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

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Abstract

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

Key words: Upper Paraná Basin, Freshwater catfish, pelvic-fin loss, taxonomy.

Introduction

Trichomycteridae is one of the most species-rich groups of freshwater fishes (de Pinna 1998) in the Neotropics with approximately 290 valid species (Eschmeyer *et al.* 2017) distributed into 41 genera and eight subfamilies. The highest species diversity of the family is concentrated in the Trichomycterinae, especially in the genus *Trichomycterus*, which currently includes about 190 valid species (Eschmeyer *et al.* 2017), most of which are characterized by restricted geographic distributions and high levels of endemism (de Pinna 1992; Malabarba *et al*. 2009).

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

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

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Material and methods

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

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

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

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

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

Trichomycterus pascuali, new species

(Figs. 1–3, Table 1)

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

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

Diagnosis. *Trichomycterus pascuali* is readily distinguished from its congeners by two remarkable features: possession of five pectoral-fin rays (*vs.* six or more) and absence of pelvic fin, girdle, and muscles (*vs.* presence). Within *Trichomycterus*, only *T. candidus* (Rio Grande basin, Brazil), *T. catamarcensis* (Laguna Blanca basin, Argentina), and *T. tropeiro* (laguna dos Patos basin, Brazil) also lack pelvic structures. *Trichomycterus pascuali* differs from these three species in having the aforementioned five pectoral-fin rays (*v*s. six, eight, and seven, respectively) and seven branchiostegal rays (*vs.* eight, five or six, and nine, respectively). It addition, it differs from *T. candidus* by the possession of anterior and posterior cranial fontanels completely separated from each other by a broad epiphyseal bar (*vs.* fontanels partially or completely fused, with epiphyseal bar absent or minute and incomplete) and 37 post-Weberian vertebrae (*vs.* 38–39 in *T. candidus*); from *T. catamarcensis* by the first pectoral fin prolonged as a small filament (*vs.* pectoral filament absent in most specimens), 14 ribs (*vs.* 18–20 in *T. catamarcensis*), 17–18 dorsal procurrent caudal-fin rays (*vs.* 12–13), and 15–16 ventral procurrent caudal-fin rays (*vs.* 11–12); and from *T. tropeiro* by the presence of pectoral filament (*vs.* absence), 17–18 dorsal procurrent caudal-fin rays (*vs.* 13–14), 15–16 ventral procurrent caudal fin rays (*vs.* 9–10), and absence of pores i_1 and i_3 (*vs.* presence).

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

Description. Morphometric data are given in Table 1. Body elongate, maximum total length 52.2 mm SL, cross section of trunk roughly cylindrical at pectoral girdle and progressively more compressed towards caudal-fin. In lateral view, dorsal profile of body straight from snout tip to interorbital region, slightly convex to dorsal-fin origin, and slightly convex to end of caudal peduncle. Ventral surface of body convex from tip of snout to anal-fin insertion. Caudal peduncle smoothly continues profile of trunk region, with dorsal and ventral profiles slightly convex. Greatest body depth at dorsal-fin origin. Head wide, longer than wide, nearly trapezoidal in dorsal view. Eyes elliptical, covered by thin and translucent skin separate from surface of eyeball. Anterior nostril larger than posterior one, surrounded by fleshy flap of integument continuous with base of nasal barbel. Posterior nostril partially surrounded anteriorly by thin flap of skin. Seven branchiostegal rays visible in c&s individuals. Mouth subterminal, its corners laterally or slightly posteriorly oriented. Lower lip with conspicuous fleshy lobes along lateral limits, lobes situated internal to base of rictal barbels. Lower lip with anterior and, to lesser degree, anteroventral surfaces covered by small papillae. Anterior margin of upper lip rounded with numerous papillae. Barbels relatively short and with larger bases. Nasal barbel thick always surpassing posterior margin of eye but not reaching opercular patch of odontodes. Maxillary barbel reaching posterior tip of interopercular patch of odontodes. Rictal barbel reaching interopercular patch of odontodes. Interopercular patch of odontodes posteriorly elongate with 11 or 12 conic odontodes imbedded in flesh. Opercular patch of odontodes very small, with 10–12 thin odontodes with thick tips.

