

Article

Comparative Osteology, Phylogeny and Classification of the Eastern South American Catfish Genus *Trichomycterus* (Siluriformes: Trichomycteridae)

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Abstract: *Trichomycterus* has been considered for a long time to be the most problematic genus of the diverse neotropical subfamily Trichomycterinae. Recently, *Trichomycterus* was restricted to a clade from eastern South America supported by molecular data, but no unique morphological character state was found to distinguish it, making it difficult to allocate new species based on morphology alone. The objectives of this study were to conduct an osteological comparative analysis comprising a large sample of valid species of *Trichomycterus*, to conduct a total evidence phylogenetic analysis, combining osteological characters and a multigene database, and to propose an intrageneric classification based on the results of the phylogenetic analysis. Fifty-two osteological characters were combined with a multigene molecular data set of 2974 bp for 44 species of *Trichomycterus*, and 21 outgroups generated a well-supported phylogenetic tree, making it possible to delimit and diagnose intrageneric lineages, of which six subgenera are recognized. The high morphological diversity of osteological structures herein first reported for *Trichomycterus* from eastern South America is possibly related to some ecological specializations. This study shows that osteological characters combined with molecular data may be useful to consistently delimit and distinguish between trichomycterines, shedding light on the still persistent problems in trichomycterine systematics.

Keywords: bone morphology; loriciarioid catfishes; molecular phylogeny; mountain biodiversity; Neotropical region; new subgenera



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1. Introduction

The spectacular diversity of South American freshwater fishes has been repeatedly reported in the recent literature [1,2]. Among the most iconic fish groups in the region is the Trichomycteridae, a neotropical family of catfishes including the popularly known candiru [3] and many other taxa with uncommon morphological and ecological specializations [4–7]. The greatest species diversity among trichomycterids occurs in the Trichomycterinae, a subfamily containing about 250 valid species [8] distributed in all the main river basins of South America [9]. The Trichomycterinae, hereafter trichomycterines, are present in a large array of freshwater environments from southern Central America to southern South America, including Lake Titicaca in the central Andes, Patagonian rivers, and Amazonian lowland streams [10]. However, trichomycterine species diversity is particularly high in rivers draining the Andes in western South America and the mountain ranges of eastern South America [11]. Among the eight genera included in this subfamily, species of *Cambeva* Katz, Barbosa, Mattos and Costa 2018, *Eremophilus* Humboldt 1805, *Hatcheria* Eigenmann 1909, *Rhizosomichthys* Miles 1943, *Silvinichthys* Arratia 1998 and *Trichomycterus* Valenciennes 1832 typically inhabit aquatic mountain environments, whereas members of the genera *Bullockia* Arratia, Chang, Menu-Marque and Rojas 1978, *Ituglanis* Costa and Bockmann 1993 and *Scleronema* Eigenmann 1917 are found in low altitude streams [10,12–14]. At least one species inhabits a phreatic environment [15], whereas several others are specialized troglomorphic species [16,17].

The taxonomy of the trichomycterine genus *Trichomycterus* has been considered the most problematic among trichomycterids [18]. Its diagnosis was limited to a combination of features allowing to include all species lacking the derived character states diagnosing the other trichomycterine genera [13,14,18]. As a result, *Trichomycterus* comprised a broad artificial assemblage of trichomycterids, occupying the whole geographical range of the family. Even after some species of *Trichomycterus* being transferred to *Ituglanis* and *Silvinichthys* [14,19], *Trichomycterus* was still considered as a paraphyletic genus, with over 100 valid species, part of them endemic to the Andes and adjacent high elevation areas of northern South America, and others from eastern South America [20,21].

Concordantly to previous predictions, recent multigene analyses have strongly indicated *Trichomycterus* as paraphyletic [9,22]. In these analyses, trichomycterines were grouped in two major clades, a well-supported clade containing taxa endemic from eastern South America, including several species of *Trichomycterus* and *Scleronema*, and another clade, weakly supported containing the remaining trichomycterine genera and several other species of *Trichomycterus* from western and northern South America. Within the inclusive eastern South American clade (hereafter CST-clade), all analyses strongly supported three subclades: one comprising several species of *Trichomycterus* from eastern Brazil, including its type species *T. nigricans* Valenciennes, 1832; another one comprising species of *Scleronema*; and a third subclade, sister to *Scleronema* comprising nominal species placed in *Trichomycterus* [9,22]. The nomenclatural solution found by Katz et al. [9] was to restrict *Trichomycterus* to the molecularly well-supported subclade including *T. nigricans* and to describe a new genus *Cambeva* Katz, Barbosa, Mattos and Costa 2018 for the subclade sister to *Scleronema*. Presently, *Cambeva* comprises 29 species [23], *Scleronema*, eight species [24] and *Trichomycterus* sensu Katz et al. [9] (hereafter *Trichomycterus* s.s. [*sensu stricto*]), 60 species [25]. *Trichomycterus* s.s. occurs in a broad area between the Rio de Contas basin, north-eastern Brazil, at about 13°30' S [26] and the rivers draining the Baía de Paranaguá basin, in southern Brazil, at about 48°15' S [27].

The classification proposed by Katz et al. [9] solved an old taxonomical problem regarding monophyly and delimitation of *Trichomycterus*, but also imposed some practical difficulties for subsequent taxonomical studies since no unique apomorphic morphological character state was found by Katz et al. [9] for *Trichomycterus* s.s. The absence of a formal unambiguous morphological diagnosis for *Trichomycterus* and the absence of intrageneric comparative morphological studies involving a broad taxon sample makes it difficult to allocate new species on the basis of morphology alone. The objective of this study was to conduct an osteological comparative analysis comprising a large sample of valid species of *Trichomycterus* s.s., integrating with a multigene dataset in order to perform the most comprehensive phylogenetic study on this clade.

History of Trichomycterus Intrageneric Classification

Due to the large number of species and absence of detailed comparative studies in *Trichomycterus*, some taxonomical studies have tried to delimit informal intrageneric groups when diagnosing new species. Costa [13] tentatively delimited unnamed groups and subgroups of *Trichomycterus* s.s. from south-eastern and eastern Brazil based on some putative apomorphic character states of the external morphology, such as the relative position of supraorbital pores s6, the relative position between the urogenital orifice and the dorsal-fin base, relative position of pelvic-fin bases, barbels extent, caudal-fin shape, and color patterns. Species were then divided into two main groups, the first one including most species presently placed in *Trichomycterus* s.s. that were described before 1992, and a second one comprising *T. reinhardti* (Eigenmann 1917) and species presently placed in the *T. brasiliensis* group (see below) and in *Ituglanis*. Species presently placed in *Cambeva* were then considered distantly related from those two groups and placed in a separate third group.

Barbosa and Costa [28] named the *T. brasiliensis* species group based on derived conditions of osteological characters and color pattern. In spite of some diagnosis changes [29,30], this group has been corroborated in all molecular phylogenetic analyses [9,22,31–34], but different names have been used to this clade, including *T. brasiliensis* species-complex [29] and the brasiliensis clade [31]. Barbosa and Costa [35] named a group of *Trichomycterus* from south-eastern Brazil as the *T. travassosi* species complex based on coloration alone, but this group has not been corroborated by molecular data [9,32]. More recently, based on osteology and molecular evidence, Costa et al. [32,36] named the *T. nigroauratus* and *T. nigricans* groups, and Costa and Katz [25] named the *T. reinhardti* group, as well as Ochoa et al. [31], based on molecular evidence, named the itatiayae clade (including *T. itatiayae* and the *T. reinhardti* group in [25]) and the immaculatus clade. The use of different names for the same clades and the use of misidentified species to name clades have made the intrageneric informal classification of *Trichomycterus s.s.* confusing. The secondary objective of this paper was to provide an intrageneric classification well supported by both morphology and DNA evidence.

2. Materials and Methods

2.1. Comparative Osteology

This study is primarily based on the examination of specimens previously cleared and stained following Taylor and Van Dyke's protocol [37] and deposited in the ichthyological collection of the Institute of Biology, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil (see list of specimens in Appendix A). A total of 53 of the 60 valid species of *Trichomycterus s.s.* were examined, comprising samples of paratypes or topotypes. The analysis also included 33 species of non-*Trichomycterus* trichomycterines; two species, *Listrura tetraradiata* Landim and Costa 2002 and *Microcambeva ribeirae* Costa, Lima and Bizerril 2004, representing the Microcambevinae, the sister group of the remaining subfamilies comprising the TSVSGM-clade, which was supported as a sister to the Trichomycterinae [7]; two species, *Copionodon pecten* de Pinna 1992 and *Trichogenes longipinnis* Britski and Ortega 1983, representing the Copionodontinae and Trichogeninae, respectively, which together form the sister group to a clade comprising all other trichomycterids [22,31]; and the nematogeniid *Nematogenys inermes* (Guichenot 1848), a basal loricarioid catfish taxon.

The comparative analysis focused on bone structure variation, which has been used in the last almost 50 years to infer phylogenetic relationships of trichomycterids [38], as well as to diagnose trichomycterine genera and species (e.g., [12,19–21,39–42]). As a consequence, the considerable amount of available data in the literature on trichomycterine osteology allows more inclusive comparisons. The comparative analysis was directed to qualitative characters potentially diagnosing distinct intrageneric clades; quantitative characters and characters with high variability within different lineages or with a high level of subjectivity were not considered in this analysis. Illustrations were made using a Zeiss Stemi SV 6 stereomicroscope with camera lucida.

Terminology for bones followed Bockmann et al. [20], except for: 'epibranchial 5 cartilage', here using 'ceratobranchial 4 accessory element' according to Carvalho et al. [43]; 'urohyal', here replaced by 'parurohyal' following Arratia and Schultze [44]; 'pleural rib', here substituted by 'rib' following Britz and Bartsch [45] that have provided evidence that there is a single rib type in teleosts. The use of the names antorbital and sesamoid supraorbital here adopted, following Bockmann et al. [20] and Adriaens et al. [46], respectively, deserves some additional explanation due to some recent controversy involving homology and terminology, as below discussed.

The anterior-most bone has been identified as lacrimal [38], tendon-bone infraorbital [19], antorbital [20], and more recently lacrimal-antorbital [47]. In trichomycterines, it consists of a small flat ossification, drop-shaped in a dorsal view, associated with a segment of the infraorbital canal; it is placed close to the base of the nasal barbel externally and the antero-lateral part of the autopalatine internally. Since this bone is placed at the same position as the antorbital bone of basal siluriforms [48], it is here called antorbital following Bockmann et al. [20] and Adriaens et al. [46]. De Pinna et al. [47] found evidence that the antorbital is a compound structure derived from the fusion between the antorbital and the lacrimal. However, evidence was based only on the examination of a single cleared and stained juvenile specimen of *T. longipinnis* Britski and Ortega 1983, 20.3 mm of standard length, thus needing confirmation since robust evidence of a fusion between these elements is still unavailable [49].

