

Phylogenetic Position of *Trichomycterus payaya* and Examination of Osteological Characters Diagnosing the Neotropical Catfish Genus *Ituglanis* (Siluriformes: Trichomycteridae)

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The Trichomycterinae is among the most diverse catfish groups in the world, with interesting ecological specializations; however, the taxonomy of Trichomycterinae is still problematic, mainly due to unclear limits and diagnoses of the eight included genera. Herein we infer the phylogenetic position and generic placement of *Trichomycterus payaya*, a recently described species from north-eastern Brazil, with unique morphological features among congeners. A multigene analysis including fragments of two nuclear and two mitochondrial genes (total of 2974 bp) for 53 trichomycterine taxa and three outgroups clearly supports inclusion of *T. payaya* in *Ituglanis*, a trichomycterine genus containing 29 valid species, which is corroborated by osteological characters. An examination of osteological characters used to diagnose *Ituglanis* in its original description indicates that a rudimentary or absent posterior cranial fontanel is synapomorphic for *Ituglanis*, but this is reversed in *I. payaya*; an anteriorly directed antero-lateral extremity of the sphenotic-prootic-pterosphenoid complex and a narrow and long lateral process of the parurohyal are synapomorphic for *Ituglanis*, but homoplastically occurring in other trichomycterids; and the presence of a deep medial concavity on the autopalatine is ambiguous to diagnose *Ituglanis* by occurring in other closely related trichomycterine taxa. *Ituglanis* is also distinguishable from other trichomycterines by a unique shape of the metapterygoid and by a reduced number of ribs. The analysis supports a clade, comprising *I. payaya* and *I. paraguassuensis*, that is endemic to the Chapada Diamantina, in the semiarid Caatinga of north-eastern Brazil, reinforcing the importance of this region for trichomycterid diversification. By integrating molecular analysis and comparative morphology, the present study provides a more solid basis for delimiting *Ituglanis*, creating a better taxonomical resolution of the Trichomycterinae, although much more research is necessary to reach a consistent generic classification for the entire subfamily.

Key words: Biodiversity, Chapada Diamantina, Rio Itapicuru, Multigene phylogeny, Osteology.

BACKGROUND

The Trichomycterinae is a diversified Neotropical catfish subfamily with about 250 species in eight genera (*Cambeva* Katz, Barbosa, Mattos and Costa, 2018, *Eremophilus* Humboldt, 1805, *Hatcheria*

Eigenmann, 1909, *Ituglanis* Costa and Bockmann, 1993, *Rhizosomichthys* Miles, 1943, *Scleronema* Eigenmann, 1917 *Silvinichthys* Arratia, 1998, and *Trichomycterus* Valenciennes, 1832) (Katz et al. 2018; Fricke et al. 2021). Most species in Trichomycterinae have been placed in a single genus, *Trichomycterus*,

which was traditionally diagnosed by a combination of plesiomorphic character states, resulting in a broad paraphyletic assemblage and causing great taxonomical problems (e.g., de Pinna 1998). Recent advances in Trichomycterinae taxonomy have been made based on molecular studies that sampled representatives of diverse trichomycterine lineages (Ochoa et al. 2017; Katz et al. 2018), making it possible to establish *Trichomycterus* limits (Katz et al. 2018; Vilardo et al. 2020) supported by accurate historical research on the type species identity (Costa et al. 2020b). However, many persistent problems, including numerous species with uncertain taxonomical placement, impede the development of deeper studies on the diversity and evolution of trichomycterines.

The species herein analysed, *Trichomycterus payaya* Sarmiento-Soares, Zanata and Martins-Pinheiro, 2011, was described from the upper Rio Itapicuru basin, in an area of north-eastern Brazil without previous records on the occurrence of *Trichomycterus* (Sarmiento-Soares et al. 2011). This region is inserted in the north-eastern part of the Chapada Diamantina, an elevated plateau region inserted within the semiarid phytogeographical province of the Caatinga, known for the high level of species endemism (Juncá et al. 2005). The only other species of *Trichomycterus* known to occur in the Chapada Diamantina is *Trichomycterus tete* Barbosa and Costa, 2011, from the Rio de Contas basin, south-western Chapada Diamantina, about 280 km south-west of the localities where *T. payaya* was found. *Trichomycterus payaya* was described as belonging to *Trichomycterus* despite presenting some morphological character states that are typical for *Ituglanis*. Its diagnosis, limited to comparisons with eastern South American species of *Trichomycterus*, included the presence of a narrow urohyal (= parurohyal) with distally sharp lateral process and a reduced number of pleural ribs (five or six). These two conditions are diagnostic for *Ituglanis* according to Costa and Bockmann (1993) and do not occur in any species of *Trichomycterus* as delimited by Katz et al. (2018). Checking osteological illustrations included in this paper, it was possible to observe the autopalatine (Sarmiento-Soares et al. 2011: figs. 2–3) and metapterygoid (Sarmiento-Soares et al. 2011: fig. 4) morphology that are also typical for *Ituglanis*, as herein discussed, but not occurring in any species of *Trichomycterus*. However, unlike species of *Ituglanis*, *T. payaya* has well-developed cranial fontanels (Sarmiento-Soares et al. 2011: fig. 2), a primitive condition highly widespread among trichomycterines, but never recorded for *Ituglanis*. Finally, the habitat where this species was found, a stream running in a plain area, with sandy bottom and dense amphibious vegetation (Sarmiento-

Soares et al. 2011: fig. 9), is also typical for *Ituglanis*, but not for *Trichomycterus*.