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

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

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

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

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

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

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

Habitat, ecological notes, and conservation status. The new species was discovered in a small stream with clear water and moderate water flow at the altitude of approximately 600 meters above sea level. The specimens were collected under rocks and aquatic vegetation in a muddy bottom area bordered by riparian vegetation. The region is highly impacted by agricultural activity, more specifically eucalyptus monoculture. Rivers in the region are poor in both alpha diversity and specimen abundance. *Trichomycterus pascuali* was the only species of fish found in the type locality and the number of individuals sampled was low. This species has a restricted area of

occupancy and its vulnerability to the threats from anthropic impacts could drive the taxon to critically endangered in a short time. According to the IUCN (2001) criteria, *T. pascuali* should be classified in the category vulnerable VU D2.

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

	Holotype	Range	Mean	SD
Standard length (mm)	48.8	31.37-49.36	40.4	6.1
Percents of Standard Length				
Head length	15.5	14.30-16.77	15.8	0.9
Predorsal length	67.3	65.24-68.31	67.2	1.1
Preanal length	73.3	68.27-73.81	71.8	1.9
Scapular girdle width	10.8	10.68-12.08	11.4	0.5
Pectoral-fin length	8.2	8.21- 12.56	10.4	1.5
Caudal peduncle length	17.5	14.49-21.33	17.8	1.9
Caudal peduncle depth	11.5	9.66-11.53	10.7	0.7
Body depth	13.7	8.19-14.87	12.5	2.0
Length of dorsal-fin base	10.9	6.57-13.13	10.3	1.8
Length of anal-fin base	8.4	5.77-9.54	8.1	1.1
Percents of Head Length				
Head width	96.0	80.66-96.03	91.2	6.4
Nasal barbel length	66.1	64.20-79.92	68.9	4.9
Maxillary barbel length	55.4	55.36-76.31	62.7	7.1
Rictal barbel length	66.0	53.30-71.43	63.2	6.9
Snout length	12.9	7.14-17.35	12.8	3.4
Interorbital	27.7	25.27-31.86	29.0	2.2
Mouth width	33.0	26.72-47.08	35.3	5.9
Supra-orbital pore distance	11.7	10.52-16.07	12.9	1.9

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

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

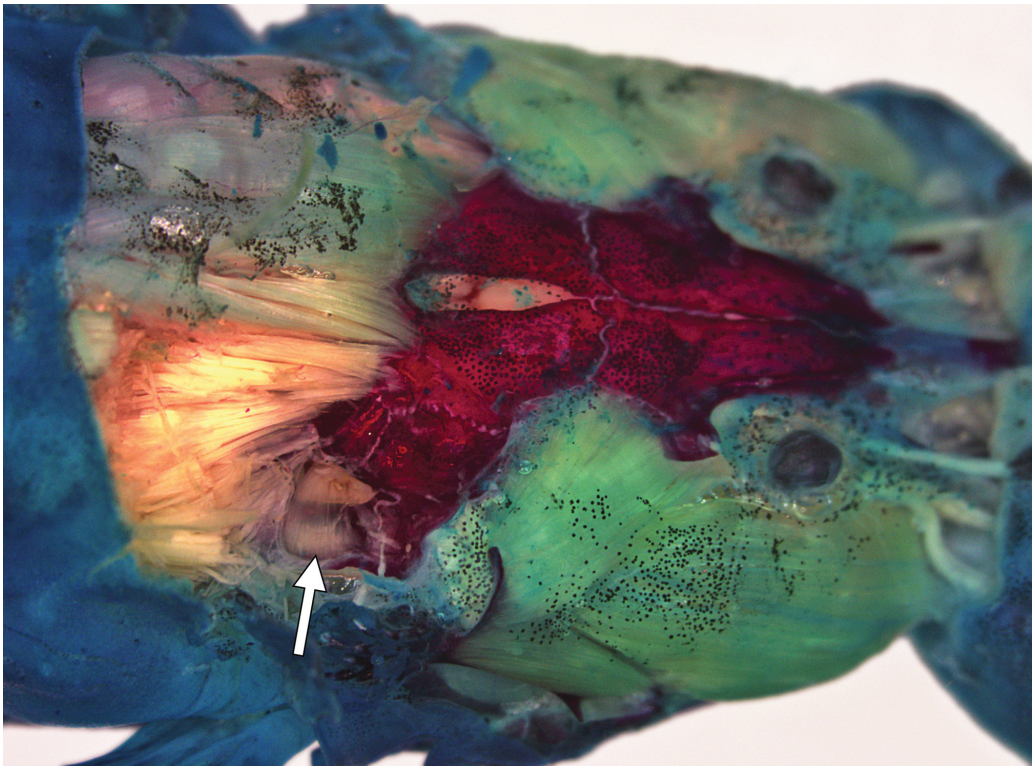

FIGURE 3. Dorsolateral view of head of *Trichomycterus pascuali*, LBP 23323 (51.2 mm SL); arrow indicates *levator internus IV* originating from the dorsal face of posttemporo-supracleithrum.