The posterior osseous structure here identified as sesamoid-supraorbital, resulting from the ossification of a ligament between the antorbital and the neurocranium [46], was first equivocally identified as a nasal bone [10]. Since it is not associated with a sensory canal as a true nasal bone, Baskin [38] considered this structure as neomorphic and a synapomorphy of a trichomycterid group (i.e., Trichomycterinae plus Sarcoglanidinae and Glanapteryginae), naming it the fronto-lacrimal tendon bone. Different names for this bone have been used in the subsequent literature, including supraorbital [12], antorbital [50], and tendon-bone supraorbital [19]. More recently, this bone was identified as a sesamoid supraorbital bone [46] and subsequently called the sesamoid supraorbital by several authors [21,36,51]. This rod-shaped bone is present both in the Trichomycterinae and in basal members of the TSVSGM-clade [7,38], but no similar structure is present in basal trichomycterids (i.e., Copionodontinae and Trichogeninae). Two of the three species of Trichogeninae, *T. longipinnis* and *T. beagle* de Pinna, Reis and Britski 2020 have a scale-like ossification attached to the dorsal surface of the lateral process of the lateral ethmoid [47] that is not present in the other species of *Trichogenes* and in any species of the Copionodontinae, the sister group of the Trichogeninae [22,31]. In fact, due to its shape and position, the presence of this bone in those two trichogenine species seems to be a unique condition among trichomycterids and other loricarioids. This scale-like ossification occurring only in trichogenines was first tentatively identified as antorbital and homologous to the rod-shaped sesamoid supraorbital of trichomycterines [50], and recently named the barbular bone [47] and again considered as homologous to the fronto-lacrimal tendon bone sense by Baskin [38] (sesamoid supraorbital). This supposed homology was justified by both bones being positioned between the antorbital and the neurocranium margin and by both not being associated with any head sensory canal. However, only in *T. beagle* among species of Copionodontinae and Trichogeninae, there is a ligament uniting the antorbital and the barbular bone [47]. On the other hand, these structures have a different shape (scale-like in *Trichogenes* vs. rod-shaped), and more importantly, they have a different position (i.e., barbular bone associated with the lateral process of the lateral ethmoid in *Trichogenes*, vs. sesamoid supraorbital associated with the dorsal surface of the autopalatine), suggesting that they are not homologous. Therefore, it would be premature to interpret the barbular bone present only in two species of the clade encompassing copionodontines and trichogenines as homologous to the sesamoid supraorbital of the clade comprising trichomycterines and the TSVSGM-clade, without developmental studies supporting such proposal.

Terminology for minor osteological structures such as processes, bone outgrowths, and articulations, mostly followed Baskin [38], Arratia [41], and Adriaens et al. [46], as described in the text, and for cephalic latero-sensory system following Arratia and Huaquin [48].

2.2. DNA Sequences

The molecular dataset comprised partial sequences of two nuclear genes, myosin heavy chain 6 (MYH6, 543 bp) and recombination activating 2 (RAG2, 821 bp), and two mitochondrial genes, cytochrome c oxidase subunit I (COI, 522 bp) and cytochrome b (CYTB, 1088 bp). Sequences were first obtained in previous studies focusing on trichomycterine phylogeny [9,25,32–34], in which sequenced specimens were, whenever possible, collected in the species type locality. Amplification of the target DNA fragments was made through the polymerase chain reaction (PCR) method, using the primers MHRAG2-F1 and MHRAG2-R1 [52]; RAG2 TRICHO F and RAG2 TRICHO R [7]; RAG2 MCF and RAG2 MCR [53]; myh6_F459 and myh6_R1322 [54]; MYH6 TRICHO F and MYH6 TRICHO R [7]; Cytb Siluri F and Cytb Siluri R [55]; L5698-ASN and H7271-COI [55]; and FISHF1 and FISHR1 [56]. The analyses were complemented with sequences obtained from GenBank; a complete list of species and GenBank accession numbers appears in Appendix B. Alignment was conducted for each gene set using Clustal W algorithm [57] implemented in MEGA 7.0 [58]; no gap was found in the analyzed alignments.

2.3. Phylogenetic Analyses

The phylogenetic analyses comprised species that were examined in the osteological comparative analysis and had available DNA data, including 44 species of *Trichomycterus s.s.* and 21 outgroups (see Appendix B for a list of species used in the phylogenetic analyses). Fifty-two characters resulted from the osteological comparative analysis (see above); a list of characters, formulated according to Sereno [59], is provided in the Appendix C, and the distribution of character states among taxa appears in the data matrix of the Appendix D. Osteological characters were combined to a set of gene fragments more commonly used in phylogenetic studies on trichomycterines (see DNA sequences above), a total of 2974 bp. Molecular data were partitioned according to the codon position for each gene, as well as morphological data were placed in a separate partition. The best-fitting models of molecular evolution for each partition was found using the Bayesian information criterion (BIC) of ModelFinder [60], implemented in IQ-TREE 1.6.11 [61], with partition models described by Chernomor et al. [62]; the list of partitions and their respective models of nucleotide substitution are provided in the Appendix E. The concatenated dataset, a total of 3026 characters for 65 taxa, was first analyzed using IQ-TREE for Maximum Likelihood (ML), applying three methods for assessing the reliability of internal branches: the Shimodaira-Hasegawa-like procedure support (SH-aLRT) [63], the Bayesian-like transformation of SH-aLRT support (aBayes) [64] and the ultrafast bootstrap support (UFBoot) [65,66], using 1000 replicates and default parameters as implemented in IQ-TREE. The concatenated dataset was also analyzed using Maximum Parsimony (MP) methods performed with TNT 1.1 [67]. The phylogenetic analysis was rooted in the nematogenyid *N. inermis*. The search for most parsimonious trees was conducted using the ‘traditional’ search algorithm and setting random taxon-addition replicates to ten, tree bisection–reconnection branch swapping, multitrees in effect, collapsing branches of zero length, characters equally weighted, and a maximum of 1000 trees saved in each replicate. Character states were treated as unordered. Branch support was assessed by bootstrap analysis [68], using a heuristic search with 1000 replicates and the same settings used in the MP search. Unambiguous diagnostic character states for the main lineages of *Trichomycterus* were obtained by character state optimization of the combined analysis tree using TNT 1.1.

2.4. Taxonomic Accounts

Subgenera appeared following a phylogenetic order, according to Figure 1. Diagnoses comprised osteological apomorphic features supporting the taxon, followed by combinations of external morphological features additionally distinguishing the taxon from other taxa; the abbreviation ‘chast’ means character state, that is numbered according to Appendix C.

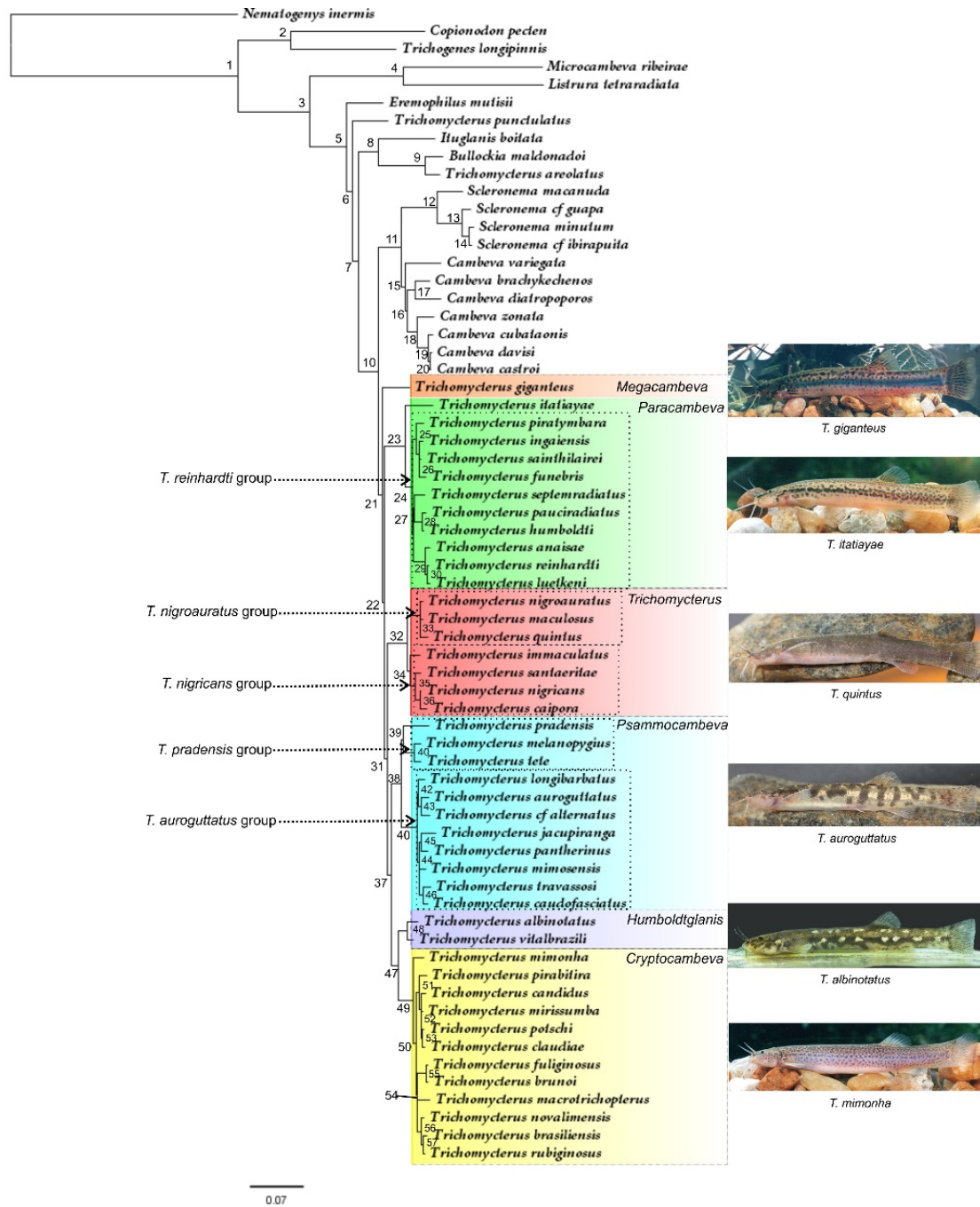


Figure 1. Phylogenetic tree generated by Maximum Likelihood analysis using IQ-tree for 44 species of *Trichomycterus* and 21 outgroups, combining four genes (COI, CYTB, MYH6, and RAG2, with a total of 2974 bp) and 52 osteological characters (the most external taxon *Nematogenys inermis* is not depicted). Asterisks indicate species endemic to the Rio Paraiba do Sul basin. For support values for each node, see Table 1.

3. Results

3.1. Comparative Osteology and Phylogeny

Fifty-two osteological characters with informative variability were obtained from the comparative analysis (see Appendix C for list of characters and Appendix D for distribution of character states among terminal taxa). The ML analysis generated a phylogenetic tree with most nodes reaching high support values (Figure 1). The single most parsimonious tree generated by the MP analysis was similar to the ML tree, but with some nodes being weakly supported (see list of support values for each node of Figure 1 in Table 1). In both analyses, the monophyly of *Trichomycterus s.s.* was corroborated (SH-aLRT support of 85.1%, aBayes support of 0.96, ultrafast bootstrap support of 92% for the ML analysis, and a bootstrap of 89% for the MP analysis).

