. alternatus*	0.154	0.001	0.003	0.002	0.000	0.000	0.001	0.000				
10. TEC8129B. *T. jacupiranga*	0.151	0.003	0.004	0.001	0.001	0.001	0.002	0.001	0.001			
11. TEC8118B. *T. alternatus*	0.148	0.003	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.000		
12. TEC8118A. *T. alternatus*	0.152	0.003	0.004	0.001	0.001	0.001	0.002	0.001	0.001	0.000	0.000	-

Species delimitation in *Trichomycterus* (Siluriformes: Trichomycteridae) from coastal drainages of South and Southeast Brazil

(Manuscrito a ser submetido para o periódico Neotropical Icthhyology)

Species delimitation in *Trichomycterus* (Siluriformes: Trichomycteridae) from coastal drainages of South and Southeastern Brazil

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Running head. Species delimitation in *Trichomycterus* from coastal drainages.

Abstract

Trichomycterus is a highly diverse genus, which contains many poorly delimitated species and a complex taxonomic history. Color patterns, which are typically used for identifying species diversity within the genus is shown to be highly variable in some instances and assessment of geographical variation within species of *Trichomycterus* is often absent. In this paper an extensive review of specimens of *Trichomycterus* from coastal drainages of South and Southeastern Brazil is used to evaluate species limit in an integrative analysis including both morphological and molecular data. We identified nine morphospecies inhabiting coastal drainages of South and Southeast Brazil based on a combination of morphological characters, three of them putatively undescribed:

Trichomycterus sp. ‘malacara’, *T. balios*, *Trichomycterus* sp. 'tubarão', T. aff. *davisi*, *T. cubataonis*, *T. davisi*, *T. guaraquessaba*, *T. tupinamba* and *T. zonatus*. The coalescent-based analysis on genetic data (GMYC) suggests the recognition of 11 clusters within the studied region and four of those matches with the morphological delimitation. Our results support the diversification of *Trichomycterus* in South and Southeast Brazil seem to be primarily associated with faunal exchanges between coastal and continental drainages secondarily constrained by paleodrainage configuration during pleistocenic sea level changes.

Keywords. Color pattern variation, Generalized Mixed Yule-Coalescent (GMYC), Headwater capture, Integrative Taxonomy, Palaeodrainages, Trichomycterinae.

Palavras-chave. Captura de cabeceira, Generalized Mixed Yule-Coalescent (GMYC), Variação de colorido, Taxonomia Integrativa, Paleodrenagens, Trichomycterinae.

Introduction

Trichomycteridae harbors a notably large diversity of small to medium size catfishes, and it is the second richest family of Siluriformes (Fricke *et al.*, 2019). The group is widely distributed in the Neotropical region from Costa Rica to Argentina and Chile, with species distributed in both cis- and trans-Andean drainages (de Pinna, Wosiacki, 2003; Fernandez, Schaefer, 2005; Malabarba, Malabarba, 2014). Trichomycterinae is the most species-rich subfamily, with more than half of the total number of species in the family (233 of 319), with unresolved inter-specific relationships (de Pinna, 1989; Wosiacki, de Pinna, 2003; Fernandez, Schaefer, 2009; Datovo, Bockmann, 2010; Ochoa *et al*., 2017; Katz *et al*., 2018). *Trichomycterus* is a systematic puzzle, highly diverse and containing species with complexes taxonomic histories (Baskin, 1973; de Pinna, 1989, 1998; Datovo, Bockmann, 2010, Ochoa *et al.*, 2017a, Katz *et al.*, 2018; Donin *et al.* in prep.). Within some of the conundrums of the study of *Trichomycterus* diversity and taxonomy, is its highly variable intraspecific morphology (Arratia *et al.*, 1978; Silva et al., 2010; Nascimento *et al.*, 2017; Donin *et al.* in prep.). Some morphological characters, such as color pattern that is typically used for identifying species diversity within the Neotropical ichthyofauna (Vari, 1991; Kullander & Lucena, 1992; Lucinda, 2008; Carvalho, Reis, 2011; Craig et al., 2017) are shown to be highly variable (Silva et al., 2010; Nascimento *et al.*, 2017; Donin *et al.* in prep.). Despite of that, assessment of geographical morphological variation within species of *Trichomycterus* is often poorly done or absent and many species are described based on specimens solely from the type locality (Costa, 1992; Triques, Vono, 2004; Wosiacki, Oyakawa, 2005; Barbosa, Costa, 2010) and with no additional information other than morphology. Despite these difficulties, *Trichomycterus* is an interesting group for studying speciation processes associated with landscape evolution because populations are often restricted to headwaters and often isolated from each other by ecological and physical barriers in the lower portions of these drainages. Most species of the genus have restricted distributions, often restricted to headwaters (Nogueira *et al.*, 2009; Ferrer, Malabarba, 2011), with moderate to rapid water flow a region usually with high number of endemic species and probably exposed to events of faunal exchange by capture of headwaters in adjacent basins (Morais-Silva *et al.*, 2017; Thomaz *et al.*, 2019). In the coastal region of southeastern Brazil two main geological processes affecting the distribution of fishes have been proposed: Faunal exchange through headwater captures between continental and coastal drainages by progressive erosion of the crystalline shield and neotectonic activity (Ribeiro, 2006; Ribeiro *et al.*,

2006) and the paleodrainage connections within the sea level fluctuations during the Pleistocene (Weitzman *et al.*, 1988; Thomaz *et al.*, 2015, 2017; Thomaz, Knowles, 2018). In this paper, an extensive review of specimens of *Trichomycterus* from coastal drainages of South and Southeastern Brazil is used to evaluate species limit in an integrative analysis including both morphological and molecular data. We also discuss how geography may be responsible for rising diversity and delineate species distributions in this area.

Material and Methods

Study region and taxon sampling. The study area comprises coastal drainages of South and Southeast of Brazil from Tramandaí River basin (South) to the Ribeira do Iguape River basin (North) (Fig. 1). This area corresponds to the distribution limits of clade D4 *sensu* Ochoa *et al*. (2017) within coastal drainages in South and Southeast Brazil. The species of *Trichomycterus* included in our analyses belongs to clade D4 *sensu* Ochoa *et al*. (2017 or *Cambeva sensu* Katz *et al.*, 2018). In addition for comparison we included several species from neighboring basins such as the Laguna dos Patos System (Ferrer, Malabarba, 2013); Uruguay River (Datovo *et al.*, 2012), Iguaçu River (de Pinna, 1992; Wosiacki, Garavello, 2004) and upper Paraná River (Nascimento *et al.*, 2017). Part of samples used in this study were examined from historical collections from the following institutions: CPUFMT, Coleção de Peixes da Universidade Federal do Mato Grosso; DZSJRP, Coleção de Peixes da Universidade Estadual Paulista “Júlio de Mesquita Filho”, São José do Rio Preto; MCN, Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre; MCP, Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do

Sul, Porto Alegre; MZUEL, Museu de Zoologia da Universidade Estadual de Londrina, Londrina; MNRJ, Museu Nacional, Rio de Janeiro; MZUSP, Museu de Zoologia da Universidade de São Paulo, São Paulo; NUP, Coleção Ictiológica do Nupélia, Universidade Estadual de Maringá, Paraná; UFRGS, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre. A large portion of the samples used this study were recently collected by field expeditions with collection permits from Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), under collection permits issued to Luiz R. Malabarba (#9318-1, 9220-1, 8796-1) and are housed at UFRGS fish collection. These recent collections were done using the electric fishing method. After capture, specimens were anesthetized and euthanized using a concentrated Eugenol solution (Lucena *et al.*, 2013). Tissue samples from fin clips or muscle were removed and preserved in 96% ethanol for molecular analysis and vouchers were fixed in 10% formaldehyde for morphological analysis (latter preserved in 70% ethanol). Some specimens were directly fixed in 96% ethanol and tissue samples for DNA extraction were removed in laboratory (whole specimens were latter preserved in 70% ethanol in the regular collection). Tissue samples are maintained in -4º C freezers. Abbreviations used in the text are: c&s (cleared and stained specimens), SL (standard length), tec (specimens with tissue sample available).