Discussion

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

3) and lacks the synapomorphies for the TSVSG clade, so that its allocation in the Trichomycterinae is unequivocal.

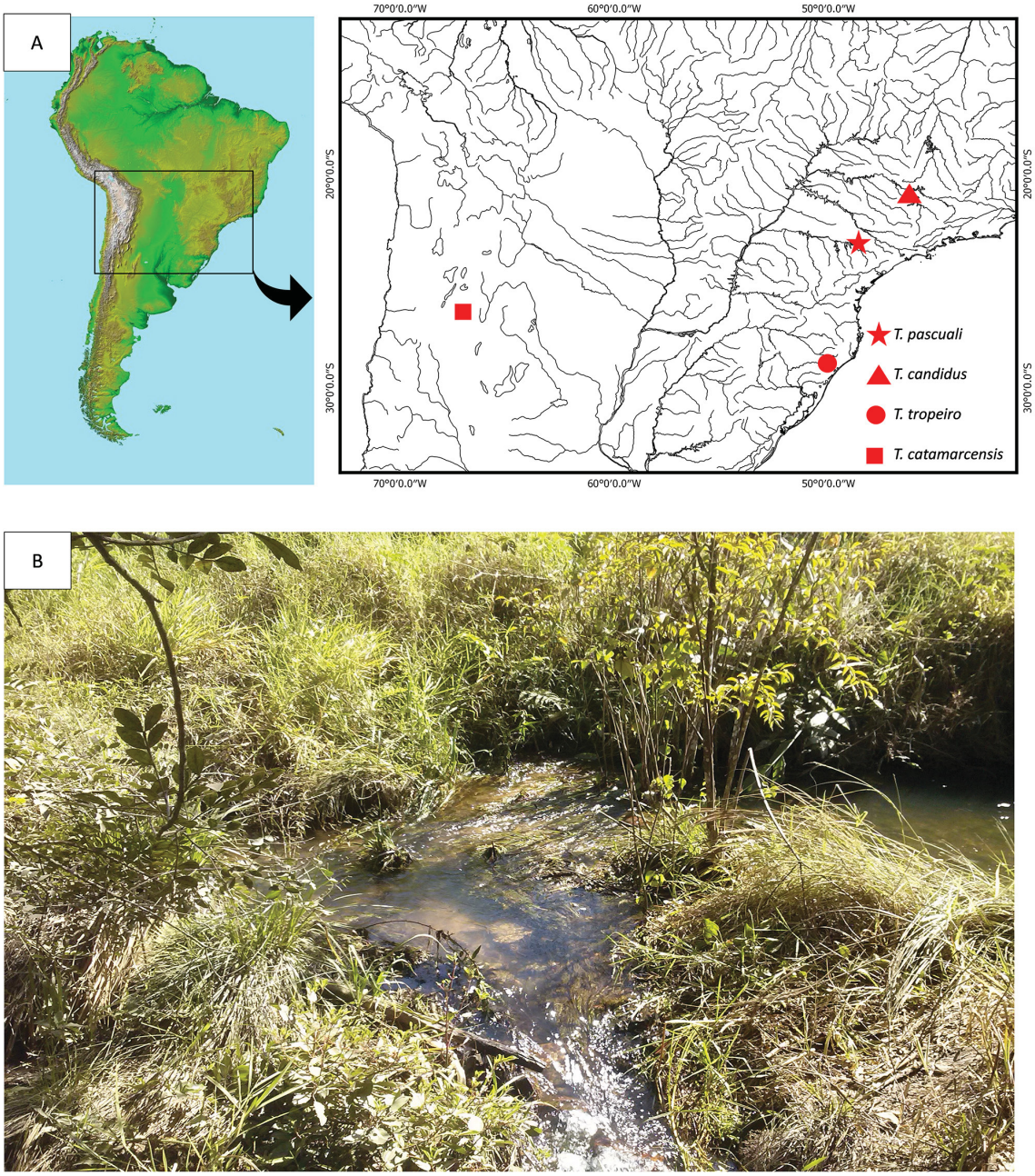

FIGURE 4. A. Map showing the type localities of *Trichomycterus pascuali*, 23°13'27.06"S 48°31'45.34"W, and congeners lacking pelvic fins and girdle. **B**. Type locality of *T. pascuali*, unnamed tributary of Tamanduá river, Paranapanema basin.