Table 1. Node support in phylogenetic analyses (Figure 1), comprising Shimodaira-Hasegawa-like procedure support (SH-aLRT) with values above 70%, the Bayesian-like transformation of SH-aLRT support (aBayes) with values above 0.7, and the ultrafast bootstrap support (UFBoot) with values above 70%, for the Maximum Likelihood (ML) analysis, and bootstrap values above 50% for the Maximum Parsimony (MP) analysis.

Node	ML			MP
	SH-aLRT	aBayes	UFBoot	Bootstrap
1	-	-	-	100
2	97.4	1	100	97
3	100	1	100	99
4 (Microcambevinae)	100	1	100	99
5 (Trichomycterinae)	98.4	1	100	98
6	84.5	1	74	-
7	85.8	1	76	-
8	99.6	1	100	78
9	100	1	100	100
10 (CST-clade)	99.8	1	100	95
11 (<i>Cambeva</i> + <i>Scleronema</i>)	99.9	1	100	99
12 (<i>Scleronema</i>)	100	1	100	100
13	99.9	1	100	100
14	98.6	1	100	100
15 (<i>Cambeva</i>)	89.0	1	95	84
16	66.7	-	79	-
17	96.6	1	95	-
18	99.1	1	100	83
19	99.4	1	100	100
20	73.3	0.8	92	-
21 (<i>Trichomycterus</i>)	85.1	1	92	89
22	78.6	0.8	82	-
23 (<i>Paracambeva</i>)	100	1	100	100
24 (<i>T. reinhardti</i> group)	97.3	1	99	99

Table 1. Cont.

Node		ML		MP
25	98.8	1	100	97
26	96.9	1	97	92
27	-	1	-	-
28	100	1	100	100
29	100	1	100	99
30	83.7	0.9	97	99
31	83.1	1	91	-
32 (sg. <i>Trichomycterus</i>)	100	1	100	100
33 (<i>T. nigroauratus</i> group)	100	1	100	100
34 (<i>T. nigricans</i> group)	86.2	1	96	72
35	98.3	1	100	94
36	97.6	1	100	100
37	91	1	93	-
38 (<i>Psammocambeva</i>)	98.9	1	100	89
39 (<i>T. pradensis</i> group)	50.6	0.7	80	-
40	87.1	1	96	-
41 (<i>T. travassosi</i> group)	100	1	100	94
42	-	-	-	-
43	92.3	1	98	-
44	85.9	1	87	-
45	92.1	1	97	-
46	-	-	85	-
47	99.2	1	100	95
48 (<i>Humboldtglanis</i>)	99.4	1	100	100
49 (<i>Cryptocambeva</i>)	100	1	100	100
50	92.4	1	99	55
51	96.2	1	99	58
52	96	1	99	92
53	89.6	1	97	97
54	73	-	95	-
55	100	1	100	100
56	63.6	-	94	92
57	92.6	1	99	-

Like in previous studies [9,32], *T. giganteus* Lima and Costa 2004, here placed in a separate subgenus, appeared as sister to a clade containing all other congeners, besides exhibiting ten unique osteological apomorphic character states, first described here (see taxonomic accounts below). The clade comprising all species of *Trichomycterus* s.s., except *T. giganteus*, contained five main subclades supported by high values in all analyses (Figure 1, Table 1), which were below recognized as subgenera.

3.2. Taxonomic Accounts

3.2.1. *Megacambeva*, New Subgenus

LSID: urn:lsid:zoobank.org:act:DD5D2BBB-A0E2-4D6F-96F1-229A811ADCE7

Type species. *Trichomycterus giganteus* Lima and Costa 2004.

Diagnosis. *Megacambeva* differs from all other subgenera of *Trichomycterus* s.s. in possessing the following apomorphic conditions: presence of a short osseous ridge on the dorsal surface of the autopalatine, between the lateral margin and the middle portion of the bone (chast.2.1, Figure 2A; vs. absence of a similar ridge, Figure 2B–F); a posteriorly directed latero-posterior process of the autopalatine (chast.4.2, Figure 2A; vs. laterally or latero-posteriorly directed, Figure 2B–F); presence of a prominent ridge on the dorsal surface of the shell-like articular facet for the lateral ethmoid, terminating in a process latero-dorsally directed and connected by ligament to a robust sesamoid supraorbital (chast.8.1, Figure 2A; vs. never a similar structure, Figure 2B–F); presence of a deep concavity in the hyomandibular outgrowth reaching the ventral portion of the bone outgrowth (chast.13.3, Figure 3A; vs. absence, Figure 3B–F); strongly curved distal portion of largest internal-most opercular and interopercular odontodes, with their tips forming an angle of about 90° with its main axis (chast.15.1, Figure 3A; vs. nearly straight or only slightly curved, Figure 3B–F); a prominent trapezoidal process on the lateral margin of the lateral ethmoid, immediately posterior to the articular zone with the autopalatine (chast.30.1, Figure 2A; vs. process absent, Figure 2B–F); a slender Weberian capsule, subtly narrowing laterally to form a long lateral extremity (chast.44.1; Figure 4A; vs. broad, pear-like, abruptly narrowing laterally to form a short lateral extremity, Figure 4B–D); a hypertrophied tree-shaped supralateral process on the scapulo-coracoid (chast.45.1; Figure 5A; vs. needle shaped and small, Figure 5B); and three anterior processes in the pelvic bone (chast.51.1, Figure 6A; vs. two, Figure 6B–G). In addition, the only species included in *Megacambeva*, *T. giganteus* is unique among congeners by reaching a larger size, surpassing 200 mm SL (vs. usually reaching about 50–75 mm SL as maximum adult size, rarely reaching about 100–120 mm SL), and by having a unique dorsal fin morphology, that is deeper than long (vs. longer than deep, Figure 1). *Megacambeva* is also distinguished from all other subgenera by the following combination of character states: nine or ten pectoral-fin rays (vs. six to eight in *Cryptocambeva*, *Humboldtglanis*, and *Paracambeva*); anal-fin origin posterior to dorsal-fin base (vs. anal-fin origin in vertical through posterior half of dorsal-fin base in *Cryptocambeva*, *Humboldtglanis*, *Paracambeva*, and *Psammocambeva*); long maxillary barbel, its tip reaching the anterior third of the pectoral fin (vs. reaching anterior margin of pectoral-fin base or shorter in *Cryptocambeva*, *Humboldtglanis*, *Paracambeva*, and *Trichomycterus*); basal portion of maxillary barbel not distinctively widened (vs. widened in *Psammocambeva*); and jaw teeth always sharply pointed (vs. at least larger adults with incisiform teeth in *Trichomycterus*).

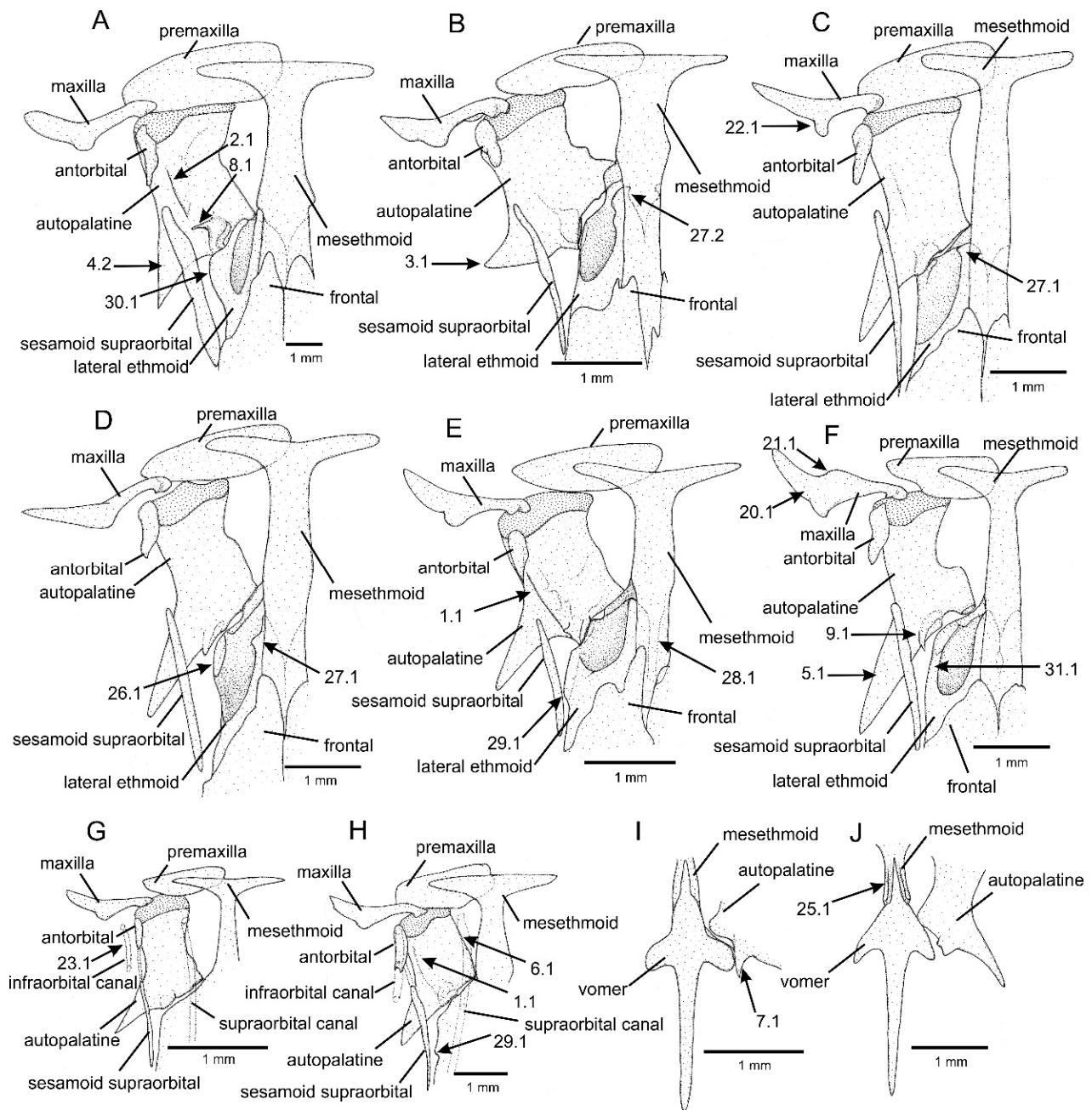


Figure 2. Mesethmoidal region and adjacent bones: (A–F) left and middle portion, detailed dorsal view: (A) *Trichomycterus (Megacambeva) giganteus*; (B) *Trichomycterus (Paracambeva) itatiayae*; (C) *Trichomycterus (Trichomycterus) immaculatus*; (D) *Trichomycterus (Trichomycterus) maculosus*; (E) *Trichomycterus (Cryptocambeva) mirissumba*; (F) *Trichomycterus (Psammocambeva) auroguttatus*. (G,H) left and middle portion, general dorsal view, including latero-sensory dermal canals: (G) *Trichomycterus (Paracambeva) septemradiatus*; (H) *Trichomycterus (Humboldtglanis) albinotatus*. (I,J) middle portion, general ventral view: (I) *Trichomycterus (Paracambeva) reinhardti*; (J) *Trichomycterus (Cryptocambeva) brasiliensis*. Larger stippling represents cartilages. The numbers are characters, followed by character states after dots, which are numbered according to Appendix C.