Ituglanis was described to include a group of species previously placed in *Trichomycterus*, considered by Costa and Bockmann (1993) to be more closely related to the subfamilies Glanapteryginae, Sarcoglanidinae, Stegophilinae, Tridentinae, Vandelliinae (i.e., TSVSG-clade, hereafter TSVSGM-clade, following Costa et al. 2020a), than to other members of the Trichomycterinae. Costa and Bockmann (1993) described three apomorphic features as diagnostic for *Ituglanis*: supraoccipital fontanel represented by a small orifice posteriorly placed; anterior extremity of the sphenotic anteriorly directed; and autopalatine with a deep concavity on its medial margin. Costa and Bockmann (1993) also described two apomorphic features for *Ituglanis* that are shared with *Scleronema* and the TSVSGM-clade: a short interopercular patch of odontodes, its length about 35–45% of the distance between the anterior extremity of the quadrate and the posterior extremity of the hyomandibula; and a pointed and elongated lateral process of the parurohyal; as well as one apomorphic condition shared only by *Ituglanis* and the TSVSGM-clade: presence of two to six ribs. In addition, Costa and Bockmann (1993) recorded a slightly reduced metapterygoid in *Ituglanis*, contrasting with the minute metapterygoid in the TSVSGM-clade and the well-developed metapterygoid of other trichomycterids. Apomorphic morphological character states used by Costa and Bockmann (1993) to diagnose a clade including *Ituglanis* and the TSVSGM-clade have been refuted due to some variability that was found (Datovo and Bockmann 2010), but their evolutionary significance was not approached.

Molecular analyses have consistently shown that *Ituglanis* and *Scleronema* are not closely related to the TSVSGM-clade, but in fact are members of the Trichomycterinae, and *Ituglanis* and *Scleronema* are monophyletic genera, but each one is related to different trichomycterine lineages (Ochoa et al. 2017 2020; Katz et al. 2018; Costa et al. 2020c; the present paper). In light of these new findings, those apomorphic character states shared by *Ituglanis* and some not closely related trichomycterids should be interpreted as potential synapomorphies for *Ituglanis* with possible homoplastic occurrence in *Scleronema* and members of the TSVSGM-clade. The objectives of this study were to infer the phylogenetic position and, consequently, the generic placement of *T. payaya* through a multigene analysis including fragments of two nuclear and two mitochondrial genes (total of 2974 bp) for 53 trichomycterine taxa and three outgroups, and re-examine diagnostic osteological character states used by Costa and Bockmann (1993).

MATERIALS AND METHODS

Taxon sampling

The phylogenetic analysis was mainly based on sequences taken from specimens captured with small dip nets (40 × 30 cm), with permits provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade; permit numbers: 38553–7). Field methods, including euthanasia using a buffered solution of tricaine methane sulphonate (MS-222) at a concentration of 250 mg/L (e.g., Leary et al. 2013), were approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit number: 065/18). Molecular data were obtained from specimens fixed and preserved in absolute ethanol. Specimens used for morphological comparisons were fixed in formalin for a period of 14 days and then transferred to 70% ethanol. All the collected material was deposited in the fish collection of the Institute of Biology, Federal University of Rio de Janeiro (UFRJ).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscle tissues of the caudal peduncle and dorsum, using the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocol. The analyses included a set of partial sequences of two nuclear genes: recombination activating 2 (RAG2) and myosin heavy chain 6 (MYH6); and partial sequences of two mitochondrial genes: cytochrome *c* oxidase subunit I (*COI*) and cytochrome *b* (CYTB). Amplification of the target DNA fragments was made through the polymerase chain reaction (PCR) method, using the primers MHRAG2-F1 (5'-TGY TAT CTC CCA CCT CTG CGY TAC C-3') and MHRAG2-R1 (5'-TCA TCC TCC TCA TCK TCC TCW TTG TA-3') (Hardman and Page 2003); RAG2 TRICHO F (5'-GAC AGY CGA GGA TGC AAT CGG AA-3') and RAG2 TRICHO R (5'-CTG TCC TSC ATY TCA TGG GGT TCA CG-3') (Costa et al. 2020a); RAG2 MCF (5'-CCG TAC ACC CAA TGA-3') and RAG2 MCR (5'-AAA TTC AGT AGA TTC TTG ACT GC-3') (Cramer et al. 2011) for recombination activating 2 (RAG2); myh6_F459 (5'-CAT MTT YTC CAT CTC AGA TAA TGC-3') and myh6_R1322 (5'-CTC ACC ACC ATC CAG TTG AAC AT-3') (Li et al. 2007); MYH6 TRICHO F (5'-ACK GAC AGA GAG AAC CAG TC-3') and MYH6 TRICHO R (5'-ACC TGA TCC ACA CCT TGG CCT TT-3') (Costa et al. 2020a) for myosin heavy chain 6 (MYH6); Cytb Siluri F (5'-CCA CCG TTG TAA TTC AAC TA-3') and Cytb Siluri R (5'-GAT TAC AAG ACC GGC GCT TT-3') (Villa-Verde et al. 2012) for cytochrome *b* (CYTB); L5698-ASN (5'-AGG

CCT CGA TCC TAC AAA GKT TTA GTT AAC-3') and H7271-*COI* (5'-GTG GTG GGC TCA TAC AAT AAA-3') (Villa-Verde et al. 2012); and FISHF1 and FISHR1 (Ward et al. 2005) for cytochrome *c* oxidase subunit I (*COI*). Double-stranded PCR amplifications were performed in 60 µL reactions with reagents at the following concentrations: 5× GreenGoTaq Reaction Buffer (Promega), 1 mm MgCl₂, 1 µm of each primer, 75 ng of total genomic DNA, 0.2 mm each of dNTP and 1 U of standard Taq polymerase or Promega GoTaq Hot Start polymerase. The thermocycling profile was as follows: initial denaturation for 2 min at 95°C; 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 48–60°C and extension for 1.2–2 min at 72°C. Negative controls without DNA were used to check for contaminations. The PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sanger Sequencing reactions were made by Macrogen Inc. (South Korea). Sequencing chromatograms and sequences were assessed using MEGA 7 (Kumar et al. 2018). The generated DNA sequences were translated into amino acids residues to verify the absence of premature stop codons or indels using the program MEGA. New sequences of *Ituglanis* species were obtained from individuals collected in their type locality areas: *I. amphipotamus*: 24°11'36"S, 47°53'26"W; *I. paraguassuensis* 12°06'44"S, 41°07'14"W; *I. payaya*: 11°19'43"S, 40°28'13"W.