Whereas the genus *Trichomycterus* is a non-monophyletic group that includes those trichomycterines lacking the diagnostic features of the remaining genera, *Eremophilus* has been traditionally distinguished by its lack of pelvic fins (Eigenmann 1918; Myers 1944). However, several studies indicate that pelvic fins were lost independently many times during the trichomycterid radiation and that species with such a feature are often more closely related to taxa with pelvic fins (de Pinna 1988, 1989; Barbosa & Costa 2003b; de Pinna & Wosiacki 2003; Datovo 2014). As a result, the genus *Eremophilus* has been eroded by the removal of some species originally assigned to this genus, namely *E. candidus* and *E. camposi* (Miranda-Ribeiro 1949; 1957) that were transferred to *Trichomycterus* (*T. candidus*, de Pinna & Wosiacki 2003) and *Listrura* (*L. camposae*, Glanapteryginae; de Pinna 1988), respectively. In the last decade, two new trichomycterines lacking pelvic fins and girdles were described and

allocated to the genus *Trichomycterus* following similar reasoning: *T. catamarcensis* (Fernandéz & Vari 2000) from Catamarca, Argentina, and *T. tropeiro* (Ferrer & Malabarba 2011) from rio das Antas, laguna dos Patos system. As a result, *Eremophilus* is currently a monotypic genus that includes only its type species, *E. mutisii*, from the Bogotá River, Colombia.

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

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

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

Comparative material

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

Brazil. *Trichomycterus variegatus* Costa 1992: LBP 10289 (3), municipality of São Roque de Minas, Córrego do Lavapés, tributary rio São Francisco, Brazil.