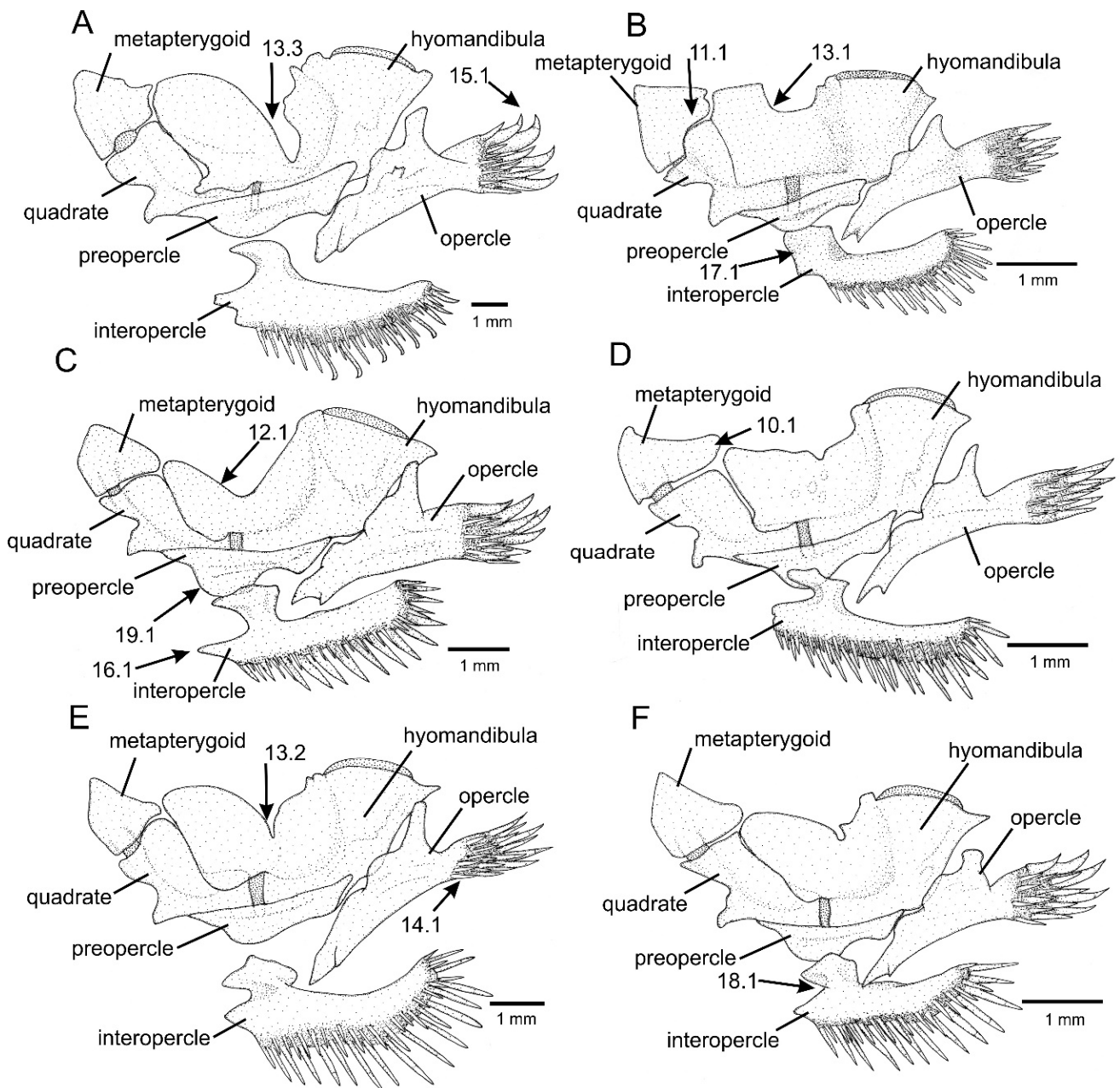


Figure 3. Left jaw suspensorium, excluding autopalatine, and opercular series, lateral view: (A) *Trichomycterus (Megacambeva) giganteus*; (B) *Trichomycterus (Paracambeva) itatiayae*; (C) *Trichomycterus (Psammocambeva) pradensis*; (D) *Trichomycterus (Trichomycterus) quintus*; (E) *Trichomycterus (Humboldtglanis) albinotatus*; (F) *Trichomycterus (Psammocambeva) auroguttatus*. Larger stippling represents cartilages. The numbers are characters, followed by character states after dots, which are numbered according to Appendix C.

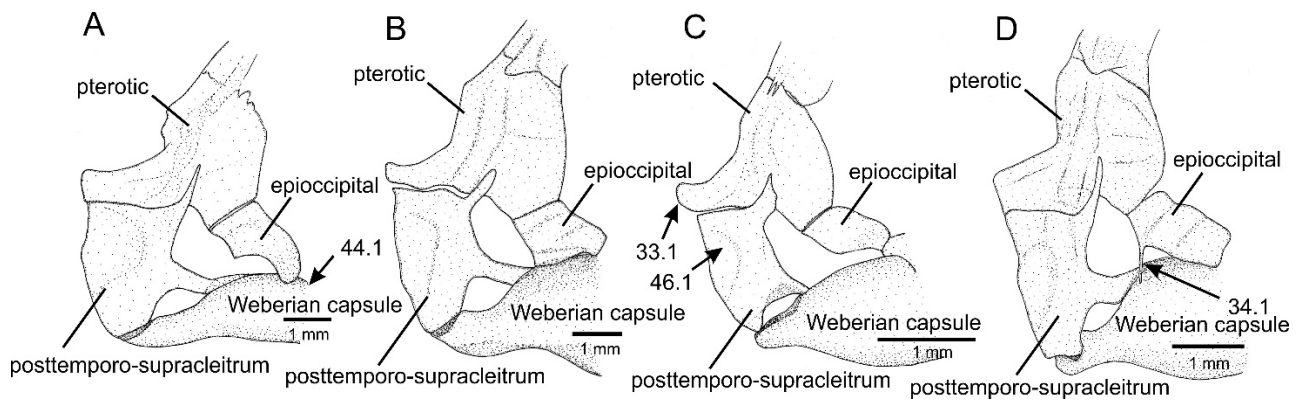


Figure 4. Postero-lateral region of neurocranium and adjacent bones, left portion, dorsal view: (A) *Trichomycterus (Megacambeva) giganteus*; (B) *Trichomycterus (Trichomycterus) maculosus*; (C) *Trichomycterus (Cryptocambeva) mirissumba*; (D) *Trichomycterus (Humboldtglanis) albinotatus*. The numbers are characters, followed by character states after dots, which are numbered according to Appendix C.

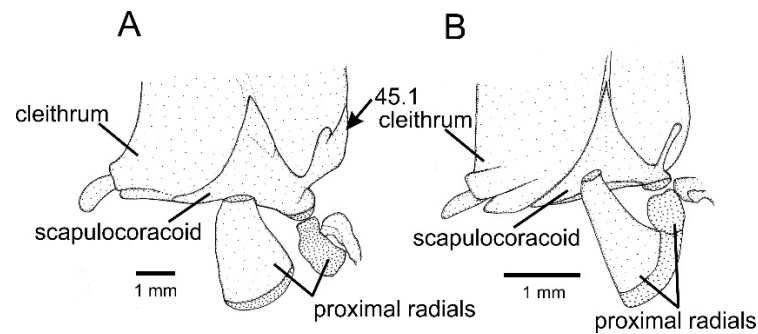


Figure 5. Left shoulder girdle, posterior portion, ventral view: (A) *Trichomycterus (Megacambeva) giganteus*; (B) *Trichomycterus (Trichomycterus) nigricans*. Larger stippling represents cartilages. The number is the character, followed by character states after the dot, which is numbered according to Appendix C.

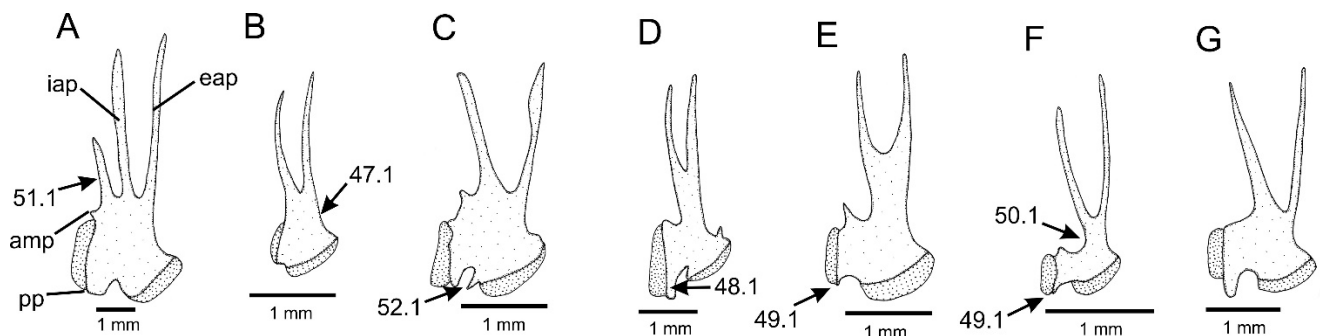


Figure 6. Left pelvic bone, ventral view: (A) *Trichomycterus (Megacambeva) giganteus*; (B) *Trichomycterus (Paracambeva) reinhardti*; (C) *Trichomycterus (Paracambeva) itatiayae*; (D) *Trichomycterus (Trichomycterus) nigrauratus*; (E) *Trichomycterus (Humboldtglanis) albinotatus*; (F) *Trichomycterus (Cryptocambeva) mirissumba*; (G) *Trichomycterus (Psammocambeva) pantherinus*. Larger stippling represents cartilages. Numbers are characters followed by character states after a dot, which are numbered according to Appendix C. Abbreviations: amp: antero-median process; eap: external anterior process; iap: internal anterior process; pp: posterior process.

Included taxa. A single species, *T. giganteus*.

Etymology. From the Greek *mega* (large), and *cambeva*, a popular name for trichomycterids in south-eastern Brazil, referring to the large size of the only included species. Gender feminine.

Distribution and ecological notes. The only included species, *T. giganteus*, is known from a single locality in the Rio Guandu-Mirim, Rio Guandu basin, an isolated small coastal basin in south-eastern Brazil. Adult specimens were found where the river forms small lakes below waterfalls, close to the river bottom, and below large rocks, about 200 cm deep or slightly more. Specimens were eventually seen swimming between bottom rocks during daylight, suggesting some diurnal activity.

3.2.2. *Paracambeva*, New Subgenus

LSID: urn:lsid:zoobank.org:act:F5037571-D95D-4091-880B-9E598AF9ED9F

Type species. *Trichomycterus itatiayae* Miranda Ribeiro 1906.