Phylogenetic analyses

Terminal taxa for the molecular phylogenetic analyses were species with available material for osteological preparations (see below) or, in a few cases, when information on osteological structures here compared was available in the literature. The phylogenetic analyses included a total of 53 trichomycterine species. Outgroups were *Listrura tetraradiata* Landim and Costa, 2002 and *Microcambeva ribeirae* Costa, Lima and Bizerril, 2004, two basal representatives of the TSVSGM-clade, the sister group of the Trichomycterinae (Costa et al. 2020a); and *Trichogenes longipinnis* Britski and Ortega, 1983, a member of the clade comprising Copionodontinae and Trichogeninae, which is sister to a clade comprising all other trichomycterids (Ochoa et al. 2017). A list of species and GenBank accession numbers is provided in table S1. Alignment was conducted separately for each gene set using the Clustal W (Chenna et al. 2003) algorithm implemented in MEGA; gaps were not found in the analysed alignments. The best partition scheme and best-fit models of substitution for the data sets for phylogenetic analyses were identified using PartitionFinder 2.1.1 (Lanfear et al. 2016) according

to the Bayesian information criterion (BIC; Schwarz 1978); partitions included each codon position for each gene; the best-fit partitions and models are provided in Table 1. The concatenated matrix comprised 2974 pb (522 bp for *COI*, 1088 for *CYTB*, 543 for *MYH6*, 821 for *RAG2*). Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were conducted using MrBayes 3.2.5 (Ronquist et al. 2012) and IQTREE 2.0 (Nguyen et al. 2015), respectively. The BI analysis was conducted with the following parameters: two independent Markov chain Monte Carlo (MCMC) runs of two chains each for 10 million generations, with a tree sampling frequency of every 1000 generations. The convergence of the MCMC chains and the proper burn-in value were assessed by evaluating the stationary phase of the chains using Tracer 1.7.1 (Rambaut et al. 2018). The BI final consensus tree and its Bayesian posterior probabilities were generated with the remaining tree samples after removing the first 25% of samples as burn-in. To test the support of the nodes in the ML analysis, 1000 ultrafast bootstrap (Minh et al. 2013), and 1000 bootstrap (Felsenstein 1985) replicates were made.

Osteology

Osteological preparations followed Taylor and Van Dyke's (1985) protocol; osteological illustrations were made using a stereomicroscope Zeiss Stemi SV 6 with camera lucida. Terminology for osteological structures followed Bockmann et al. (2004), with modifications proposed by Datovo and Bockmann (2010) and Costa et al. (2020c). A total of 95 trichomycterine taxa were examined, including 10 species of *Ituglanis*; a list of examined specimens appears in appendix 1. Osteological comparisons within *Ituglanis* were complemented by osteological characters described and illustrated for species with no material available for the present study, including Fernández and Bichuette

(2002), Bichuette and Trajano (2004), Sarmiento-Soares et al. (2006), Wosiacki et al. (2012), Lima et al. (2013), Datovo (2014), Rizzato and Bichuette (2014), Castro and Wosiacki (2017) and Ferrer and Donin (2017). Osteological data for the genera *Rhizosomichthys* and *Silvinichthys*, not available either, were taken from Arratia (1998) and Schaefer and Fernández (2009).

RESULTS

The phylogenetic analyses generated identical trees (Fig. 1), supporting the inclusion of *T. payaya* into a clade including all nominal species of *Ituglanis* here analysed, with maximum support values. In this tree topology, *T. payaya* is supported, with high values, as sister to *I. paraguassuensis* Campos-Paiva and Costa, 2007 (Fig. 1), a species endemic to the same region as *T. payaya* (Chapada Diamantina, north-eastern Brazil). The clade comprising *T. payaya* and *I. paraguassuensis* appears as sister to a weakly supported clade comprising the other analysed species of *Ituglanis*.

The comparative osteological analysis indicated that *T. payaya* shares at least five derived morphological character states with species of *Ituglanis*: an anteriorly directed antero-lateral extremity of the sphenotic-prootic-pterosphenoid complex (Fig. 2J); an approximately semi-circular metapterygoid, with an antero-dorsal convex margin (Fig. 2B); an elongated parurohyal lateral process (Fig. 2E); a hypertrophied opercle (Fig. 2B); and the presence of few ribs (five or six in *T. payaya*, two to six, rarely seven or eight in *Ituglanis*). Due to its phylogenetic position among species of *Ituglanis* and by sharing apomorphic osteological character states with other species of *Ituglanis*, *T. payaya* is here formally transferred for *Ituglanis*: *Ituglanis payaya* (Sarmiento-Soares, Zanata and Martins-Pinheiro, 2011), new combination.