Morphological procedure. Morphometric data were taken point to point with digital caliper (precision of 0.1 mm) following Costa (1992); Tchernavin (1944) for length of barbels; Wosiacki, de Pinna (2008) for length and depth of the caudal peduncle and for supraorbital pore s6 distance; Ferrer, Malabarba (2011) for scapular girdle width; and Ferrer *et al.* (2015) for length of interopercular. Nomenclature of bones and cartilage followed Bockmann *et al.* (2004). Laterosensory canal system and associated pores followed Rizzato, Bichuette (2016). Osteological information was taken from cleared and stained specimens according to the method of Taylor, van Dyke (1985). Vertebral counts excludes those in the Weberian complex and the compound caudal centrum, (PU1+U1 = urostyle), is counted as one element. Distribution map was performed with the help of Quantum GIS version 1.8.0 software (Scherman *et al.*, 2016). Principal Component Analysis (PCA) was used to check overall morphometric variation among samples and species. The measurements were treated using Aitchinson (1986) log-ratio transformation, thus the first principal component, which mostly accounts for size variation (Strauss, 1985) was maintained. The principal component analysis (PCA) of the Aitchinson-transformed data were done using Past (Hammer *et al.*, 2001). Description of coloration in life was based on photographs taken from freshly collected and anesthetized specimens using the phototank-immersion method following Sabaj Pérez (2009).

Molecular procedure. DNA extractions from ethanol preserved samples followed a modified CTAB protocol (Doyle, Doyle, 1987). Partial sequences of the two mitochondrial genes Cytochrome Oxidase I (COI) (Ivanova *et al*., 2007; Melo *et al*., 2011) and cytochrome B (CYTB) (Barros *et al*., 2015; Unmack *et al*., 2009) were amplified by polymerase chain reaction (PCR) using the following primers (see S1 of Chapter 1). Amplifications were performed in a solution with a total volume of 20 µL with 13.8 µL of ultra pure water; 2.0 µL of 10x PCR buffer; 0,2 µL each 5 mM primer; 0.2 µL of Taq DNA Polymerase Ludwig Biotec; 1.0 µL of DNA template (10-50 ng). The PCR reactions consisted of initial denaturation of 94°C for 5 min, 35 cycles with a chain denaturation of 94°C for 45s, primer hybridization of 48°C to 54°C for 1,5 min and final extension of 72°C for 10 min. PCR products were checked by electrophoresis in agarose gel, purified using ExoSap Ilustra ® Exonuclease I and Shrimp Alkaline Phosphatase- GE Healthcare) following manufacturer instructions. The purified PCR products were sequenced in both directions at Macrogen Korea. Sequences were edited in Geneious v8.0 (Biomatters Ltd., Auckland, New Zealand) and the consensus sequences were obtained. DNA sequences from samples used in molecular analysis were deposited in GenBank (see S1 of chapter 1). Additional sequences added to our analysis were taken from GenBank from the studies of Nascimento *et al*. (2017) and Ochoa *et al*., (2017). Sequences were aligned in MUSCLE (Edgar, 2004) algorithm within Geneious v8.0 under default parameters. The genes COI and CYTB were concatenated into a single matrix for phylogenetic analysis. The single matrix was partitioned by gene and codon positions to determine codon-specific models and best partition scheme of molecular evolution in PartitionFinder v1.1.1 (Lanfear *et al.*, 2012) under the Bayesian Information Criterion (BIC). Phylogenetic relationships were inferred by Bayesian Inference using the uncorrelated relaxed molecular clock (lognormal) in BEAST v.1.7 (Drummond *et al*., 2012), programmed to run for 10 million generations of MCMC chains (saving one best tree every 10 thousand generations), under a Birth-Death prior. The first one million generations (10%) were discarded as burn-in and the remaining trees were used to summarize the results the Bayesian analysis using the Maximum Clade Credibility Tree in TreeAnnotator. The concatenated data set was analysed assuming an evolutionary rate of 0.01/site/Myr following rates previously proposed to mitochondrial markers in fishes (Bermingham *et al*., 1997; Reeves & Bermingham, 2006; Ornelas-Garcıa *et al*., 2008), including studies in the Atlantic Rainforest region (Hirschmann *et al*., 2015; Thomaz *et al*., 2015).

Species delimitation. Species assessment was done primarily by evaluating discrete morphological characters typically used in taxonomic papers of *Trichomycterus* (*e.g.*, laterosensory canal system and associated pores; number of pectoral-fin rays, ribs and vertebrae; and color pattern). For evaluating the primary morphological species delimitation we used a coalescence-based method, GMYC (Generalized Mixed Yule-Coalescent; Fujisawa, Barraclough, 2013). We also used morphometric comparison to evaluate selected cases where there was incongruence between both types of analyses (primary morphological assessment and molecular analysis using GMYC). In cases of species tree/gene tree incongruence (Maddison, 1997), the final delimitation was guided by the morphological approach. The GMYC method required an ultrametric tree estimated from single locus sequences and aims to detect the transition in the tree where the branching pattern switches from being attributed to speciation (to one lineage per species) to when it can be attributed to the intra-species coalescent process (multiple lineages per species) (Michonneau, 2016). We performed the GMYC analysis using a ultrametric tree generated in BEAST v.1.7 (Drummond *et al*., 2012) derived from concatenated mitochondrial matrix (COI +CYTB). For the GMYC analysis we used the package “splits” (Species Limits by Threshold Statistics) (http://rforge. r-project.org/projects/splits) with a single threshold method, performed in the program R version 3.0.0 (R Core Team, 2013).

Results

Morphological approach. We identified nine morphospecies inhabiting coastal drainages of South and Southeast Brazil based on a combination of morphological characters (Tab. 1; Fig. 1) described below. **1.** *Trichomycterus* sp. ‘malacara’ (Fig. 2) is the southernmost species within the study area and inhabits the upper portions from Maquiné (south limit), to Araranguá (north limit) rivers drainages (Fig. 1). This species is distinguished from all its congeners from coastal drainages of South and Southeast Brazil by the low number of pectoral-fin rays (modally I+5 *vs.* I+6 or more). We investigated morphometric differences among allopatric populations of *Trichomycterus* sp. ‘malacara’ given cluster assignment from the GMYC analysis (see also results below) from Maquiné, Três Forquilhas, Mampituba, and Araranguá rivers. The PCA show overlap between populations from Três Forquilhas and Araranguá rivers, while specimens of Mampituba and Maquiné rivers formed two distinct groups without overlapping each other (Fig. 3).

2. *Trichomycterus balios* is present in the upper portions of Mampituba River and widespread in the Itajaí River basin (Fig. 1). Within coastal species of the evaluated area, *T. balios* can be diagnosed by its unique color pattern with two layers in the skin composed of large and small round black blotches in the inner and outer layer, respectively (Fig. 4). We analyzed these two allopatrically distributed populations (Itajaí and Mampituba) and compared to specimens from upper portions of the Laguna dos Patos System (type locality). Principal component analyses (PCA) showed overlap of these populations in the morphospace (Fig. 5). The population from Laguna dos Patos System overlaps with the population of *T. balios* from Itajaí River basin, both slightly distinct from *T. balios* from Mampituba River basin (Fig. 5). Other consistent differences in meristic data and color pattern were not detected. PC1 explains 44.69% and PC2 20.23% of the variation for analyzed specimens.

3. *Trichomycterus* sp. 'tubarão' is found in the Araranguá (south) and Tubarão (north) river drainages (Fig. 1) and has a unique color pattern that differs from other congeners in the study area: one layer of coloration composed of not coalescent round small blotches (Fig. 6). This color pattern is somewhat similar in the coloration pattern presented by juveniles of *T. balios*, however *Trichomycterus* sp. 'tubarão' has a higher number of dorsal procurrent caudal-fin rays dorsally (17-21 *vs.* 14-17 in *T. balios*).