Diagnosis. *Paracambeva* is supported by two apomorphic conditions: the dorsal process of the interopercle being close to the anterior margin of the odontode patch (chast.17.1, Figure 3B; vs. dorsal process more posterior placed, Figure 3A,C–F; independently occurring in *Cambeva* and *Scleronema* according to the analysis) and a slender parapophysis of the second free vertebrae, since its proximal portion is to the distal portion (chast.38.1, Figure 7C; vs. robust, Figure 7D–F). Species of *Paracambeva* also differ from all other species of the *Trichomycterus* s.s. by having a unique color pattern consisting of a continuous black stripe between the snout and the caudal-fin base, becoming dispersed and spotted in larger specimens (Figure 1) [25] (Figures 1 and 2). *Paracambeva* is also distinguished from all other subgenera of *Trichomycterus* s.s. by the following combination of character states: six or seven pectoral-fin rays (vs. eight in *Humboldtglanis*, eight or nine in *Psammocambeva* and *Trichomycterus*, and nine or ten in *Megacambeva*); anal-fin origin in vertical through the middle or posterior portion of the dorsal-fin base (vs. posterior to end of the dorsal-fin base in *Megacambeva*); basal portion of maxillary barbel not distinctively widened (vs. widened in *Psammocambeva*); distal tip of the maxillary barbel not reaching the pectoral-fin base (vs. reaching first pectoral-fin base third in *Megacambeva*); eye small, iris with an indistinct or small light area (vs. iris light area broad in *Psammocambeva*).

Included taxa. *Paracambeva* comprises two main lineages. One lineage, containing only *T. itatiayae*, is characterized by the following apomorphic character states: a rudimentary and curved latero-posterior process of the autopalatine (chast.3.1, Figure 2B; vs. well-developed, about straight, Figure 2A,C–F); a deep concavity on the metapterygoid accommodating an expansion of the antero-dorsal process of the quadrate (chast. 11.1, Figure 3B; vs. never a similar morphology, Figure 3A,C–F); a deep anterior outgrowth of the hyomandibula, with the dorsal margin nearly horizontal anteriorly and a distinctive U-shaped concavity posteriorly (chast. 13.1, Figure 3B; vs. never a similar morphology, Figure 3A,C–F); a medially directed antero-dorsal extremity of the lateral ethmoid (chast.27.2, Figure 2B; vs. anteriorly or laterally directed, Figure 2A,C–F); and the presence of a small process close to the cartilage supporting fin rays on the postero-medial portion of the pelvic bone (chast.52.2, Figure 6C; vs. absence, Figure 6A,B,D–G). The other lineage, the *T. reinhardti* species group, contains ten species: *T. anaisae* Katz and Costa 2021, *T. funebris* Katz and Costa 2021, *T. humboldti* Costa and Katz 2021, *T. ingaiensis* Katz and Costa 2021, *T. luetkeni* Katz and Costa 2021, *T. pauciradiatus* Alencar and Costa 2006, *T. piratymbara* Katz, Barbosa and Costa 2013, *T. reinhardti* (Eigenmann 1918), *T. sainthilairei* Katz and Costa 2021, and *T. septemradiatus* Katz, Barbosa and Costa 2013. The *T. reinhardti* species group is supported by the following apomorphic character states: a lengthened ventral flap of the autopalatine articular facet for the lateral ethmoid (chast.7.1, Figure 2I; vs. short or rudimentary, Figure 2J); infraorbital canal not attached to antorbital (chast.23.1, Figure 2G; vs. attached, Figure 2H); and a rudimentary pelvic girdle, with a narrow proximal region, without vestiges of the antero-medial and posterior processes (chast.47.1, Figure 6B; vs. pelvic girdle well-developed, Figure 6A,C–G). Species of the *T. reinhardti* group are also distinguished from *T. itatiayae* by having pointed jaw teeth (vs. incisiform).

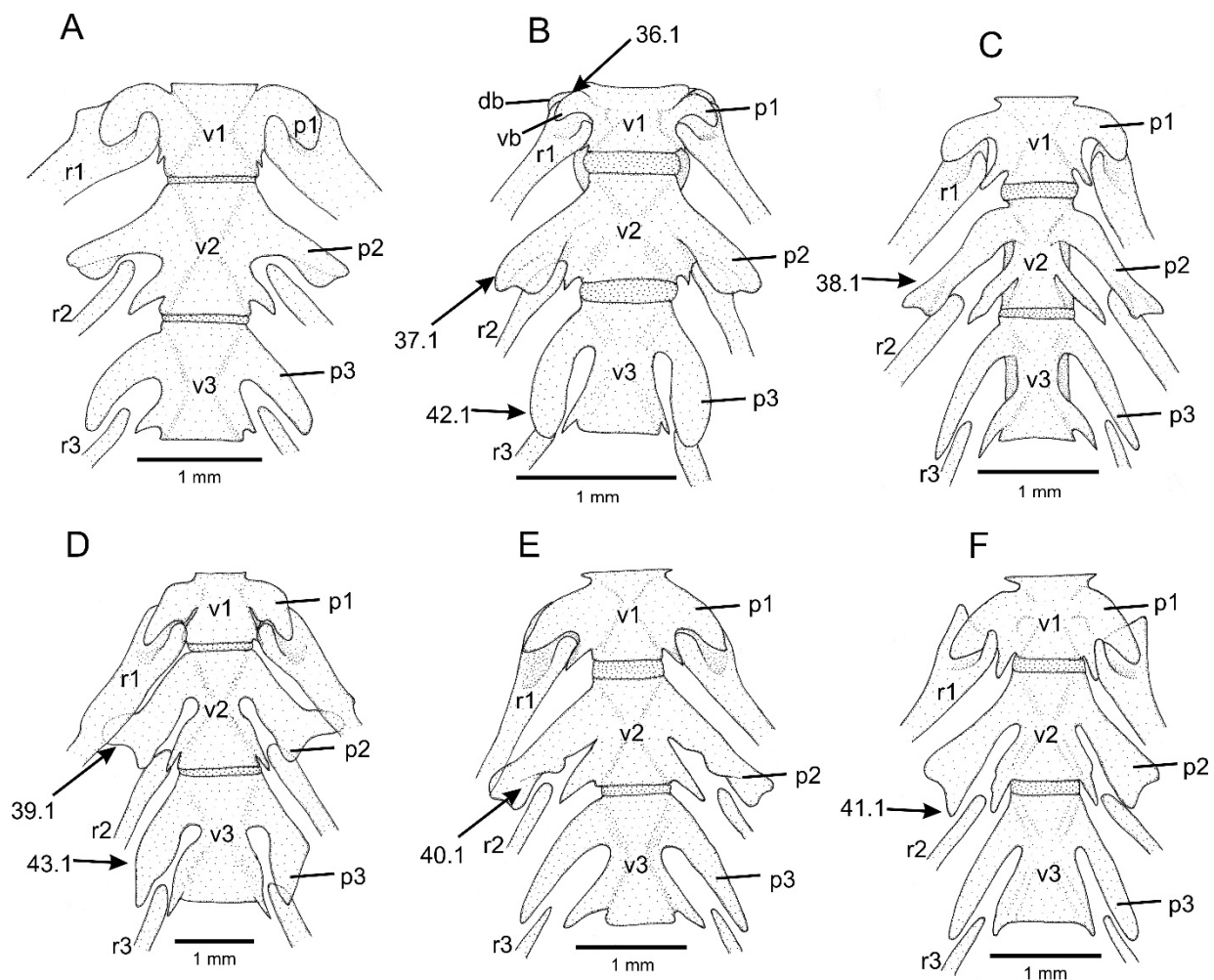


Figure 7. Three anterior-most free vertebrae, ventral view: (A) *Trichomycterus punctulatus*; (B) *Cambeva davisii*; (C) *Trichomycterus (Paracambeva) itatiayae*; (D) *Trichomycterus (Trichomycterus) nigrauratus*; (E) *Trichomycterus (Cryptocambeva) mirissumba*; (F) *Trichomycterus (Psammocambeva) auroguttatus*. Larger stippling represents cartilages. The numbers are characters followed by character states after dots, which are numbered according to Appendix C. Abbreviations: db: parapophysis dorsal branch; p1–3: parapophysis 1–3; r1–3: rib 1–3; v1–3: free vertebra 1–3; vb: parapophysis ventral branch.

Distribution and ecological notes. *Trichomycterus itatiayae* is endemic to the upper Rio Paraíba do Sul basin, south-eastern Brazil; it was found associated with leaf litter, occasionally swimming above it during daylight [69]. The *T. reinhardti* group occurs in a wide geographical range, including the upper Rio Paraná, upper Rio São Francisco, upper Rio Doce, and middle Rio Paraíba do Sul basin, south-eastern Brazil. During daylight field works, species of *Paracambeva* are found hidden within roots of marginal vegetation in small shallow streams, indicating to have nocturnal habits [25].

Etymology. The name *Paracambeva* is derived from the Greek prefix *para*, meaning resembling, and *cambeva*, a popular name for trichomycterids in south-eastern and southern Brazil and a nominal trichomycterine genus, in reference to the superficial resemblance with some species of *Cambeva* (e.g., *Cambeva poikilos* (Ferrer and Malabarba 2013)). Gender feminine.

3.2.3. Subgenus *Trichomycterus* Valenciennes 1832

Type species. *Trichomycterus nigricans* Valenciennes 1832.

Diagnosis. The subgenus *Trichomycterus* is supported by three apomorphic character states: the presence of a posterior expansion in the metapterygoid (chast.10.1, Figure 3D; vs. absence, Figure 3A–C,E,F); a long lateral process of the sphenotic-prootic-pterosphenoid

complex (chast.32.1, Figure 8A; vs. short, Figure 8B); and the presence of an expansion on the antero-distal portion of the second free vertebra parapophysis, overlapping the adjacent rib that is widened in its sub-proximal portion (chast.39.1, Figure 7D; vs. absence of expansion, rib not widened in its sub-proximal portion, Figure 7C,E–G). The subgenus *Trichomycterus* is distinguished from all other subgenera of *Trichomycterus s.s.* by having the opercular odontode patch more posteriorly placed, with its posterior limit reaching a vertical through the pectoral-fin base (vs. anterior to pectoral-fin base). The subgenus *Trichomycterus* also differs from all other subgenera of *Trichomycterus s.s.* by the following combination of character states: eight or nine pectoral-fin rays (vs. six or seven in *Cryptocambeva* and *Paracambeva*); opercular odontodes transversely arranged (vs. diagonally in *Cryptocambeva* and *Humboldtglanis*); dorsal fin longer than deep (vs. deeper than long in *Megacambeva*); basal portion of maxillary barbel not distinctively widened (vs. widened in *Psammocambeva*).