Table 1. Best-fitting models of molecular evolution

Partition	Base pairs	Evolutionary model
<i>COI</i> 1 st	174	TRNEF+H+G
<i>CYTB</i> 2 nd <i>COI</i> 2 nd	537	TRN+H+G
<i>COI</i> 3 rd	174	TIM+G
<i>CYTB</i> 1 st	363	K80+H+G
<i>CYTB</i> 3 rd	362	GTR+G
<i>MYH6</i> 1 st , <i>MYH6</i> 2 nd	362	JC+I+G
<i>RAG2</i> 2 nd , <i>MYH6</i> 3 rd	455	TVMEF+G
<i>RAG2</i> 1 st , <i>RAG2</i> 3 rd	547	K80+G

DISCUSSION

Original diagnostic features of *Ituglanis*

Cranial fontanels: According to Costa and Bockmann (1993: fig. 3A), a synapomorphic condition supporting *Ituglanis* was the reduced cranial fontanels, with the anterior fontanel being absent and the posterior one restricted to a small posterior orifice on the posterior portion of the parieto-supraoccipital, thus highly contrasting with the plesiomorphic

condition for trichomycterines, in which there is a short anterior fontanel between frontals and a longer posterior fontanel extending from the posterior part of frontal to the middle-posterior portion of the parieto-supraoccipital (e.g., Bockmann et al. 2004: fig. 4). However, subsequent studies have shown that in some species of *Ituglanis*, the anterior fontanel was present, whereas the posterior fontanel was minute or absent in some congeners (Table 2). Consequently, the absence or presence of an anterior cranial fontanel was not considered as diagnostic for *Ituglanis*, but a possible

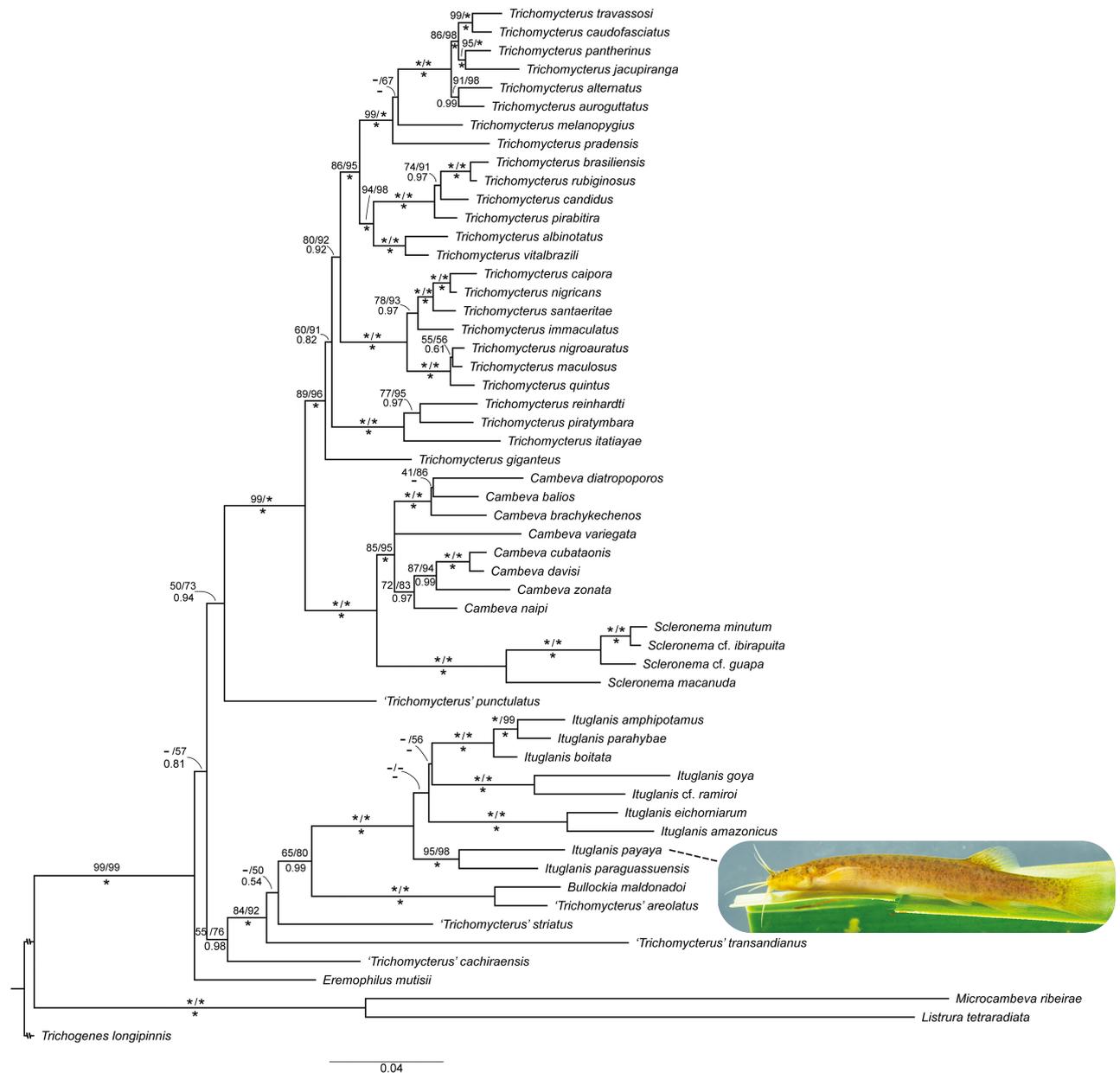


Fig. 1. Phylogenetic tree generated by Maximum Likelihood analysis for 53 Trichomycterinae and three outgroups (*COI*, *CYTb*, *MYH6* and *RAG2*, total of 2974 bp); numbers above the branches are bootstrap values and fast bootstrap values, respectively, separated by a bar; percentages under the branches are posterior probabilities from the Bayesian Inference; asterisks represent maximum values.

retention of a plesiomorphic trait (e.g., Campos-Paiva and Costa 2007). On the other hand, among the 24 species of *Ituglanis* with available information about neurocranium fontanels (Table 2), a rudimentary or absent posterior fontanel (Fig. 2I) occur in all species of *Ituglanis* except *I. payaya* (Sarmiento-Soares et al. 2011: fig. 2; Fig. 2J). No other trichomycterine has rudimentary or absent posterior fontanel, except ‘*Trichomycterus*’ *cachiraensis* Ardila Rodríguez, 2008 (Ardila Rodríguez 2008: fig. 4) and at least *Cambeva brachykechenos* (Ferrer and Malabarba, 2013) has a short posterior fontanel (Ferrer and Malabarba 2013:

fig. 2d). However, these two species are distantly related to *Ituglanis* (Fig. 1) and consequently cranial fontanel reduction in these taxa are considered as independent acquisitions. The highly supported sister group relationships between *I. payaya*, with broad posterior fontanel and *I. paraguassuensis* that has a minute posterior fontanel, and their inclusion in a clade comprising species of *Ituglanis* analysed with posterior fontanel rudimentary or absent, indicate that this condition is synapomorphic for *Ituglanis*, with a reversal in *I. payaya*.