4. *Trichomycterus* aff. *davisi* is distributed from Cubatão Sul River (south) to Cubatão Norte River (north), including the Florianópolis Island, but not found in the Itajaí River basin (Fig. 1). This species can be distinguished from congeners in the study region by a combination of characters, such as the modally number of pectoral-fin rays (i+7), the antorbital segment of infraorbital of the laterosensory canal absent, and the variable color pattern. This variation goes from a mottled color pattern with (Fig. 7 A-B) to a blotched color pattern with dark marks varying in shape and size (Fig. 7. C-E). Populations of this species showed this polymorphic color pattern when comparing specimens from either the same drainage or among drainages. *Trichomycterus* aff. *davisi* can be distinguished from *T. davisi* (*sensu* Nascimento *et al.*, 2017) by the number of ribs and branchiostegal rays (11-12 *vs.* 13-15 and 7-8 *vs.* 9-10, respectively), and the insertion of the first pterygiophore of dorsal fin relate to the neural spines of 16th to 18th free vertebrae in the former *vs.* 19th free vertebra in the latter.

5. *Trichomycterus cubataonis* is found from the Itapocu River (south) to the tributaries of the Paranaguá Bay (north) (Fig.1). This species can be distinguished by a combination of characters, such as the antorbital segment of the laterosensory system present (absent only in populations of the Guaratuba River basin), six branched rays, and the mottled color pattern composed by blotches variable in shape and forming marked saddles dorsally (some specimens with large dark areas merged giving a overall dark color pattern; Fig. 8).

6. *Trichomycterus guaraquessaba* is endemic to rivers and streams that drains to the northern portion of the Paranaguá Bay (Fig. 1) and can be distinguished by congeners from study area by the presence of a narrow dark mid-lateral stripe from opercle to vertical through anal-fin origin (Fig. 9a,b).

7. *Trichomycterus tupinamba* is endemic to the Ribeira de Iguape River basin (Fig. 1) being distinguished from congeners from the study region by the lateral surface of body with a longitudinal row of black spots forming a stripe in at the anterior region (Fig. 9c,d). *Trichomycterus tupinamba* and *T. guaraquessaba* are morphologically similar and can be distinguished by the color pattern (Fig. 9a-d). Furthermore, *T. guaraquessaba* and T. *tupinamba* are also morphologically distinct by the number of free vertebrae (37 *vs*. 39 to 40, respectively), insertion of dorsal-fin and anal-fin pterygiophores in relation to free vertebrae (18th-20th *vs* 22th-25th; respectively) and by the number of odontodes associated with the interopercular and opercular patch (22-23 *vs* 34-36; and 18-19 *vs.* 23-26; respectively) (*cf*. Wosiacki & Oyakawa, 2005 and data examined herein). Due to their genetic similarity (see section below), we also performed an morphometric analysis contrasting their allopatric populations from tributaries of the Paranaguá Bay (*T. guaraquessabe*) and Ribeira de Iguape River basin (*T. tupinamba*) (Tab. 1). Principal component analyses (PCA) on the Aitchinson-transformed data show a separation between the two species, forming two distinct groups (Fig. 10). PC1 explains 32.70% and PC2 22.45% of the variation for analyzed specimens.

8. *Trichomycterus zonatus* is restricted to a small area of the Ribeira de Iguape River basin, the Iporanga River and its tributaries (Fig. 1). This species can be distinguished by some external characters as the pectoral fin with six branched rays with the first never prolonged as a filament, laterosensoy system with the antorbital segment of infraorbital canal with the pores “i1” and “i3” absent, two pores “s6” in the supraorbital canal and a color pattern composed by well defined and larger black blotches, not forming marked saddles (Fig. 11). *Trichomycterus zonatus* and *T. davisi* are similar externally, however, some osteological aspects are distinct, such as the number of branchiostegal rays (8 *vs.* 9-10) and the first pterygiophore of the anal fin position (anterior to haemal spine of 21th vertebra *vs.* anterior to haemal spine of 22-23th vertebra). Additionally, it is possible to diagnose the two species analyzing the color patter of adults (Fig. 12): *T. davisi* has the lateral surface with smaller and more coalescent black blotches forming a mottled pattern (Fig. 12a) whereas *T. zonatus* has well defined and larger black blotches, if coalescent, only in the midline of the caudal peduncle (Fig. 12b).

9. Few specimens from the headwaters of the Ribeira de Iguape basin is herein tentatively identified as *Trichomycterus davisi* based on their morphological similarity with those analyzed by Nascimento *et al.* (2017) from the Iguaçu River basin (see also chapter 1).

Phylogenetic reconstruction and biogeography. We investigated the phylogenetic position and genetic distinctiveness of *Trichomycterus* species (in clade D4) from coastal drainages of South and Southeast Brazil based on two mitochondrial molecular markers (Fig. 13). The concatenated matrix has 1,866 bp. (COI - 789 bp. and Cytb – 1,077 bp.). In the concatenated dataset, 567 sites were variable, 445 were parsimony informative and 1,299 were invariant. The nucleotide composition was of 28.8% (T), 28.4% (C), 25.4% (A) and 17.5% (G). The best-fit model of nucleotide evolution estimated by PartitionFinder by gene and coding positions (see Tab. 2 of chapter 1). Species investigated are part of the clade D4 of Ochoa *et al*. (2017; or *Cambeva* genus *sensu* Katz *et al*., 2018). *Trichomycterus* sp. ‘malacara', is endemic to the Tramandaí-Mampituba ecoregion (*sensu* Abell *et al*., 2008) ocurring in the Maquiné, Três Forquilhas, Mampituba and Araranguá drainages. Populations of this morphospecies showed a strong genetic structure according to its drainage basin. *Trichomycterus* sp. ‘malacara’ is included in a well supported clade (PP=1.0) that has also species from Laguna dos Patos System and Uruguay River basins: *T. poikilos*, *T. brachychechenos* and *T. perkos,* this last species being polyphyletic in our analyses. *Trichomycterus balios* is well supported (PP=1.0) as sister to the upper Taquari River endemic *T. tropeiro*. Populations of *Trichomycterus balios* inhabit the upper portions of the Laguna dos Patos System (type locality) but also the inland Uruguay River basin and two coastal drainages the Mampituba and the Itajaí river basins. *Trichomycterus* sp. 'tubarão' is found in both Araranguá and Tubarão river basins and is moderately supported (PP=0.76) as the sister group to a population composed by two small specimens collected with *T. balios* (putatively assigned as juveniles of *T. balios*) in the Itajaí River basin, but not aligned genetically with others populations of *T. balio*s *sensu stricto*. Lineages morphologically identified as *Trichomycterus davisi* were recovered as polyphyletic, appearing at least in three independent portions within *Trichomycterus* phylogeny. The morphospecies *Trichomycterus* aff. *davisi*, which is highly supported as nested within lineages of *T. davisi* from Iguaçu and Paranapanema rivers widely distributed between tributaries from the Cubatão Sul River basin, (southernmost limit) to Cubatão Norte River basin (northernmost limit) is closely related with populations of *T. davisi* from Iguaçu and Tibagi River basins (sequences from Nascimento *et al*., 2017). Populations of *Trichomycterus davisi* restricted to few records in the headwaters of Ribeira de Iguape River basin, near to its watershed limits with its neighbors Paranapanema and Iguaçu river basins are well supported as a monophyletic group. *Trichomycterus zonatus*, endemic to Betari River basin is sister group to one population of *T. davisi* from Iguaçu River basin. *Trichomycterus tupinamba,* endemic to Ribeira de Iguape River basin is nested within samples of *T. guaraquessaba* from tributaries of the

Paranaguá Bay. *Trichomycterus cubataonis*, which is closely related to one population of *T. stawiarski* from Iguaçu River basin, is widely distributed to Cubatão Norte, Babitonga, Guaratuba river basins and Paranaguá Bay.