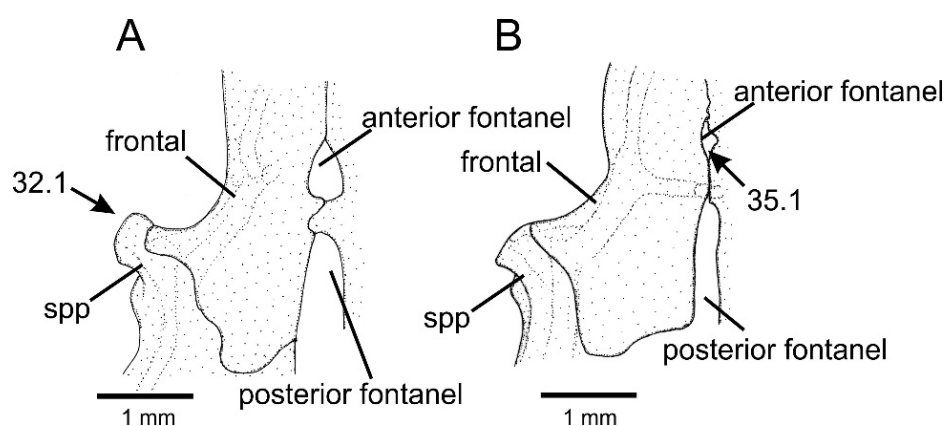


Figure 8. Mid-lateral region of neurocranium, left portion, dorsal view: (A) *Trichomycterus* (*Trichomycterus*) *maculosus*; (B) *Trichomycterus* (*Psammocambeva*) *auroguttatus*. The numbers are characters followed by character states after dots, which are numbered according to Appendix C. Abbreviation: spp: sphenotic-prootic-pterosphenoid complex.

Included taxa. Eight valid species in two groups: the *T. nigricans* group, including *T. caipora* Lima, Lazzarotto, and Costa 2008, *T. immaculatus* (Eigenmann and Eigenmann 1889), *T. nigricans*, and *T. santaeritae* (Eigenmann 1918), supported by the presence of an enlarged posterior process of the maxilla (chast.22.1, Figure 2C; vs. not enlarged, Figure 2A,B,D–G); and the *T. nigroauratus* group, including *T. maculosus* Barbosa and Costa 2010, *T. nigroauratus* Barbosa and Costa 2008, and *T. quintus* Costa 2020, supported by the presence of a folded laminar expansion of the lateral ethmoid border, just above the articular facet for the autopalatine (chast.26.1, Figure 2D; vs. absence, Figure 2A–C,E–G); and an elongate pelvic bone, with a narrow and straight posterior process (chast.48.1, Figure 6D; vs. never a similar shape, Figure 6A–C,E–G). Species of the *T. nigricans* group are also distinguished from species of the *T. nigroauratus* group by having eight pectoral-fin rays (vs. nine) and posterior margin of the caudal fin straight to slightly convex (vs. concave at least in juveniles).

Distribution and ecological notes. Species of the subgenus *Trichomycterus* are endemic to an area comprising the Rio Paraíba do Sul basin and the neighboring smaller coastal basins. Most species have cryptical habits during the day, suggesting that they are nocturnal, but *T. santaeritae* is a specialized diurnal psammophilic species [32,36].

3.2.4. *Psammocambeva*, New Subgenus

LSID: urn:lsid:zoobank.org:act:4ED967A8-D2DB-43F1-9918-6A3384652165

Type species. *Trichomycterus travassosi* Miranda Ribeiro, 1949.

Diagnosis. *Psammocambeva* is supported by the presence of a long maxilla, that is longer than the premaxilla (chast.20.1, Figure 2F; vs. shorter, Figure 2A–E,G,H), a derived condition independently acquired in a subclade of the subgenus *Trichomycterus*. *Psammocambeva* is distinguished from other subgenera of *Trichomycterus* s.s. by the presence of a widened basal portion of the maxillary and rictal barbels (Figure 9B; vs. not widened, Figure 9A,C,D) and a broad ventral expansion of the light portion of the iris (Figure 9B; vs. light portion of the iris not ventrally widened, Figure 9A,C,D). Distinguished from *Paracambeva* also by the anterior segment of the infraorbital latero-sensory canal being always present and close to the nostrils (vs. separated by an interspace from the nostrils when present); from *Cryptocambeva* and *Paracambeva* by the presence of eight or nine pectoral-fin rays (vs. six or seven); from *Cryptocambeva* and *Humboldtglanis* by the opercular odontodes being transversely arranged, vs. diagonally); from *Humboldtglanis* by the absence of white spots on the flank (vs. presence); from *Trichomycterus* by the opercular odontode patch not extending posteriorly above the pectoral-fin base (vs. extending); and from *Megacambeva* by the dorsal fin being longer than deep (vs. deeper than long).

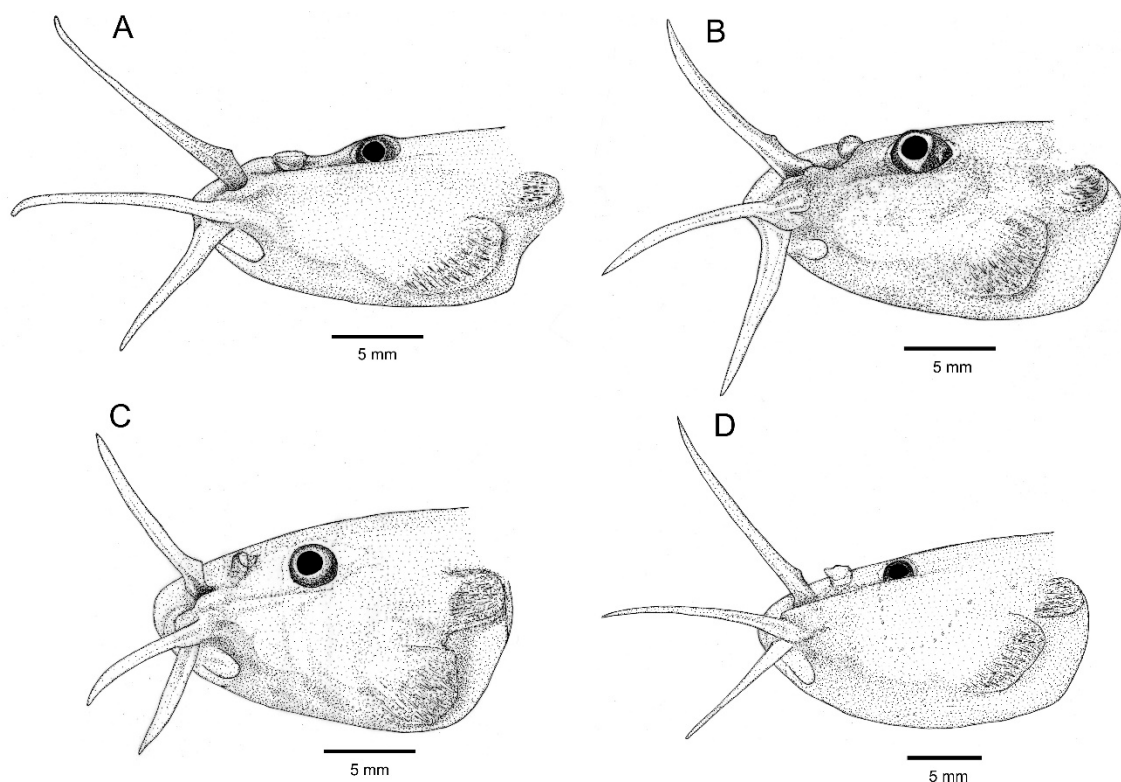


Figure 9. Schematic representation of the head in live specimens, left lateral view, of four sympatric species: (A) *Trichomycterus* (*Trichomycterus*) *quintus* (B) *Trichomycterus* (*Psammocambeva*) *auroguttatus* (C) *Trichomycterus* (*Humboldtglanis*) *albinotatus* (D) *Trichomycterus* (*Cryptocambeva*) *mirissumba*.

T. melanopygius Reis, dos Santos, Britto, Volpi, and de Pinna 2020, *T. pradensis* Sarmento-Soares, Martins-Pinheiro, Aranda, and Chamon 2005, and *T. tete* Barbosa and Costa 2010, is characterized by two apomorphic conditions: a shallow anterior hyomandibular outgrowth (chast.12.1, Figure 3C; vs. deep, Figure 3A,B,D–G); and an expanded ventral preopercular flap (chast.19.1, Figure 3C; vs. short, Figure 3A,B,D–G). Three species not available for the present study, showing great external morphological similarity, are probably members of this clade: *T. itacambirussu* Triques and Vono 2004, *T. jequitinhonhae* Triques and Vono 2004,

T. landinga Triques and Vono 2004, and *T. trefauti* Wosiacki 2004. The *T. travassosi* group, comprising *T. auroguttatus* Costa 1992, *T. cf. alternatus* (Eigenmann 1917), *T. caudofasciatus* Alencar and Costa 2004, *T. gasparini* Barbosa 2013, *T. goeldii* Boulenger 1896, *T. jacupiranga* Wosiacki and Oyakawa 2005, *T. longibarbatulus* Costa 1992, *T. macrophthalmus* Barbosa and Costa 2012, *T. mimosensis* Barbosa 2013, *T. pantherinus* Alencar and Costa 2004, *T. puriventris* Barbosa and Costa 2012, and *T. travassosi* Miranda Ribeiro 1949, is supported by: a widened lateral margin of the lateral ethmoid, (chast.31.1, Figure 2F; vs. not widened, Figure 2A–E).

Trichomycterus astromycterus Reis, de Pinna, and Pessali 2020, not available to the present study, probably is a member of the *T. travassosi* group. Although considered to be possibly closely related to the Chilean monotypic genus *Bullockia* [70], *T. astromycterus* exhibits the derived character states supporting *Psammocambeva* and the *T. travassosi* group, as well as three synapomorphies supporting a subclade comprising *T. cf. alternatus*, *T. auroguttatus*, and *T. goeldii*: a long autopalatine postero-lateral process, its length nearly equal or slightly longer than the autopalatine longitudinal length excluding the postero-lateral process (chast.5.1, Figure 2F; vs. distinctively shorter, Figure 2A–E); a slightly folded maxilla (chast.21.1, Figure 2F; vs. curved or straight, Figure 2A–E); and the anterior cranial fontanel represented by a minute aperture (chast.35.1, Figure 8B; vs. broad aperture, Figure 8A), besides all having a protruded orbit (Figure 9B).

Etymology. From the Greek, *psammos* (sand), and *cambeva*, a popular name for trichomycterids in south-eastern and southern Brazil and a nominal trichomycterine genus, an allusion to the psammophilic habits of the type species and other included species. Gender feminine.

Distribution and ecological notes. *Psammocambeva* occupies the entire geographical distribution of *Trichomycterus s.s.*, from the Rio de Contas basin in north-eastern Brazil to the rivers associated with the Baía de Paranaguá in southern Brazil. Field studies indicate that species of *Psammocambeva* are always found associated with gravel or sand substrate [35], but at least some species (e.g., *T. auroguttatus* and *T. cf. alternatus*) are specialized psammophilic species, often found completely buried in sand stretches on the middle of river bottom [13]. At least *T. pradensis* has migrating behavior, which is supported by field studies conducted in February 2014, when hundreds of juvenile specimens of this species, about 20 mm of total length, were seen forming a continuous upstream flow in the lower Rio Jucuruçu (16°23'34" S 39°17'09" W), eastern Brazil (person. observ.).

3.2.5. *Humboldtglanis*, New Subgenus

LSID: urn:lsid:zoobank.org:act:09886ED2-C1BC-4A84-8824-07E6EEA8B510

Type species. *Trichomycterus albinotatus* Costa 1992.