Sphenotic-prootic-pterosphenoid complex:

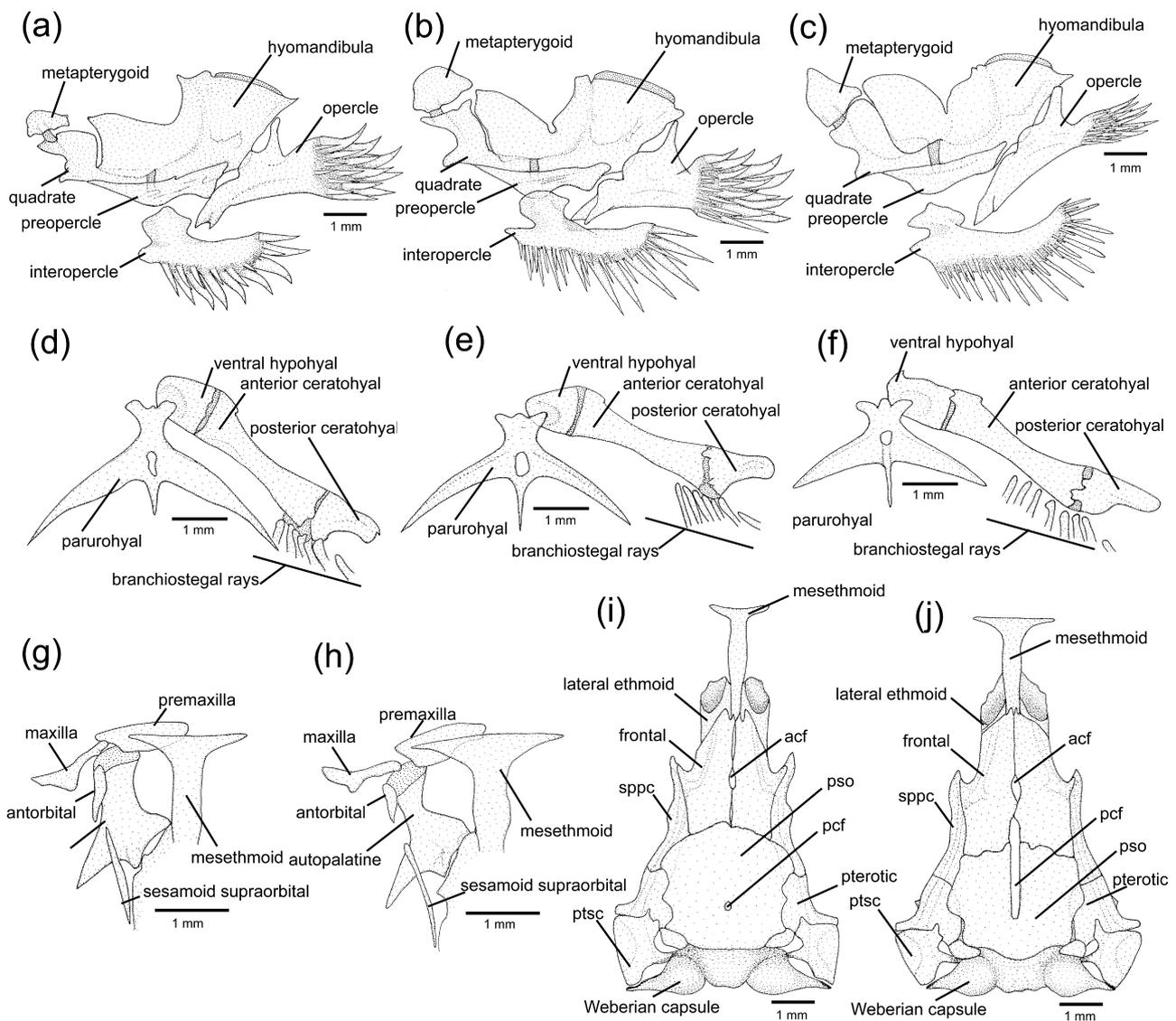


Fig. 2. Osteological features in some trichomycterine species. (a–c), left suspensorium and opercular apparatus, lateral view, of (a), *Ituglanis boitata*; (b), *Ituglanis payaya*; (c), *Trichomycterus albinotatus*. (d–f), hyoid arch, middle and left portions, ventral view, of (d), *I. boitata*; (e), *I. payaya*; (f), *Trichomycterus itatiayae*. (g–h), mesethmoidal region, middle and left portions, dorsal view, of (g), *I. payaya*; (h), *I. boitata*. (i–j), neurocranium, dorsal view, of (i), *I. boitata*; (j), *I. payaya*. Abbreviations: acf, anterior cranial fontanel; pcf, posterior cranial fontanel; ptsc, posttemporosupracheithrum; sppc, sphenotic-prootic-pterosphenoid complex. Larger stippling represents cartilages.