Coalescent-based species delimitation. The results suggested the recognition of 15 clusters (= putative species) and a confidence interval ranging from 12 to 16 clusters (Fig. 13). Additional seven entities containing a single individual were recognized (likelihood of null model = 255.8706 and likelihood of the GMYC model 265.0193) (see SX for more details). The entities (cluster and single individual entities) identified by the GMYC model were 22 (15 cluster + 7 single individual entities), 13 of those 22 match with the morphological delimitation (see results below).

Our results indicate nine morphogroups representing species of *Trichomycterus* from coastal drainages of South and Southeast Brazil (Fig. 13): *T. balios*, *T. cubataonis*, *T. davisi*, *T. guaraquessaba*, *T. tupinamba*, *T. zonatus*, *Trichomycterus* aff. *davisi*, *Trichomycterus* sp. 'malacara', *Trichomycterus* sp. 'tubarão'. The GMYC analysis supported three entities (2 clusters and 1 single individual) within *Trichomycterus* sp. 'malacara', one cluster include specimens from Mampituba and Três Forquilhas rivers; a second includes specimens from Araranguá River basin and the third entity is a single individual from Maquiné River. Individuals representing *Trichomycterus balios*, *T. cubataonis*, and *Trichomycterus* sp. 'tubarão' were recognized as unique clusters. Coalescence was not identified to some species recognized by morphology and in a few instances two morphospecies were identified as single clusters. Individuals of *Trichomycterus tupinamba* and *T. guaraquessaba* form a single cluster. In addition, the species *T. zonatus* form a cluster together with a single specimen of *T. davisi* from Iguaçu River basin. The specimens recognized as *Trichomycterus davisi* from Ribeira de Iguape River basin form a unique cluster with individuals of *T. stawiarski* from Iguaçu River, and individuals of *Trichomycterus* aff. *davisi* form a single cluster with *T. davisi* from Tibagi River basin.

Discussion

Phylogenetic inferences and Coalescent-based species delimitation

The Generalized mixed Yule- Coalescence method (GMYC) has been one of the most popular approaches for species delimitation on single locus phylogenetic data sets (Talavera *et al*., 2013, Roxo *et. al*., 2017, Serrano *et al*., 2018). A good prospect of this methodology is its exploratory capacity, not requiring previous information on species assignment and being an important tool to explore groups with uncertain and problematic taxonomy (Talavera *et al*., 2013), as is the case of the genus *Trichomycterus*. Despite the increasing number of works using this methodology with several taxonomic groups, evaluation of this methodology in *Trichomycterus* is scarce (Mesa *et al*., 2018). Therefore, our results represent a comprehensive revision work for the species of the genus *Trichomycterus* from coastal drainages of South and Southeast using GMYC method for species delimitation.

In general, the GMYC species delimitation, as a complementary tool to morphology proved, to be efficient and congruent for most *Trichomycterus* species delimitated by morphology. However, the results also show some incongruences between the molecular data and morphological in species delimitation, discussed below. One example of incongruence occurs in the putative new species *Trichomycterus* sp. ‘malacara’. Although populations referred as *T.* sp. ‘malacara’ compose a monophyletic entity, the GMYC analysis supported the presence of three distinct taxonomic groups, suggesting strong genetic structure within *Trichomycterus* sp. 'malacara' populations and potential isolation by drainage. Similar results, regarding genetic data and none or feeble morphological distinction, are also found by other studies within the same region (e.g. *Diapoma itaimbe* in Hirschmann *et al*., 2015). Although the GMYC has a strong theoretical basis, empiric studies have demonstrated that GMYC typically recognize more units than traditional approaches using morphology-based species delimitation (Esselstyn *et al*., 2012; Talavera *et al*., 2013; Camelier *et al*., 2018; Serrano *et al*., 2018) as it seem to be the case of *Trichomycterus* sp. 'malacara'. Similar resolution also has already been reported for some Neotropical freshwater species of fishes (Costa-Silva *et al*., 2015; Henriques *et al*., 2015; Camelier *et al*., 2018; Serrano *et al*., 2018). Another possible explanation that is also reported in other studies (Pereira *et al*., 2013, Henriques *et al*., 2015) is that mutations can be accumulated and genetic discrimination is possible without morphological differentiation, these entities are also known as cryptic species (de Queiroz, 2007).

A distinct case of incongruence occurs when species are delimitated morphologically and GMYC does not recover these groups. As it is in four cases within the species examined: *Trichomycterus tupinamb*a and *T. guaraquessaba*; *T. zonatus* and *T. davisi* from Iguaçu River basin, *T. davisi* from Ribeira de Iguape River basin and *T. stawiarski* from Iguaçu River basin, and *T.* aff. *davisi* and *T. davisi* from Tibagi River basin. The low genetic divergence observed between these species is possibly related to the recent speciation process recognized to *Trichomycterus* (Ochoa *et al*., 2017) and incomplete lineage sorting. Thus, these species are not distinguished by a coalescent-based approach as previously reported for other groups with complex taxonomic history (Pereira *et al*., 2010; Henriques *et al*., 2015, Costa-Silva, 2015).

Finally, we agree that inferences regarding species boundaries based on genetic data alone are likely inadequate, and robust species delimitation should be conducted with consideration of other sources of data, such as morphology, in an integrative approach (Pereira *et al*., 2013, Serrano *et al*., 2018, Camelier *et al*., 2018). In this sense, incongruence across the results from different methods of species delimitation is relatively common and inferences and taxonomical acts drawn from these results should be conservative. Thereby, in most contexts it is better to fail to delimit species than it is to falsely delimit entities that do not represent independent evolutionary lineages (Carstens *et al*., 2013).

Geography of diversification

The drainages of South and Southeast Brazil are among the most endemic regions with regard to freshwater fish (Abell *et al.*, 2008; Albert *et al*., 2011). In this region freshwater fish evolution and distribution pattern has been shaped mainly by changes in the surface of the Earth involving changes in river courses and sea level fluctuations (Ribeiro, 2006; Thomaz *et al*., 2015).

Trichomycterus species from the coastal region are often closely related with population/species from the continental drainages such as Uruguay, Iguaçu and Upper Paraná. From the nine morphotypes delimitated by our taxonomic study six have their closest relatives within continental drainages. Therefore, our results support that some lineages of *Trichomycterus* have dispersed between coastal and continental drainages by headwater capture. *Trichomycterus* species diversity within coastal drainages seems to be associated with many geological events that may have promoted faunal exchanges during the Quaternary (Ribeiro 2006; Ribeiro *et al*., 2016). In this sense neotectonic processes in this region may be influencing riverine landscape evolution and therefore delineating ichthyofaunal distribution and speciation in this region (Ribeiro 2006;

Ribeiro *et al.*, 2016).

Herein we suggest that there was an exchange of *Trichomycterus* species between continental and coastal drainages by headwater capture, (*e.g.*, populations of *T. balios* in Laguna dos Patos system, Uruguay, Mampituba, and Itajaí rivers basin and *T. davisi* in Iguaçu River, upper Paraná River, and Ribeira de Iguape River basin). Headwater capture events are complex process associated with the main fault systems, which are more susceptible to erosion. In this process, portions of the tributaries of a river in one hydrographic basin are captured by adjacent basins resulting in isolated populations. This process occurred several times in the formation of the drainage basin in the southeastern Brazilian Shield (Ribeiro, 2006; Ribeiro et al., 2006).