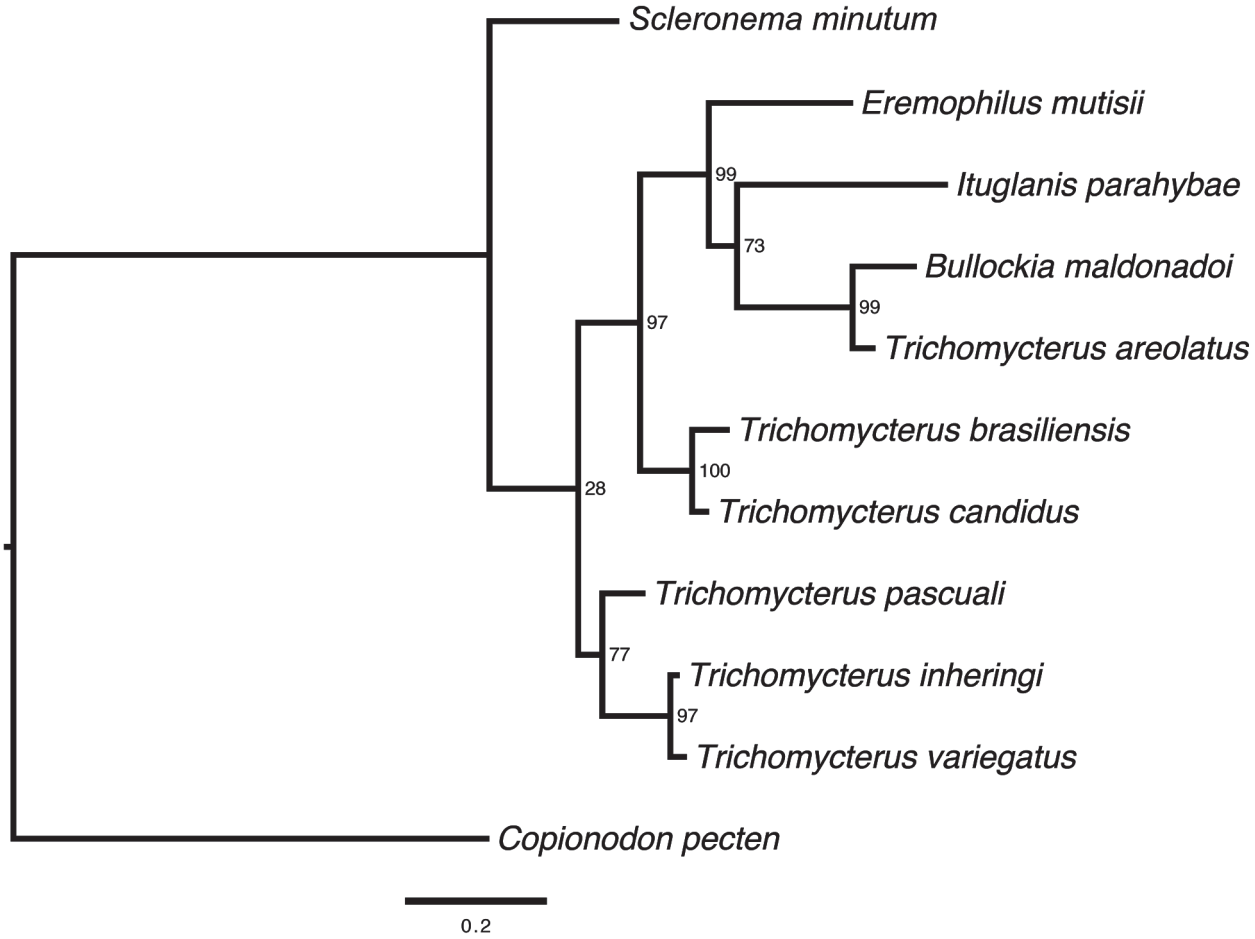

FIGURE 5. Maximum likelihood tree built with concatenated matrix using cytochrome oxidase sub-united I (COI) and 16S genes, showing the relationships of *Trichomycterus pascuali* within Trichomycterinae. Numbers on branches of tree denote bootstrap (B) values.

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References

Barbosa, M.A. & Costa, W.J.E.M. (2003a) *Trichomycterus potschi* (Siluriformes: Loricarioidei): a new trichomycterid catfish from coastal streams of southeastern Brazil. *Ichthyological Exploration of Freshwaters*, 14, 281–287.

Barbosa, M.A. & Costa, W.J.E.M. (2003b) Validade, relações filogenéticas e redescrição de *Eremophilus candidus* Ribeiro, 1949 (Teleostei, Siluriformes, Trichomycteridae). *Arquivos do Museu Nacional do Rio de Janeiro*, 61, 179–188.

Barbosa, M.A. & Costa, W.J.E.M. (2010) Seven news species of catfish genus *Trichomycterus* (Teleostei: Siluriformes: Trichomycteridae): from southeastern Brazil and re-description of *T. brasiliensis*. *Ichthyological Exploration of Freshwaters*, 21, 97–122.

Baskin, J.N. (1973) *Structure and relationships of the Trichomycteridae*. Unpublished Ph.D. dissertation, City University of New York, New York, 409 pp.

Bockmann, F.A., Casatti, L. & de Pinna, M.C.C. (2004) A new species of trichomycterid catfish from the Rio Paranapanema basin, southeastern Brazil (Teleostei: Siluriformes), with comments on the phylogeny of the family. *Ichthyological Exploration of Freshwaters*, 15, 225–242.

Costa, W.J.E.M. (1992) Description de huit nouvelles espèces du genre *Trichomycterus* (Siluriformes: Trichomycteridae), du Brésil oriental. Revue Française d'Aquariologie et Herpétologie, 18, 101–110.

Datovo, A. (2014) A new species of *Ituglanis* from the Rio Xingu basin, Brazil, and the evolution of pelvic fin loss in trichomycterid catfishes (Teleostei: Siluriformes: Trichomycteridae). *Zootaxa*, 3790 (3), 466–476. https://doi.org/10.11646/zootaxa.3790.3.5

Datovo, A. & Bockmann, F.A. (2010) Dorsolateral head muscles of the catfish families Nematogenyidae and Trichomycteridae (Siluriformes: Loricarioidei): comparative anatomy and phylogenetic analysis. *Neotropical Ichthyology*, 8, 193–246. https://doi.org/10.1590/S1679-62252010000200001

Datovo, A., Carvalho, M. & Ferrer, J. (2012) A new species of the catfish genus *Trichomycterus* from the La Plata River basin, southern Brazil, with comments on its putative phylogenetic position (Siluriformes: Trichomycteridae). *Zootaxa*, 3327, 33–44.