Diagnosis. Species of *Humboldtglanis* are distinguished from all other congeners by the following apomorphic features: a slightly convex medial margin of the autopalatine in larger individuals (above about 45 mm SL; chast.6.1, Figure 2H; vs. concave), steep anterior and posterior portions of the dorsal margin of the hyomandibula anterior outgrowth, converging to a pronounced V-shaped notch (chast.13.1, Figure 3E; vs. outgrowth never with similar morphology, Figure 3A–D,F), and a sharp process in the latero-posterior margin of the epioccipital (chast.34.1, Figure 4E; vs. process absent, Figure 4A–C). *Humboldtglanis* is also distinguished from other subgenera, except *Cryptocambeva*, by the following apomorphic features: presence of a long and narrow ridge on the dorsal surface of the autopalatine, close to its lateral margin (chast.1.1, Figure 2E,H; vs. absence, Figure 2A–D,F,G); an oblique arrangement of odontodes of the basal portion of the opercular odontode patch (chast.14.1, Figure 3E; vs. vertical, Figure 3A–D,F); ventral surface of the lateral margin of the mesethmoid ventrally folded around the vomer margin (chast.25.1, Figure 2J; vs. not folded, Figure 2I); lateral ethmoid medially separated from its symmetrical homologous (chast.28.1, Figure 2E; vs. in contact, Figure 2A–D,F); presence of a lateral expansion of the lateral ethmoid margin close to the sesamoid supraorbital (chast.29.1, Figure 2E; vs. absence, Figure 2A–D,F); the presence of a small notch in the posterior margin of the parapophysis of the second free vertebra, in an area close to the proximal

extremity of the adjacent rib (chast.40.1, Figure 7E; vs. absence, Figure 7A–D,F); and a rudimentary posterior process of the pelvic bone (chast.49.1, Figure 6E,F; vs. well-developed, Figure 6A,C,D,G). Species of *Humboldtglanis* are also distinguished from other congeners by the presence of rows of small white spots along the flank in specimens about 50 mm SL or less (Figure 1; vs. white spots absent). *Humboldtglanis* is also distinguishable from other subgenera of *Trichomycterus* s.s. by the following combination of characters: the anal-fin origin is placed in a vertical through the posterior portion of the dorsal-fin base (vs. anal-fin origin posterior to the dorsal-fin base in *Megalebias*), eight pectoral-fin rays (vs. six or seven in *Cryptocambeva* and *Paracambeva*, nine or ten in *Megacambeva*), dorsal fin longer than deep (vs. deeper than long in *Megacambeva*, Figure 1); presence of an anterior infraorbital canal attached to the antorbital, externally close to nostrils (vs. anterior infraorbital canal when present is not attached to the antorbital and is externally separated from nostrils by an interspace in *Paracambeva*); never having a continuous black stripe between the snout and the caudal-fin base, becoming disperse and spotted in larger specimens (vs. always present in *Paracambeva*); orbital diameter about 12–16% of the head length in specimens between about 40 and 60 mm SL (vs. about 7–11% of the head length in *Cryptocambeva*), by having the basal portion of maxillary barbel not distinctively widened (vs. widened in *Psammodambeva*); the light portion of iris not extending ventrally (vs. broad, extending ventrally in *Psammodambeva*, Figure 9); and the posterior margin of the opercular patch of odontodes placed in a vertical anterior to pectoral-fin base (vs. in vertical through the pectoral-fin base in *Trichomycterus*).

Included taxa. Two species, *T. albinotatus* and *T. vitalbrazili* Vilardo, Katz, and Costa 2020.

Etymology. The name *Humboldtglanis* was used in honor of the Prussian naturalist Friedrich Wilhelm Heinrich Alexander von Humboldt (1769–1859), for his valuable and pioneering contribution in studies on distribution, ecology, and conservation of mountain organisms; the name *glanis*, is from the ancient Greek and is commonly used for generic catfish names. The name is also an allusion to several species of *Humboldtglanis* only inhabiting mountain rivers 1000 m above sea level. Gender masculine.

Distribution and ecological notes. *Humboldtglanis* is endemic to the Rio Paraíba do Sul basin, south-eastern Brazil, occurring in areas about 1100 m above sea level. Field observations indicate that species are diurnal, swimming actively during daylight a few centimeters above gravel substrate and small rocks, but larger specimens about 60 mm SL or more, are mostly found hidden among stones, only sporadically swimming around this habitat [13,33].

3.2.6. *Cryptocambeva*, New Subgenus

LSID: urn:lsid:zoobank.org:act:E0BE3FF5-ABAE-4881-9875-5FA5C23FE2C4

Type species. *Trichomycterus mirissumba* Costa 1992.

Diagnosis. Species of *Cryptocambeva* are distinguished from all other congeners by the following apomorphic features: a rounded, narrow, and elongated extremity of the pterotic lateral process (chast.33.2, Figure 4C; vs. pterotic lateral process truncate and not elongated, Figure 4A,B,D), a small posttemporo-supracleithrum, resulting in a broad interspace between the posttemporo-supracleithrum and adjacent bones, and a short postero-medial process attached on the distal portion of the Weberian capsule (chast.46.1, Figure 4C; vs. interspace narrow, postero-medial process attaching on the proximal portion of the Weberian capsule, Figure 4A,B,D). Species of *Cryptocambeva* also differs from all other congeners by having a distinctively narrowed area adjacent to the base of the anterior processes of the pelvic bone (chast.50.1, Figure 6F), but this character state is not applicable to diagnose one species *T. candidus* (Miranda Ribeiro 1949), in which pelvic fin and girdle are absent. *Cryptocambeva* is also distinguished from other subgenera, except *Humboldtglanis*, by the following apomorphic features: presence of a long and narrow ridge on the dorsal surface of the autopalatine, close to its lateral margin (chast.1.1, Figure 2E,H; vs. absence, Figure 2A–D,F,G); an oblique arrangement of odontodes of the basal portion of the opercular odontode patch (chast.14.1, Figure 3E; vs. vertical, Figure 3A–D,F); ventral surface of the lateral margin of

the mesethmoid ventrally folded around the vomer margin (chast.25.1, Figure 2J; vs. not folded, Figure 2I); lateral ethmoid medially separated from its symmetrical homologous (chast.28.1, Figure 2E; vs. in contact, Figure 2A–D,F); presence of a lateral expansion of the lateral ethmoid margin close to the sesamoid supraorbital (chast.29.1, Figure 2E; vs. absence, Figure 2A–D,F); the presence of a small notch in the posterior margin of the parapophysis of the second free vertebra, in an area close to the proximal extremity of the adjacent rib (chast.40.1, Figure 7E; vs. absence, Figure 7A–D,F); and a rudimentary posterior process of the pelvic bone (chast.49.1, Figure 6E,F; vs. well-developed, Figure 6A,C,D,G). Species of *Cryptocambeva* are distinguished from all other congeners by a color pattern consisting of minute dark brown dots scattered over all the body (Figure 1; vs. never a similar color pattern). Species of *Cryptocambeva* are also distinguishable from species of all other subgenera by the following combination of character states: six or seven pectoral-fin rays (vs. nine or ten in *Megacambeva*, eight in *Humboldtglanis*, eight or nine in *Psammocambeva* and *Trichomycterus*), the anal-fin origin in a vertical through the posterior portion of the dorsal-fin base (vs. anal-fin origin posterior to the dorsal-fin base in *Megacambeva*), dorsal fin longer than deep (vs. deeper than long in *Megacambeva*); never having a continuous black stripe between the snout and the caudal-fin base, becoming disperse and spotted in larger specimens (vs. always present in *Paracambeva*); jaw teeth always sharply pointed (vs. at least larger adults with incisiform teeth in *Trichomycterus*); the basal portion of maxillary barbel not distinctively widened (vs. widened in *Psammocambeva*); and an inconspicuous light portion of the iris (vs. broad in *Psammocambeva*).

Included taxa. *Cryptocambeva* comprises fifteen valid species: *T. brasiliensis* Lütken 1874, *T. brunoi* Barbosa and Costa 2010, *T. candidus*, *T. claudiae* Barbosa and Costa 2010, *T. fuliginosus* Barbosa and Costa 2010, *T. maracaya* Bockmann and Sazima 2004, *T. mariamole* Barbosa and Costa 2010, *T. mimonha* Costa 1992, *T. mirissumba*, *T. macrotrichopterus* Barbosa and Costa 2010, *T. novalimensis* Barbosa and Costa 2010, *T. pirabitira* Barbosa and Azevedo-Santos 2012, *T. potschi* Barbosa and Costa 2003, *T. rubiginosus* Barbosa and Costa 2010, *T. vermiculatus* (Eigenmann 1917), and at least three undescribed species. *Trichomycterus argos* Lezama and Triques e Santos 2012, not available for the present study, was considered to belong to the *T. brasiliensis* group by Lezama et al. [71], but the morphology of the neurocranium and adjacent bones schematically illustrated in its original description [71] (Figure 3) suggests that is not a member of this group. In this illustration, the two derived conditions relative to the pterotic and posttemporo-supracleithrum seem to be not present.

Etymology. From the Greek *cryptos* (hidden) and *cambeva*, a popular name for trichomycterids in south-eastern and southern Brazil and a nominal trichomycterine genus, referring to the cryptic habits of species of this group during daylight collections (see below). Gender feminine.

Distribution and ecological notes. Species of *Cryptocambeva* occur in a broad area of south-eastern Brazil, encompassing the upper courses of the Rio São Francisco, Rio Itabapoana, Rio Paraíba do Sul, and Rio Paraná basins, besides small isolated coastal basins emptying in the Baía de Sepetiba. Field studies indicate that species of the *T. brasiliensis* group are nocturnal, with adults being found deeply buried in river banks and juveniles in leaf litter deposits during the day [13,28].

This section may be divided into subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

4. Discussion

4.1. Phylogeny

The phylogenetic tree obtained in the present analysis was similar to those generated when using only molecular data [32–34], supporting the same intrageneric clades here recognized as subgenera and species groups. The only exception was the *T. pradensis* group, here supported as monophyletic, but with relatively low support values, whereas two lineages formed a paraphyletic lineage in previous studies, but also with weakly supported

nodes. These discrepant results are due to the inclusion of unique osteological character states herein assigned to the *T. pradensis* group (see taxonomical accounts above).

4.2. Morphological Diversity of Osteological Structures

A high morphological diversity of osteological structures in different lineages of *Trichomycterus s.s.* was herein first recorded and described. This diversity, mainly concentrated in bones of the mesethmoidal region and jaw suspensorium, is comparable or superior to that found among closely related genera of other trichomycterid groups. For example, the morphological divergence among the subgenera of *Trichomycterus s.s.* and included groups is conspicuously greater than that reported to distinguish the trichomycterine genera *Bullockia* and *Hatcheria* [12], and the copionodontine genera *Copionodon* de Pinna 1992 from *Glaphyropoma* de Pinna 1992 [72].