Another example to illustrate this headwater captures observed in the distribution of *Trichomycterus* sp. 'malacara'. *Trichomycterus* sp. 'malacara' is related with species that inhabit the upper portions of the neighboring Laguna dos Patos System (e.g. *T. brachychekenos* and *T. poikilos*) and Uruguay River basin (*T. perkos*). This species is distributed in isolated drainages, comprising Maquiné, Três Forquilhas, Mampituba, and Araranguá Rivers basin, that compose the Tramandaí-Mampituba Freshwater Ecoregion (unit 335 *sensu* Abell *et al.*, 2008), an area of high endemism and with congruent distribution patterns among many species (Ferrer *et al.*, 2015; Hirschmann *et al.*, 2015). *Trichomycterus* sp. 'malacara' populations are restricted to high portions, suggesting that the species is limited to headwater environment, probably because of its ecological requirements (*e.g.*, clear, cold, and rapid flowing waters over rocks substrates). Besides that, for species adapted to upstream environments, the lowland environments may represent a barrier to gene flow among adjacent drainages, seems to be the case for *Trichomycterus* sp. 'malacara'. Thus, with the sea level retreats during glaciation periods would have created temporary connections among presently isolated basins, allowing fishes to disperse between them (Weitzman *et al.*, 1988; Thomaz *et al.*, 2015, Hirschmann *et al.*, 2015). Regarding the genetic structure of *Trichomycterus* sp. “malacara” within these basins, similar results are also found to another rapid water fish specialist in this region (*e.g.*, *Diapoma itaimbe* in Hirschmann *et al*., 2015; *Bryconamericus lethostigmus* in Hirschmann *et al*., 2017). In these species, populations of Mampituba River basin are also related or have shared haplotypes with Três Forquilhas River. Therefore, this suggests that a recent gene flow due to headwater capture is responsible by the recent genetic mixture between Três Forquilhas and Mampituba populations in the rivers of the coastal region.

Contrasting with river capture events the genetic molded by palaeodrainages can be illustrated in *Tricomycterus* aff. *davisi* and *Trichomycterus cubataonis*. These taxa have distributions on lower portions of Santa Catarina and Paraná States coastal drainages (Cubatão Sul, Biguaçu, Tijucas, Ilha de Florianópolis, Itapocú and Cubatão Norte rivers basin; Itapocu River basin, Cubatão Norte River basin, Guaratuba River basin, Babitonga and Paranaguá Bays). The past connection among these drainages is potentially associated to past geological events in marine regression events, that permited dispersal among the fishes due network of rivers united in lower portions of continental plataform (Thomaz *et al.*, 2015; Thomaz & Knowles, 2018).

Therefore, the diversification in *Trichomycterus* of South and Southeast Brazil seem to be associated with both geological events associated with geographical distributions of fishes in this region. Primarily by faunal exchanges occurred between coastal and continental drainages and secondarily constrained by palaeodrainage connection formed within coastal basins during the pleistocene. This relatively recent diversification processes may be reflected in the delimitation of distinct lineages, therefore making it necessary a integrative analysis approach to understand evolutive/biogeographical mecanisms promoting diversification and constraining species distributions.

Material examined.

Trichomycterus alternatus: FMNH 58082, holotype; FMNH 58083, 8, paratypes. *Trichomycterus balios*: UFRGS 16229, holotype; UFRGS 16341, 3 (1 c&s), paratype; UFRGS 16233, 3 (1 c&s), paratype; CPUFMT 337, 2; DZSJRP 3256, 1; MCP 16522, 15(5); MNRJ 18526, 4(2); NUP 10465, 8(5); NUP 010471, 13(6); UFRGS 9180, 1; UFRGS 18104, 29; UFRGS 18095, 2; UFRGS 20118, 8; UFRGS 22939, 33(3); UFRGS 23182, 29; UFRGS 23495, 52; UFRGS 24527, 2; UFRGS 24558, 4. *Trichomycterus cubataonis*: MNRJ 12490, holotype; MNRJ 12491, 9 paratypes. MCP 10641, MNRJ 32940, MNRJ 41029, UFRGS 24543, 8; UFRGS 24549, 11; UFRGS 24552, 7; UFRGS 24556, 5; UFRGS 24567, 30; UFRGS 24311, 7. *Trichomycteus davisi*: FMNH 60309, holotype FMNH 52242, 8, paratypes; NUP 6711, 1; MZUEL 17202, 30; MZUEL 17213, 4. *Trichomycterus* aff. *davisi*: MCP 16570, 1; MCP 17477, 2; MNRJ 13191, 1; UFRGS 20914, 1; UFRGS 20915, 4(2) 1 c&s; UFRGS 22906, 29(17), 2 c&s; UFRGS 22907, 2; UFRGS 22932, 6, 2 c&s; UFRGS 23183, 4; UFRGS 23235, 1; UFRGS 24296, 8(3); UFRGS 24311, 7; UFRGS 24567, 30. *Trichomycterus guaraquessaba*: MHNCI 7916, 3, paratypes; MHNCI 7916, 1; MNRJ 40846, 1; MPEG 7916, holotype; MZUSP 85531, 2, paratypes; UFRGS 24554, 1; UFRGS 24541, 1. *Trichomycterus jacupiranga*: MZUSP 67818, holotype; MZUSP 67821, paratype; MZUSP 67820, paratype; MZUSP 67892, paratype; MZUSP 84095, paratype; MZUSP 67819, paratype. *Trichomycterus tupinamba*: MPEG 7895, 5, paratypes; MZUSP 61686, 1; MZUSP 62382, holotype; MZUSP 84094, 4, paratypes; MZUSP 62362, paratype; MZUSP 63495, paratype; MZUSP 61686, 2; UFRGS 24550, 3; UFRGS

25094, 2; UFRGS 24563, 2. *Trichomycterus zonatus*: DZSJRP 13630, 1; FMNH 58573, holotype. FMNH 58572, 3, paratypes; MCP 20621, 1; MZUSP 28415, 1; MZUSP 38784, 1; MZUSP 62380, MZUSP 61696, 1; MZUSP 61715, 1; MZUSP 82685, 1; MZUSP 82771, 1; MZUSP 82932, 1; MZUSP 83139, 1; MZUSP 83714, 1; MZUSP 83724, 1; MZUSP 83730, 1; MZUSP 83751, 1; 1; UFRGS 11420, 2; UFRGS 24538, 1. *Trichomycterus* sp. ‘malacara’: MCN 18587, 1; MCP 10646, 7(4); MCP 23623, 2; MCP 25375, 1; MCP 25395, 1; MCP 29143, 2(1); MCP 29147, 1; MCP 29694, 2; MZUEL 07547, 2; UFRGS 10651, 1 (c&s); UFRGS 17220, 2; UFRGS 17228, 1; UFRGS 17575, 1; UFRGS 19149, 5; UFRGS 19169, 1; UFRGS 19178, 1; UFRGS 21346, 1; UFRGS 22211, 1; UFRGS 22962, 6(4), 1 c&s; UFRGS 23494, 1; UFRGS 29117, 1. *Trichomycterus* sp. ‘tubarão’: CPUFMT 79, 11; CPUFMT 86, 2; CPUFMT 106, 1; MCP 10642, 37(10); UFRGS 22964, 19(7), 2 c&s; UFRGS 23184, 2; UFRGS 23185, 7(3); UFRGS 24553, 12(1).

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ANEXOS

Tab. 1. Summary of variable characters within species of *Trichomycterus* inhabiting coastal basins of Southern Brazil.

	***T*. sp. 'malacara'**	***T. balios***	***T*. sp. 'tubarão'**	***T*. aff. *davisi***	***T. davisi***	***T. cubataonis***	***T. zonatus***	***T. guaraquessaba***	***T. tupinamba***
Pectoral-fin rays: number	I,5 (19); I,6 (2)	I,6*; I,7	I,6	I,6 ; I,7	I,5*; I,6	I,6*	I,6*	I,7	I,7
Dorsal procurrent caudal-fin rays: number	14-17 (2)	14-17 (14)	17-21 (3)	17-22 (4)	17*	17-21 (4)	13-15* (4)	15 (2)	15-16 (2)
Ventral procurrent caudal-fin rays: number	10-12 (2)	10-13 (14)	12-14 (3)	10-12 (4)	11*	12-13 (4)	9-12* (4)	8 (2)	10 (2)
Branchiostegal rays: number	8-9 (2)	8-9 (14)	7-8 (3)	7-8 (6)	9-10 (2)	9 (4)	8/9 (1)	8 (2)	7-8 (2)
Vertebrae: number	36-39 (2)	38-41 (14)	38-40 (3)	36-37 (6)	37-38 (2)	38-40 (4)	37* (4)	37 (2)	39-40 (2)
Ribs: number	12-14 (2)	12-15 (14)	14-15 (3)	11-12 (6)	13-15 (2)	12-15 (4)	13 * (4)	14-15 (2)	14-15 (2)
Dorsal-fin Insertion: first pterygiophore: position of relate to neural spine of vertebrae	17-20 (2)	19-22 (14)	19 (3)	16-18 (6)	19 (2)	18-20 (4)	18*-19 (4)	18 (2)	19-20 (2)
Anal-fin Insertion: first pterygiophore: position of relate to haemal spine of vertebrae	20-22 (2)	22-25 (14)	22-23 (3)	21-22 (6)	22-23 (2)	22-24 (4)	21* (4)	22 (2)	23-25 (2)
Antorbital segment of the infraorbital canal (pores 'i1', 'i3')	absent	absent	absent	absent	absent	present*	absent	present	present
Skin layers of coloration: number	one	two	one	one	two	one	one	one	one