In trichomycterines, the sphenotic, prootic and pterosphenoid are fused to form a single bone complex (Baskin 1973). According to Costa and Bockmann (1993), the antero-lateral extremity of this bone complex, corresponding to the sphenotic in position, is anteriorly directed in *Ituglanis* (Fig. 2I), instead of laterally directed as in other trichomycterines. This apomorphic character state has been confirmed to occur in all species of *Ituglanis* (Table 2). According to Sarmento-Soares et al. (2006), this apomorphic condition does not occur in *I. payaya*, but the present examination of six cleared and stained specimens from the same locality have shown that the antero-lateral extremity of the sphenotic is in fact anteriorly

directed (Fig. 2J), not differing from other congeners (Fig. 2I). It is important to note that at least one species, '*Trichomycterus*' *cachiraensis* Ardilla Rodríguez, 2008 from the Colombian Andes, has an anteriorly directed sphenotic as illustrated in its original description (Ardilla Rodríguez 2008: fig. 4). However, as above discussed, this species is distantly related to *Ituglanis* and the condition is considered homoplastic.

Autopalatine: Costa and Bockmann (1993) described a deep concavity on the medial margin of the autopalatine as diagnostic for *Ituglanis*. In all species of *Ituglanis*, including *I. payaya* (Fig. 2G–H), the medial concavity makes the autopalatine very narrow in its middle region. Subsequent osteological reports

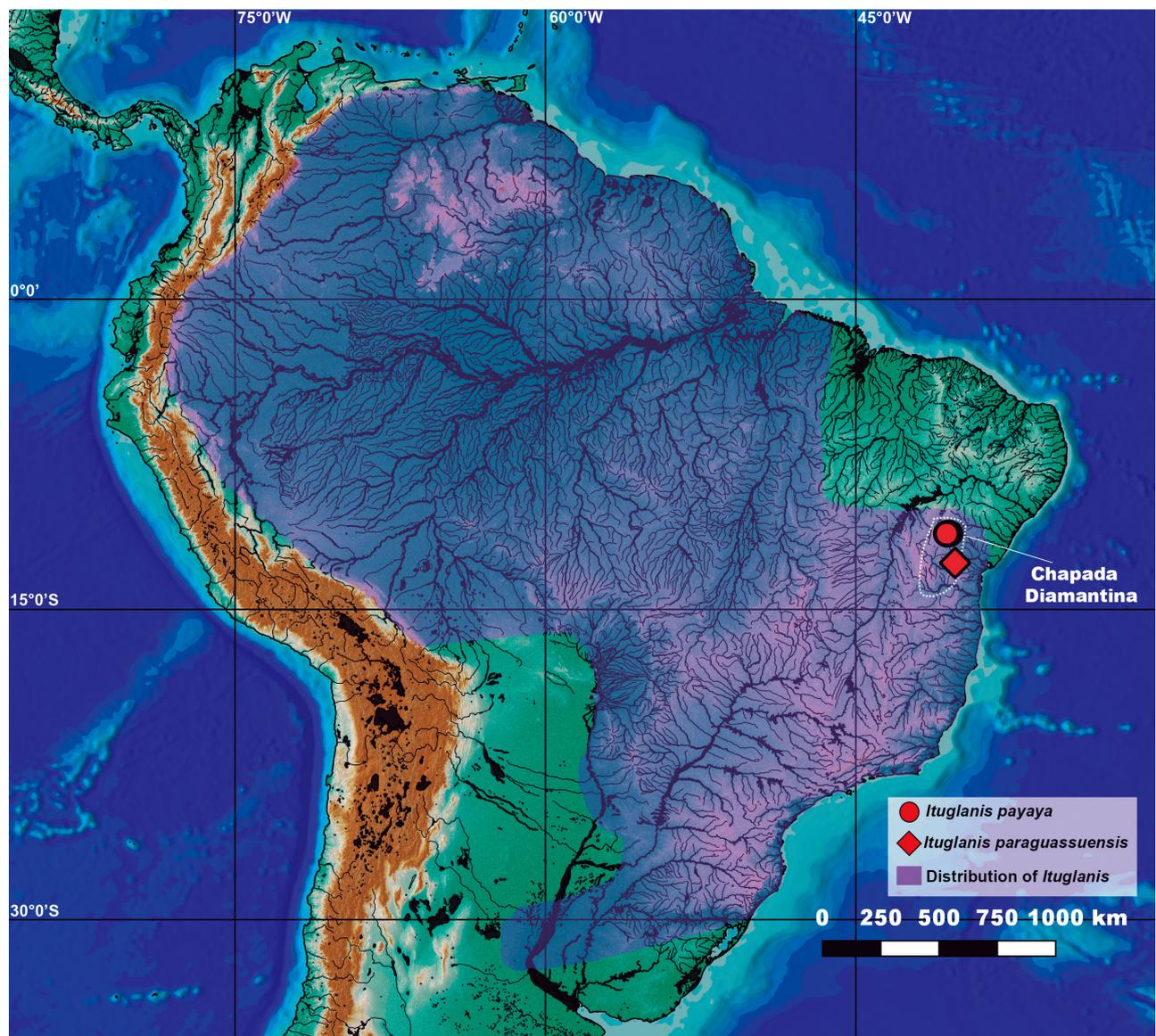


Fig. 3. Geographical distribution of *Ituglanis*, indicating type localities of *Ituglanis payaya* and *I. paraguassuensis*.

and illustrations have corroborated the presence of this autopalatine morphology in all other species placed in *Ituglanis*, described after the original description of the genus (Datovo and Landim 2005: fig. 2; Sarmiento-Soares et al. 2006: fig. 2; Campos-Paiva and Costa 2007: fig. 2; Wosiacki et al. 2012; Lima et al. 2013: fig. 2; Datovo 2014; Datovo and de Pinna 2014; Rizzato and Bichuette 2014; Ferrer et al. 2015: fig. 2; Datovo et al. 2016; Castro and Wosiacki 2017: fig. 3; Ferrer and Donin 2017: fig. 3; Mendonça et al. 2018). However, a narrowed middle portion of the autopalatine is also present in other trichomycterines, making this supposedly apomorphic condition ambiguous to diagnose *Ituglanis*.