Dutra, G.M., Wosiacki, W.B. & de Pinna, M.C.C. (2012) *Trichomycterus anhanga*, a new species of miniature catfish related to *T. hasemani* and *T. johnsoni* (Siluriformes: Trichomycteridae) from the Amazon basin, Brazil. *Neotropical Ichthyology*, 10, 225–231.? https://doi.org/10.1590/S1679-62252012000200001

Edgar., R.C. (2004) MUSCLE: multiple sequence aligment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797. https://doi.org/10.1093/nar/gkh340

Eschmeyer, W.N., Fricke, R. & van der Laan, R. (2017) Catalog of fishes: genera, species, references. Electronic Version. Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed 24 January 2017)

Eigenmann, C.H. (1918) The Pygidiidae, a family of South American catfishes. *Memoirs of the Carnegie Museum*, 7, 259–398.

Fernández, L. & K. Osinaga. (2006) A new *Trichomycterus* (Siluriformes: Trichomycteridae) from Aguarague National Park of the Bolivian preandean region, with comments on relationships within of the genus. *Environmental Biology of Fishes*, 75, 385–393. https://doi.org/10.1007/s10641-005-5065-4

Fernández, L. & Vari, R.P. (2000) New species of *Trichomycterus* (Teleostei: Siluriformes: Trichomycteridae) lacking a pelvic fin and girdle from the Andes of Argentina, *Copeia*, 4, 990–996.

Ferrer, J. & Malabarba, L.R. (2011) A new *Trichomycterus* lacking pelvic fins and pelvic girdle with a very restricted range in Southern Brazil (Siluriformes: Trichomycteridae). *Zootaxa*, 2912, 59–67.

Garcia-Melo, L.J., Villa-Navarro, F.A. & DoNascimiento, C. (2016) A new species of *Trichomycterus* (Siluriformes: Trichomycteridae) from the upper río Magdalena basin, Colombia. *Zootaxa*, 4117 (2), 226–40. https://doi.org/10.11646/zootaxa.4117.2.6

Hebert, P.D.N., Cywinska, A., Ball, S.L. & De Waard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Science*s, 270, 313–321.

IUCN (2001) *IUCN Red list categories and criteria: version 3.1*. IUCN Species Survival Commission. IUCN, Gland, 30 pp.

Lima, S.M.Q. & Costa, W.J.E.M. (2004) *Trichomycterus giganteus* (Siluriformes: Loricarioidea: Trichomycteridae): a new catfish from the Rio Guandu basin, southeastern Brazil. *Zootaxa*, 761, 1–6.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120. https://doi.org/10.1007/BF01731581

Malabarba, L.R., Fialho, C.B., Anza, J.A., Ferrer, J. & Mendes, G.N. (2009) Peixes. *In*: Boldrini, I.L. (Org.), *Biodiversidade dos Campos do Planalto das Araucárias*. Ministério do Meio Ambiente, Brasília, pp. 131–156.

Miller, M.A., Pfeiffer, W. & Schwarts, T. (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees". *Proceeding of the Gateway Computing Enviroments Workshop (GCE)*, 14 Nov. 2010, New Orleans, LA, pp 1–8.

Miranda-Ribeiro, P. (1949) Duas novas especies de peixes na coleção ictiológica do Museu nacional (Pisces, Callichthyidae et Pygidiidae). *Revista Brasileira de Biologia*, 9, 143–145

Miranda-Ribeiro, P. (1957) Notas para estudo dos Pygidiidae brasileiros (Pisces-Pygidiidae) VI. *Papeis Avulsos do Departamento de Zoologia da Secretaria de Agricultura de São Paulo*, 13, 71–73

Myers, G.S. (1944) Two extraordinary new blind nematognath fishes from the Rio Negro, representing a new subfamily of Pygidiidae, with a rearrangement of the genera of the family, and illustrations of some previously described genera and species from Venezuela and Brazil. *Proceedings of the California Academy of Sciences*, Series 4, 591–602.