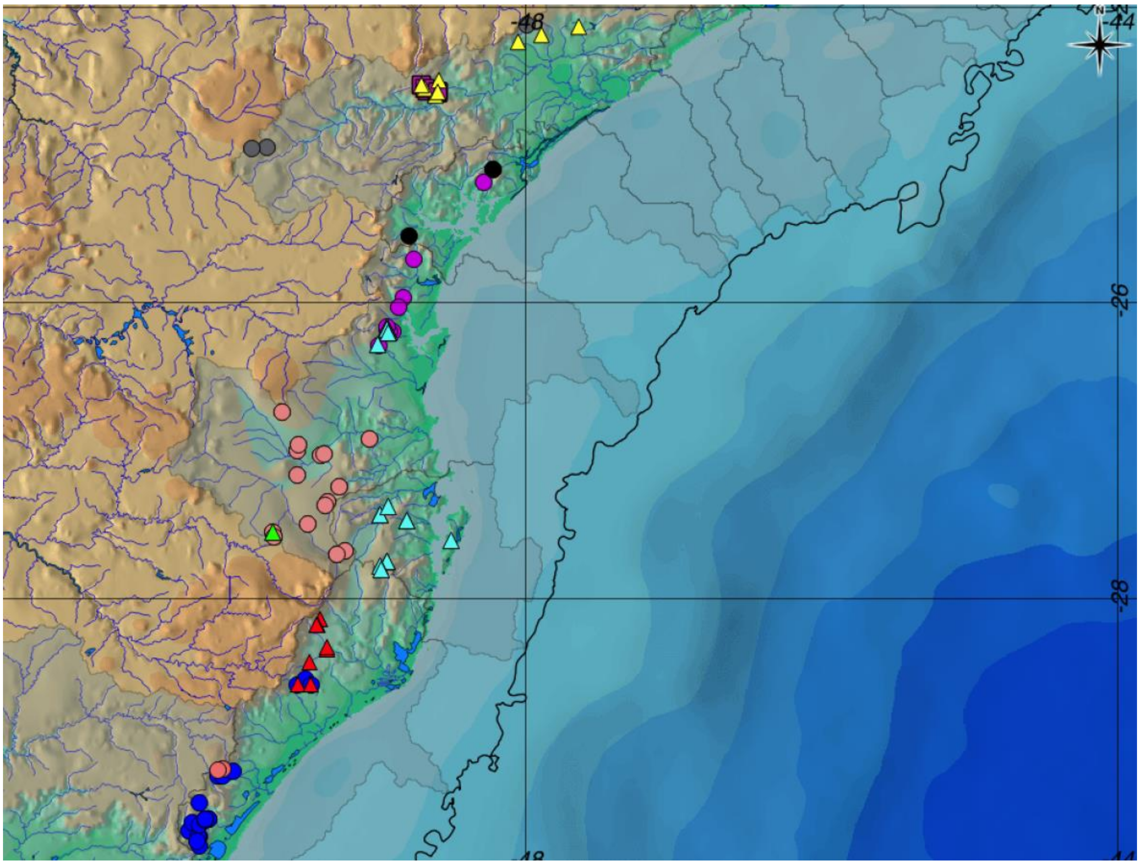

Fig. 1. Distribution of *Trichomycterus* species through coastal drainages of South and Southeast Brazil from Tramandaí River basin (South) to the Ribeira do Iguape River basin (North). Blue circles: *T.* sp. “*malacara”*; pink circle: *T.balios*; red triangles: *T.* sp. “tubarão”; green tringle: T. sp “itajaí”; light blue triangles: *T. aff*. davisi; purple circles: *T. cubataonis*; gray circles: *T. davisi*; black circles: *T. guaraquessaba*; yellow triangles: *T. tupinamba*; and rectangular purple: *T. zonatus*.

Fig. 2. Populations of *Trichomycterus* sp. "malacara" from **A.** Maquiné River, UFRGS 22211, 58.6 mm SL, **B.** Mampituba River, MCP 23623, 55.7 mm SL; and **(C-D)** Araranguá River, UFRGS 22962, 45.2-59.8 mm SL.

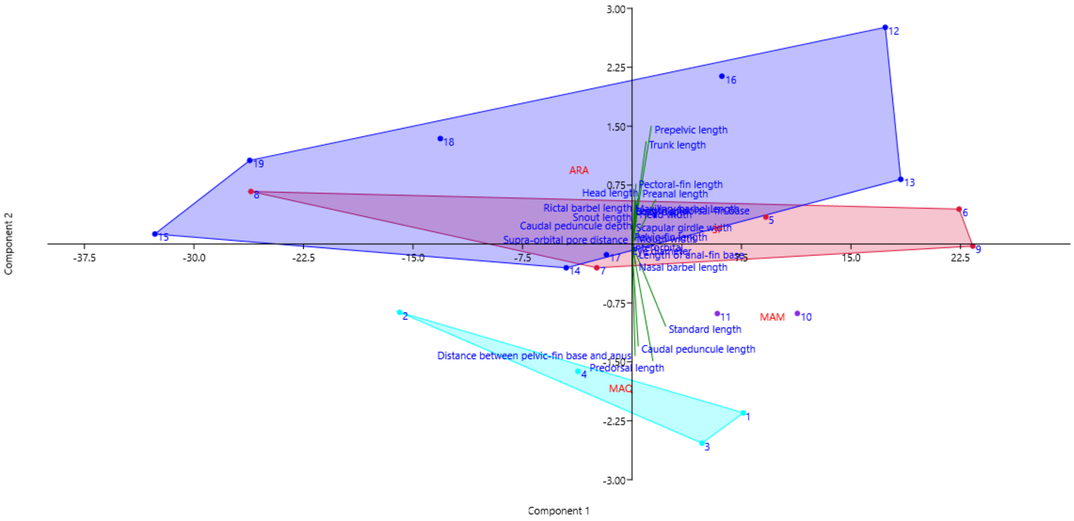

Fig. 3. Morphometric differences among allopatric populations of *Trichomycterus* sp. ‘malacara’ from Maquiné (light blue), Três Forquilhas (red), Mampituba (purple) and Araranguá (blue) rivers basin obtained by Principal component analysis (PCA)

Fig. 4. Variation on the coloration *in vivo* and ontogenetic series of *Trichomycterus balios* from Itajaí River basin **(A-F**), showing a unique color pattern with two layers in the skin composed of large and small round black blotches in the inner and outer layer.