Metapterygoid: Costa and Bockmann (1993: fig. 4) described a slightly reduced metapterygoid for *Ituglanis* (see introduction above). In fact, the metapterygoid morphology occurring in *Ituglanis* strongly differs

from other trichomycterines, not only by the size that may be variable among species, but mainly by its uncommon shape, approximately semi-circular with an antero-dorsal convex margin (Fig. 2A). Among the 18 species of *Ituglanis* with available information on the metapterygoid morphology (Table 2), all have this apomorphic condition, including *I. payaya* (Fig. 2B), indicating that it is synapomorphic for *Ituglanis*. In other trichomycterines, there is never a similar metapterygoid morphology (Fig. 2C). Therefore, this apomorphic metapterygoid morphology may be used as diagnostic for *Ituglanis*.

Interopercular and opercular patch of odontodes: Costa and Bockmann (1993) considered a short interopercular patch of odontodes as evidence of *Ituglanis* and *Scleronema* being more closely related to the TSVSGM-clade than to other members of the Trichomycterinae. Datovo and Bockmann (2010) noted

Table 2. Distribution of diagnostic osteological character states among species of *Ituglanis* with available osteological information. A: Autopalatine, medial margin, deep concavity: (0) absent; (1) present. B: Metapterygoid, shape: (0) subtriangular to subtrapezoidal; (1) semi-circular. C: Neurocranium, posterior fontanel, development: (0) well-developed, anteriorly extending to posterior part of frontal; (1) rudimentary, restricted to small posterior orifice on posterior portion of parieto-supraoccipital. D: Neurocranium, sphenotic-prootic-pterosphenoid complex, antero-lateral extremity, direction: (0) lateral; (1) anterior. E, Parurohyal, lateral process, shape: (0) compact; (1) long. F, ribs, number: (0) 10–18; (1) 2–8. G, Opercle, odontode patch, depth relative to length of dorsal hyomandibular articular facet: (0) smaller; (1) larger. (?) means structure not or unclearly recorded in the literature

Species	A	B	C	D	E	F	G	References
<i>Ituglanis agreste</i> Lima, Neves & Campos-Paiva, 2013	1	1	1	1	1	1	1	Lima et al. (2013)
<i>Ituglanis amazonicus</i> (Steindachner, 1882)	1	1	1	1	1	1	1	This paper
<i>Ituglanis amphipotamus</i> Mendonça, Oyakawa & Wosiacki, 2018	1	1	1	1	1	1	1	Mendonça et al. (2018); this paper
<i>Ituglanis apteryx</i> Datovo, 2014	1	1	1	1	?	?	?	Datovo (2014)
<i>Ituglanis australis</i> Datovo & de Pinna, 2014	1	1	1	1	1	1	?	Datovo and de Pinna (2014); this paper
<i>Ituglanis bambui</i> Bichuette & Trajano, 2004	1	?	1	1	1	1	?	Bichuette and Trajano (2004)
<i>Ituglanis boitata</i> Ferrer, Donin & Malabarba, 2015	1	1	1	1	1	1	1	Ferrer et al. (2015); this paper
<i>Ituglanis boticario</i> Rizzato & Bichuette, 2014	1	1	1	1	1	1	0	Rizzato and Bichuette (2014)
<i>Ituglanis cahyensis</i> Sarmiento-Soares, Martins-Pinheiro, Aranda & Chamon, 2006	1	1	1	1	1	1	1	Sarmiento-Soares et al. (2006)
<i>Ituglanis compactus</i> Castro & Wosiacki, 2017	1	1	1	1	1	1	1	Castro and Wosiacki (2017)
<i>Ituglanis eichorniarum</i> (Miranda Ribeiro, 1912)	1	1	1	1	1	1	1	This paper
<i>Ituglanis epikarsticus</i> Bichuette & Trajano, 2004	1	?	1	1	1	1	?	Bichuette and Trajano (2004)
<i>Ituglanis goya</i> Datovo, Aquino & Langeani, 2016	1	1	1	1	0	1	0	Datovo et al. (2016); this paper
<i>Ituglanis ina</i> Wosiacki, Dutra & Mendonça, 2012	1	?	1	1	?	1	?	Wosiacki et al. (2012)
<i>Ituglanis inusitatus</i> Ferrer & Donin, 2017	1	1	1	1	1	1	?	Ferrer and Donin (2017); this paper
<i>Ituglanis macunaima</i> Datovo & Landim, 2005	1	1	1	1	1	1	1	Datovo and Landim (2005); this paper
<i>Ituglanis mambai</i> Bichuette & Trajano, 2008	1	?	1	1	?	1	?	Bichuette and Trajano (2008)
<i>Ituglanis nebulosus</i> de Pinna & Keith, 2003	1	?	1	1	?	1	?	de Pinna and Keith (2003)
<i>Ituglanis paraguassuensis</i> Campos-Paiva & Costa, 2007	1	1	1	1	1	1	1	Campos-Paiva and Costa, 2007; this paper
<i>Ituglanis parahybae</i> (Eigenmann, 1918)	1	1	1	1	1	1	1	Costa and Bockmann (1993); this paper
<i>Ituglanis passensis</i> Fernández & Bichuette, 2002	1	1	1	1	1	1	1	Fernández and Bichuette (2002)
<i>Ituglanis payaya</i> (Sarmiento-Soares, Zanata & Martins-Pinheiro, 2011)	1	1	0	1	1	1	1	Sarmiento-Soares et al. (2011); this paper
<i>Ituglanis proops</i> (Miranda Ribeiro, 1908)	1	1	1	1	1	1	1	This paper
<i>Ituglanis ramiroi</i> Bichuette & Trajano, 2004	1	?	1	1	1	1	?	Bichuette and Trajano (2004)

that a short interopercular patch of odontodes was not present in all species of *Ituglanis*, as well as a short patch was found in a few other trichomycterine taxa. The present comparative analysis has shown that a relatively short interopercular patch like that occurring in most species of *Ituglanis* is also present in several trichomycterine taxa, making it not useful to diagnose trichomycterine genera. On the other hand, most species of *Ituglanis* have a hypertrophied opercular patch of odontodes (Fig. 2A–B). Of the 15 species of *Ituglanis* with available data for opercle morphology, 13 have a broad opercular patch of odontodes (Table 2). Although not occurring in all species of *Ituglanis*, besides eventually occurring in other trichomycterines, this is an apomorphic condition, possibly supporting an intrageneric clade, thus deserving some attention in future studies.