Palumbi, S.R. (1996) Nucleic acids II: the polymerase chain reaction. *In*: Hillis, D.M., Moritz, C. & Mable, B.K. (Eds.), *Molecular Systematic*. Sinauer & Associates Inc., Sunderland, Massachusetts, pp. 205–247.

de Pinna, M.C.C. (1988) A new genus of trichomycterid catfish (Siluroidei, Glanapteryginae), with comments on its phylogenetic relationships. *Revue Suisse de Zoologie*, 95, 113–128. https://doi.org/10.5962/bhl.part.79642

de Pinna, M.C.C. (1989) A new sarcoglanidine catfish, phylogeny of its subfamily, and an appraisal of the phyletic status of the Trichomycterinae (Teleostei, Trichomycteridae). *America Museum Novitates*, 2950, 1–39

de Pinna, M.C.C. (1998) Phylogenetic relationships of Neotropical Siluriformes: historical overview and synthesis of hypotheses. *In*: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M.S. & Lucena, C.A.S. (Eds.), *Phylogeny and*

classification of Neotropical fishes. EDIPUCRS, Porto Alegre, pp. 279–330.
de Pinna, M.C.C. & Wosiacki, W.B. (2003) Family Trichomycteridae (Pencil or parasitic catfishes). *In*: Reis, R.E., Kullander, S.O. & Ferraris, C.J. (Eds.), *Check List of the Freshwater Fishes of South and Central America*. EDIPUCRS, Porto Alegre, pp. 270–290.
Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood- based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.
https://doi.org/10.1093/bioinformatics/btl446
Tamura, K., Stecher, G., Peterson, D., Filipski, A & Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.
https://doi.org/10.1093/molbev/mst197
Taylor, W.R. & Van Dyke, G.C. (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium*, 9, 107–119.
Tchernavin, V.V. (1944) A revision of some Trichomycterinae based on material preserved in the British Museum (Natural History). *Proceeding of the Zoological Society of London*, 114, 234–275.
https://doi.org/10.1111/j.1096-3642.1944.tb00219.x
Wosiacki, W.B. (2002) *Estudo das relações filogenéticas de Trichomycterinae (Teleostei, Siluriformes, Trichomycteridae) com uma proposta de classificação*. Unpublished Ph.D. dissertation, Universidade de São Paulo, São Paulo, 324 pp.
Wosiacki, W.B. & de Pinna, M.C.C. (2008) *Trichomycterus igobi*, a new catfish species from the rio Iguaçu drainage: the largest head in Trichomycteridae (Siluriformes: Trichomycteridae). *Neotropical Ichthyology*, 6, 17–23.
https://doi.org/10.1590/S1679-62252008000100003
Xia, X., Xie, Z., Salemi, M., Chen, L. & Wang, Y. (2003) An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution*, 26, 1–7.
https://doi.org/10.1016/S1055-7903(02)00326-3
Xia, X. & Lemey, P. (2009) Assessing substitution saturation with DAMBE. *In*: Lemey, P., Salemi, M. & Vandamme, A.M. (Eds.), *The Phylogenetic Handbook: a practical approach to DNA and protein phylogeny. 2nd Edition*. University Press, Cambridge, pp. 615–630.
https://doi.org/10.1017/CBO9780511819049.022
Xia, X. (2013) DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution*, 30, 1720–1728.
https://doi.org/10.1093/molbev/mst064

SUPPLEMENTARY 1. Voucher information and GenBank accession numbers for the samples analyzed in the present study

Genus	Species	Specimen Catalog #	GenBank	
			COI	16S
Copionodon	*Copionodon pecten*	LBP 17357	KY857929	KY807205
Bullockia	*Bullockia maldonadoi*	LBP 3112	KY857926	KY807202
Scleronema	*Scleronema minutum*	LBP 3310	KY857957	KY807234
Ituglanis	*Ituglanis parahybae*	LBP 10730	KY807219	
Eremophilus	*Eremophilus mutisii*	ANSP 11306	KY857931	KY807207
Trichomycterus	*Trichomycterus pascuali*	LBP 23323	MF034463	MF034462
	Trichomycterus areolatus	LBP 3118	KY857964	KY807241
	Trichomycterus brasiliensis	LBP 11778	KY857993	KY807271
	Trichomycterus candidus	LBP 11630	KY857965	KY807242
	Trichomycterus iheringi	LBP 4512	KY858008	KY807286
	Trichomycterus variegatus	LBP 10289	KY857991	KY807269