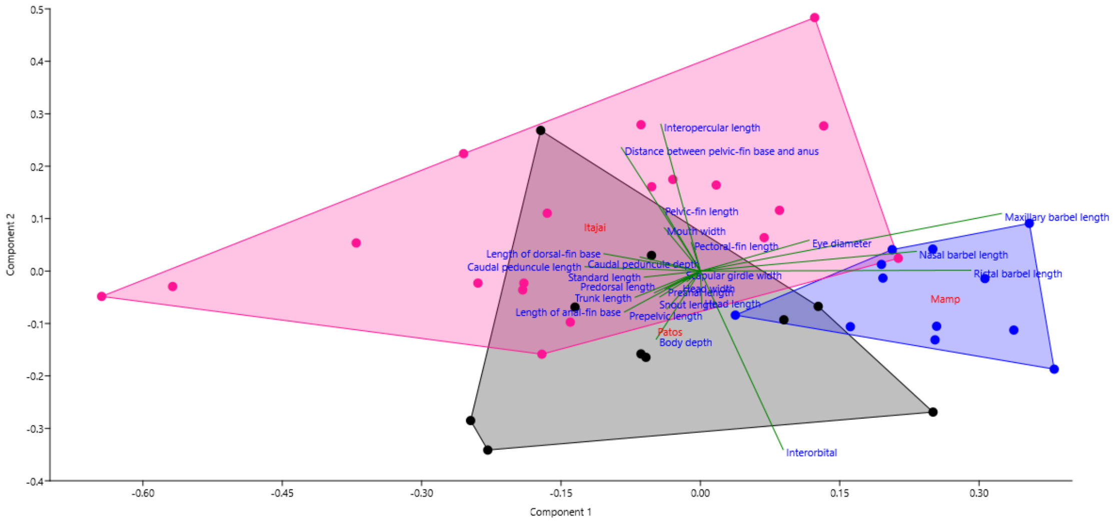

Fig. 5. Morphometric differences among allopatric populations of *Trichomycterus balios* from Mampituba (blue) and Itajaí (pink) coastal drainages and specimens from upper portions of the Laguna dos Patos System (type locality; gray).

Fig. 6. Variation on the coloration in *Trichomycterus* sp. “tubarão” from (**A-C**) Tubarão and (**D-E**) Araranguá river drainages, showing a unique color pattern that differs from other congeners in the study area: one layer of coloration composed of not coalescent round small blotches.

Fig. 7. Polymorphic color pattern within of *Trichomycterus* aff. *davisi* ranging from a mottled color pattern with (Fig. 7 **A-B**) to a blotched color pattern with dark marks varying in shape and size (Fig. 7. **C-E**).

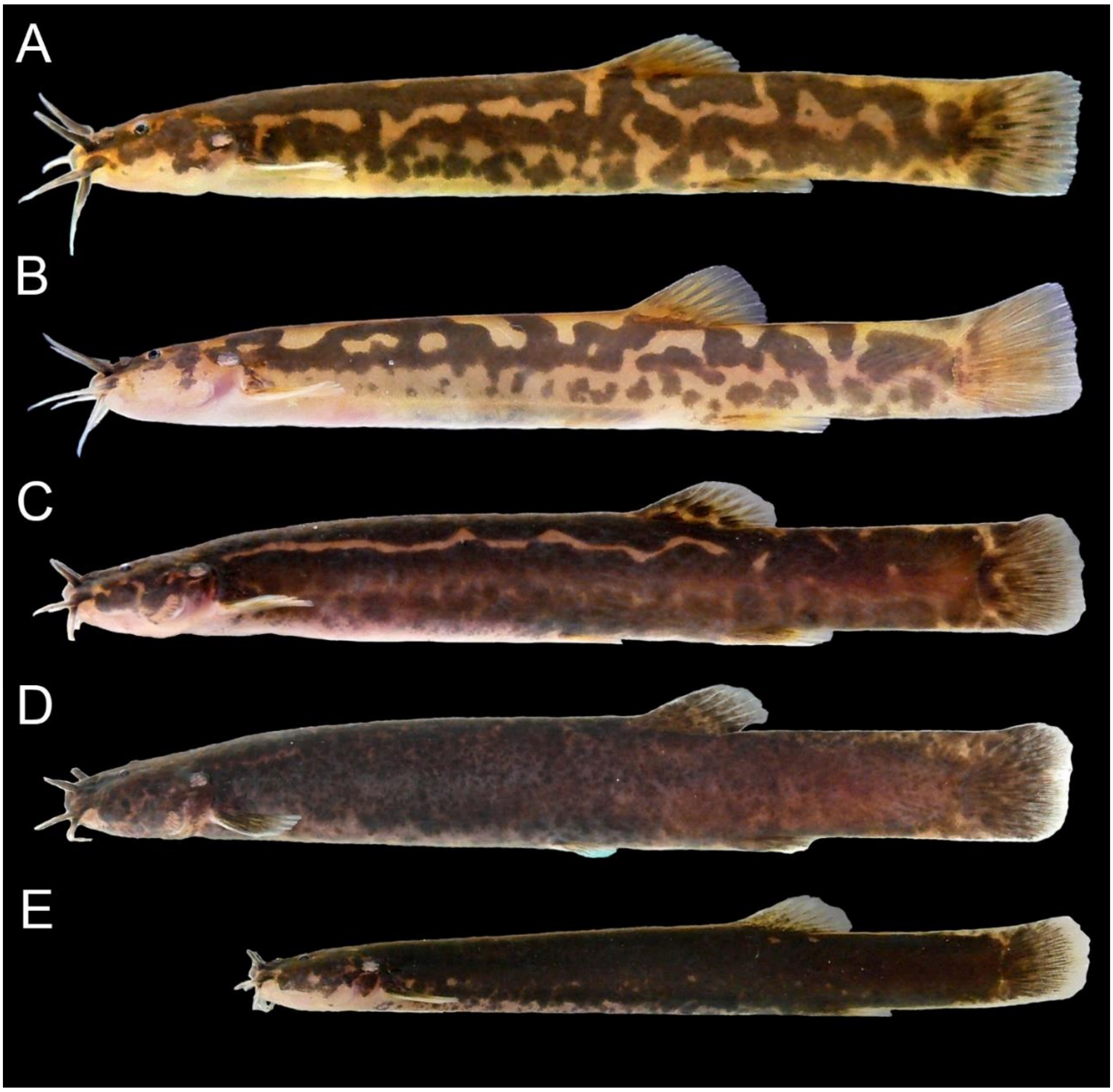

Fig. 8. Variation on the coloration in *Trichomycterus cubataonis*, showing a mottled color pattern composed by blotches variable in shape and forming marked saddles dorsally, (**C-E**) specimens with large dark areas merged giving a overall dark color pattern. Specimens from Guaratuba River basin (**A**) UFRGS 24549, 53.1 mm SL; (**B**) UFRGS 24556, 60.3 mm SL; and Babitonga, UFRGS 24552, 77.2 mm SL, 85.7 mm SL, and 61.6 mm SL; respectively.

Fig. 9. Coloration *in vivo* of *Trichomycterus* species. **A-B.** *Trichomycterus guaraquessaba*, UFRGS 24554, 37.0 and 36.95 mm SL, tributary of Guaraqueçaba River, Paranaguá River basin. **C-D.** *T. tupinamba*, UFRGS 24550, 63.0 mm SL and 39.4 mm SL, rio Betari, Ribeira de Iguape River basin.

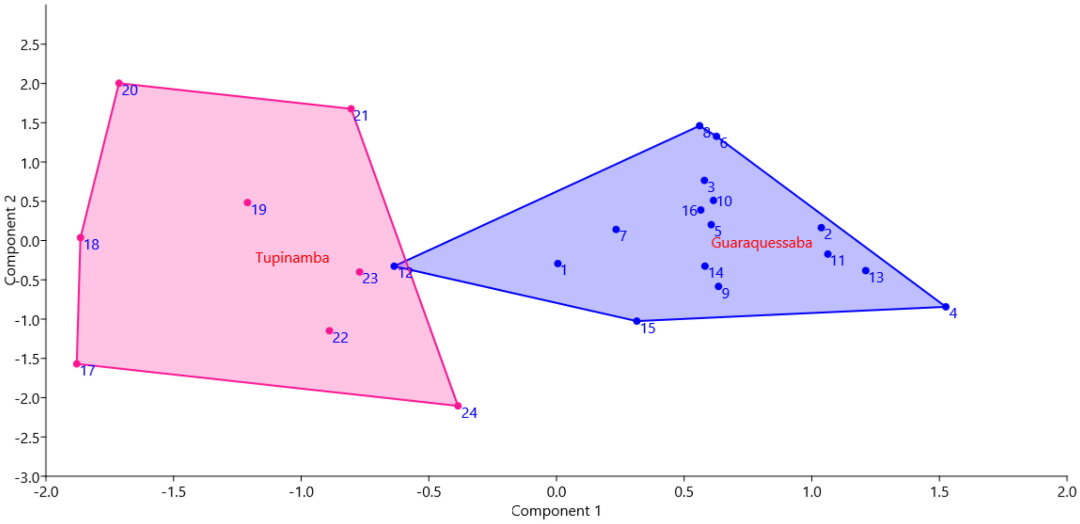

Fig.10. Principal component analysis of morphometric data between *Trichomycterus guaraquessaba* (blue) and *T. tupinamba* (purple).