Parurohyal: Costa and Bockmann (1993) reported an elongated lateral process of the parurohyal in *Ituglanis* and *Scleronema* (Fig. 2D), which was then interpreted as evidence of close relationships of these genera with the TSVSGM-clade. Datovo and Bockmann (2010) and Datovo et al. (2016) recorded the presence of a less slender parurohyal in one species of *Ituglanis*, *I. goya*, not much differing from that morphology occurring in other trichomycterines, which was here confirmed. Datovo and Bockmann (2010) also reported the occurrence of a parurohyal similar to that described by Costa and Bockmann (1993) for *Ituglanis* in some species presently placed in *Cambeva* and *Trichomycterus*. In addition, they cited an even more elongated and thinner lateral process in *Trichomycterus* cf. *itatiayae*, thus rejecting the informative phylogenetic content of this character state, without evaluating its relevance for trichomycterine systematics. However, all other recent studies confirmed the presence of an elongated lateral process of the parurohyal in *Ituglanis*, that is present in 19 of the 20 species with available information about parurohyal morphology, thus not occurring only in *I. goya* (Table 2). On the other hand, this apomorphic condition was not found in other trichomycterines, including *T. itatiayae* topotypes (Fig. 2F), with the exception of *Scleronema*, as already described by Costa and Bockmann (1993). The more robust and compact urohyal present in *I. goya*, could be interpreted either a plesiomorphic condition, in case of this species being sister to all other congeners, or a reversal, in case of it being a member of a clade comprising other congeners. The latter hypothesis is likely to be occurred due to the phylogenetic position *I. goya* (Fig. 1). Therefore, the apomorphic elongated morphology should be considered as a synapomorphy for *Ituglanis*, with a reversal in *I. goya*, besides occurring homoplastically in the distantly related genus

Scleronema.

Ribs: Costa and Bockmann (1983) described the presence of six or fewer ribs in *Ituglanis* as a unique condition among trichomycterines, which usually have more than ten ribs. Later, a slightly higher number of ribs was recorded for some species of *Ituglanis*, including seven for *I. passensis* (Fernández and Bichuette, 2002), seven or eight for *I. boticario* (Rizzato and Bichuette, 2014), and four to seven for *Ituglanis goya* (Datovo et al., 2016). However, these values are still lower than counts found in other trichomycterines, therefore considered as an apomorphy supporting monophyly of *Ituglanis*. In *I. payaya*, there are five or six ribs, supporting its inclusion in *Ituglanis*.

The clade *Ituglanis payaya* plus *Ituglanis paraguassuensis*

Ituglanis is a geographically widespread genus, occurring in most cis-Andean lowland river basins of South America (Fig. 3). Although a phylogenetic analysis among species of *Ituglanis* was not within the scope of this paper, the present analysis including nine species representing different lineages of the genus highly supported *I. payaya* as sister to *I. paraguassuensis*. Both *I. payaya* and *I. paraguassuensis* are endemic to the same region: rivers draining into the Chapada Diamantina (Fig. 3), being the only species of the genus occurring in the Caatinga, a semi-arid region comprising the seasonally dry forests of north-eastern Brazil. The Chapada Diamantina has is considered to be an important biodiversity centre, with numerous endemic taxa (Juncá et al. 2005). Among trichomycterids, the whole Copionodontinae, comprising two genera and seven species, is endemic to the Chapada Diamantina (de Pinna 1992; Campanario and de Pinna 2000; Bichuette et al. 2008; de Pinna et al. 2018a b), whereas a recent study reported the occurrence of a new endemic species of the sarcoglanidine genus *Ammoglanis* Costa, 1994, previously known only from the Amazon (Costa et al. 2020d). Thus, in supporting a trichomycterine clade endemic to the Chapada Diamantina, this study corroborates that this area is an important centre for trichomycterid diversification.

CONCLUSIONS

Trichomycterinae is among the most diversified clades of catfishes in the world, exhibiting numerous specializations for different lifestyles still poorly documented. Major advances in evolutionary studies have been impeded by insufficient knowledge on phylogenetic relationships and species composition

of genera, with numerous nominal species without an accurate generic placement. This paper contributes to recent efforts to better understand the diversity and relationships of trichomycterines (Ochoa et al. 2017; Katz et al. 2018; Costa et al. 2020b). In integrating molecular analysis and comparative morphology, the present study provides an additional step for a better taxonomical resolution of this important catfish group, although much more research is necessary to reach a consistent generic classification for the whole Trichomycterinae.

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Consent for publication: Not applicable.

Ethics approval consent to participate: This study follows all legal requirements; collection permits were provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade; permit number: 38553-7); field methods were approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit number: 065/18).

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Supplementary materials

Table S1. Terminal taxa used in the phylogenetic analysis and their respective GenBank accession numbers. New sequences are in bold. (download)

Appendix 1. List of specimens belonging to the Trichomycterinae used in osteological comparisons (all specimens cleared and stained for osteological examination). MNRJ, Museu Nacional, Rio de Janeiro; MUSM, Museo Universidad San Marcos, Lima; MZUSP, Museu de Zoologia, Universidade de São Paulo; UFRJ, Institute of Biology, Federal University of Rio de Janeiro; USNM, National Museum of Natural History, Smithsonian Institution, Washington D.C. Species of *Trichomycterus* are below listed according to the main lineages designated in this study. (download)