Fig. 11. Coloration in vivo of specimens of *Trichomycterus zonatus*, (**A-C**) UFRGS 24538, (A) 51.1 mm SL, (B) 48.4 mm SL, rio Betari, Ribeira de Iguape River basin.

Fig. 12. *Trichomycterus* species from Ribeira de Iguape River basin. A. *Trichomycterus davisi*, MZUEL 17202, 72.8 mm SL, fixed in alcohol. B. *Trichomycterus zonatus*, UFRGS 24538**,** 48.4 mm SL, *in vivo*.

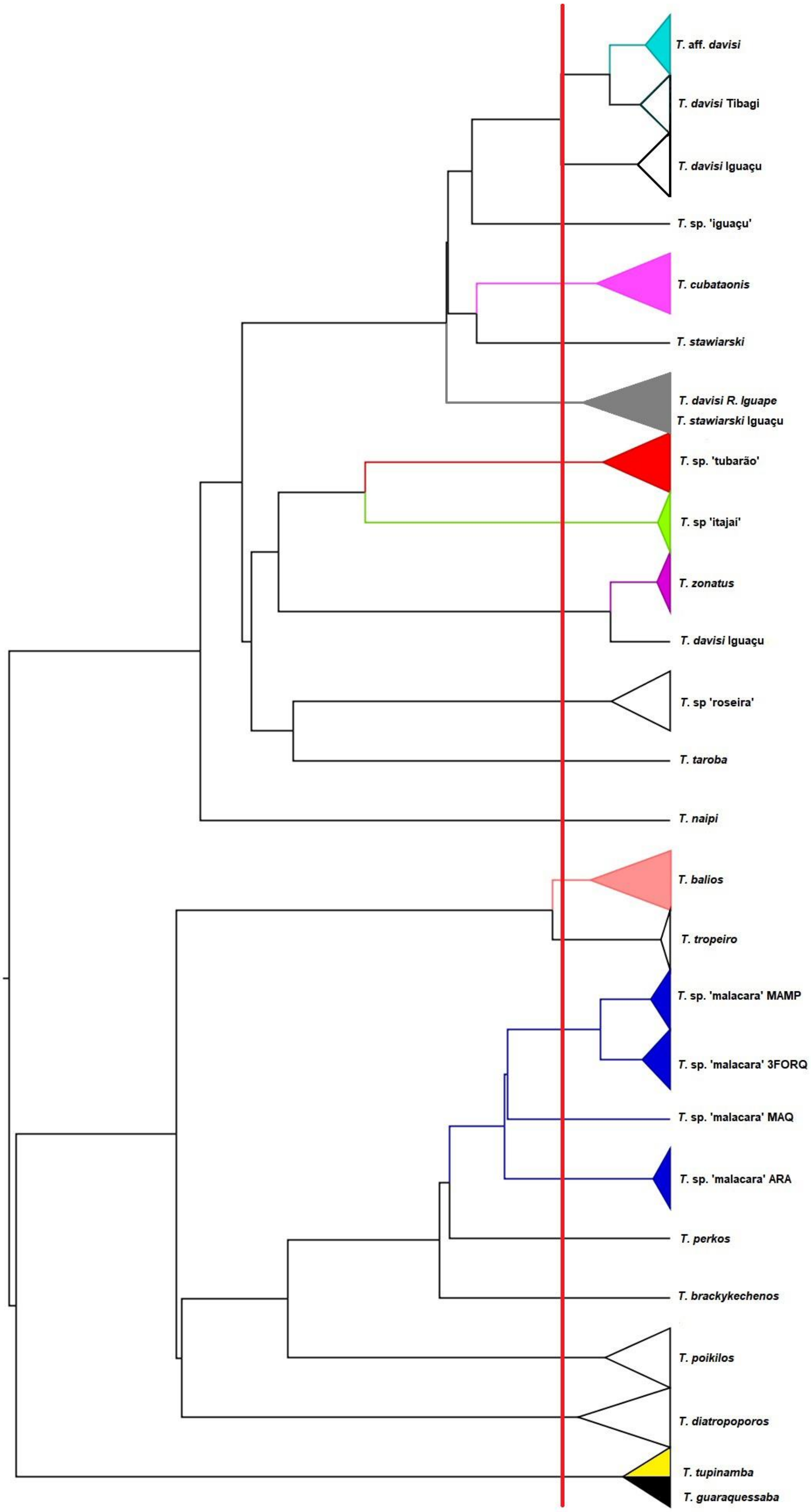
T. aff. davisi
T. davisi Tibagi
T. davisi Iguaçu
T. sp. 'iguaçu'
T. cubataonis
T. stawiarski
T. davisi R. Iguape
T. stawiarski Iguaçu
T. sp. 'tubarão'
T. sp 'itajai'
T. zonatus
T. davisi Iguaçu
T. sp 'roseira'
T. taroba
T. naipi
T. balios
T. tropeiro
T. sp. 'malacara' MAMP
T. sp. 'malacara' 3FORQ
T. sp. 'malacara' MAQ
T. sp. 'malacara' ARA
T. perkos
T. brackykechenos
T. poikilos
T. diatropoporos
T. tupinamba
T. guaraquessaba

Fig. 13. Bayesian phylogenetic tree of *Trichomycterus* species from South and Southeast Brazil obtained from concatenated mitochondrial matrix (COI +CYTB). The vertical red line shows the coalescent branching process estimated by using the single-threshold model in the GMYC test. Color corresponds to *Trichomycterus* species of the study area and matches map of figure 1.

CONSIDERAÇÕES FINAIS

O presente trabalho é uma importante contribuição no estado de conhecimento de *Trichomycterus*, especificamente nas espécies costeiras do Sul e Sudeste do Brasil, através de uma extensa revisão taxonômica integrando dados morfológicos e moleculares. Foram reconhecidos 12 morfotipos presentes na área de estudo, quatro destes como prováveis novas espécies: *Trichomycterus* sp. 'malacara', *Trichomycterus* sp 'ribeira', *Trichomycterus* sp. 'tubarão', *Trichomycterus* aff. *davisi*, *T. alternatus*, *T. balios*, *T. cubataonis*, *T. davisi*, *T. guaraquessaba*, *T. jacupiranga*, *T. tupinamba* and *T. zonatus*.

O uso do *Generalized Mixed Yule-Coalescent* (GMYC) como ferramenta adicional de delimitação, mostrou-se satisfatório para a maioria das espécies de *Trichomycterus* analisadas, no entanto apresentou algumas inconsistências, quando contrastado com morfologia. As inferências na delimitação das espécies devem ser feitas com cautela e limites de espécies baseado apenas em um conjunto de dados é por vezes insatisfatório.

Nossos resultados sugerem que a diversificação das espécies de *Trichomycterus* do Sul e Sudeste do Brasil esta associada a ambos os eventos promovedores de troca de fauna nessa região: capturas de cabeceira entre drenagens costeiras e continentais e dispersão e vicariância promovidos pela formação de paleodrenagens na plataforma costeira. Finalmente, esses processos de diversificação relativamente recentes podem ser refletidos no reconhecimento e delimitação das linhagens, portanto, assim se ressalta a importância de uma abordagem integrativa para entender os mecanismos promovedores de diversificação e restrição da distribuição das espécies.