Although field reports on trichomycterines from eastern Brazil are sparse and limited to a few notes in taxonomical papers, some data suggest that the high morphological diversity here reported for *Trichomycterus s.s.* is possibly related to some ecological specializations found in different lineages. *Trichomycterus giganteus*, the only species of *Megacambeva* and supported as sister to the clade comprising all other congeners (Figure 1), highly differs from other taxa of the CST-clade by unique osteological character states (see results above). However, *T. giganteus* also differs from other congeners by its larger size and habitat. This species reaches about 200 mm of standard length (SL) [73], a size never recorded for any member of the CST-clade, which rarely reach about 120 mm SL. *Trichomycterus giganteus* is found in small lake-like areas formed between river waterfalls, where larger specimens may be seen swimming about 200 cm below the water surface (person. observ.). Other congeners are typically found in fast-flowing, shallow streams, in depths about 100 cm or less. Interestingly, a few other trichomycterine taxa reaching large size are found in Andean lakes, such as *Eremophilus mutisii* Humboldt 1805 reaching about 300 mm SL and *Trichomycterus rivulatus* Valenciennes 1846 reaching about 350 mm SL [11], suggesting that life in lacustrine habitats may favor the development of larger sizes.

Some sister lineages seem to exhibit divergent ecological specializations. Within *Paracambeva*, *T. itatiayae* has diurnal habits and is occasionally observed swimming on the river bottom during daylight, close to leaf litter [69], thus differing from species of the sister group, the *T. reinhardti* species group, that are only found buried in the marginal vegetation of small shallow streams during daylight collections [25]. Similarly, species of *Cryptocambeva* are typical nocturnal catfishes, with adult specimens found deeply buried in riverbanks during daylight field studies, whereas juveniles are often found buried in leaf litter [13,28,30]. This behavior highly contrasts with the diurnal activity recorded for juveniles and smaller adults of species of *Humboldtglanis* [13,33], the sister group of *Cryptocambeva*. In the upper Rio Preto drainage, where populations of five species of *Trichomycterus* have been observed in the field by the author since 1979, including monthly field observations in the period between 2017 and 2020, *T. albinotatus* (subgenus *Humboldtglanis*) was easily seen actively swimming just above the gravel substrate during daylight. On the other hand, the sympatric species *T. mirissumba* (subgenus *Cryptocambeva*) was found hidden in holes below the marginal vegetation during daylight but commonly fished at night by the local people in any part of the river bottom, making obvious its nocturnal habits. These observations are consistent with sporadic field observations involving other species of both subgenera [28,30,33]. Interestingly, in species of *Cryptocambeva*, the eye is smaller and darker, without a conspicuous light iris (Figure 9D), contrasting with the larger eye with light iris area around pupil in *Humboldtglanis* (Figure 9C), conditions probably related to their nocturnal and diurnal habits, respectively. The other sympatric congeners are also equally specialized, with *T. auroguttatus*, a psammophilic species of *Psammocambeva* showing high diurnal activity (Figure 9B), and *T. quintus*, a typically nocturnal species of the subgenus *Trichomycterus*, only found buried into the bottom leaf litter among rocks during the daytime, in places with strong water flow (Figure 9A) [32].

Species of the subgenera *Psammocambeva* and *Trichomycterus* apparently have more variability regarding habitat preferences. Species of the latter subgenus typically have predominant overall dark brown to black coloration, which is probably related to their cryptic habits during daylight [32]. Species of the *T. nigroauratus* group have been reported to be associated with the bottom leaf-litter [32,69], and most species of the *T. nigricans* group are commonly found below large rocks during day light [74], but *T. santaeritae*, a species of the *T. nigricans* group, highly differs by having lighter coloration (i.e., light yellow with dark brown spots), is diurnal and psammophilic [36]. Similarly, field studies have indicated different habitats preferences for species of *Psammocambeva*, with many having diurnal habits [13,35,75] and some others nocturnal [76]. Most species of this clade have been found associated with gravel substrate [35,75], but some species such as *T. auroguttatus* are highly associated to sandy bottom [13], exhibiting a relatively large eye that is slightly dorsally protruded (Figure 6B). Field studies have shown that *T. auroguttatus* has an intense diurnal activity, being usually found swimming close to sandy stretches on the river bottom, in parts with intense waterflow; when disturbed, specimens quickly bury into the sand, about 3–5 cm below sand surface (person. observ. 2017–2020). However, detailed field studies on other species of *Psammocambeva* are necessary to make some inferences about possible ecological specializations.

4.3. CST-Clade and *Trichomycterus s.s.* Diagnosis

Using criteria traditionally accepted for genus delimitation, such as monophyly, morphological gaps, and ability to discriminate, it would be justifiable to divide *Trichomycterus s.s.* in different genera. However, in order to preserve the nomenclatural stability of included species that are consistently placed in this genus for a long time, the option here adopted was to divide *Trichomycterus s.s.* in subgenera. The morphological gaps found between *Trichomycterus s.s.* subclades was compatible with the relatively long genetic distances found among these subclades [32] (Figure 1). However, no unique morphological character state was found to be synapomorphic for *Trichomycterus s.s.* On the other hand, the comparative osteological analysis provided some evidence relative to vertebra morphology supporting both the CST-clade and the clade comprising *Cambeva* and *Scleronema*.

All taxa belonging to the CST-clade have the parapophysis main axis of the second free vertebra postero-laterally directed (chast.37.1; Figure 7B–F), instead of being more laterally directed as in other trichomycterines here examined (Figure 7A). However, a supposedly similar postero-laterally directed parapophysis may be convergently present in other trichomycterines not examined in this study since reconstruction of the Colombian trichomycterine *Rhizosomichthys totae* (Miles 1942) [42] (Figure 2C) showed a similar morphology.

Species of *Cambeva* and *Scleronema* differ from species of *Trichomycterus s.s.* by having a branched parapophysis of the first free vertebra, with a dorsal branch in contact with the dorsal surface of the articular zone of the first rib, and a ventral branch ventrally supporting the proximal extremity of the rib (chast.36.1; Figure 7B), instead of being unbranched as in *Trichomycterus s.s.* (Figure 7C–F) and other trichomycterids (Figure 7A), and by the morphology of the parapophysis of the third free vertebra that is posteriorly directed (chast.42.1; Figure 7B), instead of being postero-laterally directed as in other trichomycterines (Figure 7A,C–F). It is important to note that the latter condition, in which the whole parapophysis is posteriorly directed with its main axis reaching a position nearly parallel to the vertebral column axis, differs from that occurring in some species of *Ituglanis*, in which the parapophysis is curved, and only its extremity is posteriorly directed [77] (Figure 2B). Therefore, the combination of an apomorphic postero-laterally directed parapophysis main axis of the second free vertebra and the plesiomorphic unbranched parapophysis of the first free vertebra and a postero-laterally directed parapophysis of the third free vertebra would tentatively characterize *Trichomycterus s.s.*

5. Conclusions

The Trichomycterinae has been considered a problematic taxon (see introduction above), but recent studies are quickly changing this scenario. Major advances have resulted from studies on phylogenetic relationships [9,22,31,32], but some problems were not still addressed. The present study provides morphological evidence supporting and making it possible to distinguish between *Trichomycterus* lineages previously supported only by molecular data, thus allowing future unequivocal placement of new species without molecular data. Furthermore, this study has shown that osteological characters combined with molecular data may be useful to consistently delimit and distinguish between trichomycterine groups that cannot be properly distinguished using only external morphological features. More importantly, this integrative approach sheds light upon the way of solving still persistent problems in trichomycterine systematics, including a formal generic placement for the numerous Andean and Guianan incertae sedis taxa [9,31,78].

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Data Availability Statement: DNA sequences used in this study are deposited in GenBank.

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Conflicts of Interest: The author declares no conflict of interest.

Appendix A

List of specimens 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.

Trichomycteridae

Genus *Trichomycterus*: subgenus *Megacambeva*: *T. giganteus* Lima & Costa, 2004: UFRJ 5732, 2 (paratypes); UFRJ 5733, 2 (paratypes); **subgenus *Paracambeva*:** *T. itatiayae* Miranda-Ribeiro, 1906: UFRJ 5155, 2; UFRJ 5222, 1; ***T. reinhardti* group:** *T. anaisae* Katz & Costa, 2021: UFRJ 12679, 2; *T. funebris* Katz & Costa, 2021: UFRJ 9975, 1; UFRJ 9829, 1; *T. humboldti* Costa & Katz, 2021: UFRJ 4555, 1; UFRJ 4557, 1; UFRJ 10008, 1; UFRJ 10018, 1; *T. ingaiensis* Katz & Costa, 2021: UFRJ 9334, 2; UFRJ 9997, 1; *T. luetkeni* Katz & Costa, 2021: UFRJ 12681, 3; *T. pauciradiatus* Alencar & Costa, 2006: UFRJ 5808, 6 (paratypes); *T. piratymbara* Katz, Barbosa & Costa, 2014: UFRJ 8595, 5 (paratypes); *T. reinhardti* (Eigenmann 1917): UFRJ 9995, 2; *T. saintthilairei* Katz & Costa, 2021: UFRJ 9971, 5; *T. septemradiatus* Katz, Barbosa & Costa, 2014: UFRJ 8385, 3 (paratypes); **subgenus *Trichomycterus*: *T. nigricans* group:** *T. caipora* Lima, Lazzarotto & Costa, 2008: UFRJ 7614, 3 paratypes; *T. immaculatus* (Eigenmann & Eigenmann, 1889): UFRJ 7265, 1; UFRJ 12051, 1; UFRJ 10489, 1; UFRJ 7272, 2; *T. nigricans* Valenciennes, 1832: UFRJ 11897, 3; UFRJ 11898, 1; UFRJ 11899, 2; *T. santaeritae* (Eigenmann, 1918): UFRJ 12589, 3; UFRJ 646, 1; ***T. nigroauratus* group:** *T. maculosus* Barbosa & Costa, 2010: UFRJ 5168, 1 paratype; UFRJ 5169, 2 paratypes; *T. nigroauratus* Barbosa & Costa, 2008: UFRJ 5166, 1; UFRJ 5594, 3; UFRJ 5167, 2 paratypes; UFRJ 4552, 1 (paratype); UFRJ 5154, 1 paratypes; *T. quintus* Costa, 2020: UFRJ 11844, 3 (paratypes); **subgenus**

Humboldtglanis: *T. albinotatus* Costa 1992: UFRJ 0583, 2 (paratypes); UFRJ 5628, 3; *T. vitalbrazili* Vilarde, Katz & Costa, 2020: UFRJ 12128, 1 (paratype); UFRJ 12150, 2 (paratypes); **subgenus *Cryptocambeva*:** *T. brasiliensis* Lütken, 1874: UFRJ 4834, 3; *T. brunoi* Barbosa & Costa, 2010: UFRJ 5658, 5 (paratypes); *T. candidus* (Miranda-Ribeiro, 1949): UFRJ 4928, 5; *T. claudiae* Barbosa & Costa, 2010: UFRJ 5685, 3 (paratypes); *T. fuliginosus* Barbosa & Costa, 2010: UFRJ 5207, 2 (paratypes); MNRJ 18177, 4 (paratypes); *T. giarettai* Barbosa & Katz, 2016: UFRJ 9739, 3 (paratypes); *T. mariamole* Barbosa & Costa, 2010: UFRJ 5400, 3 (paratypes); UFRJ 5401, 3 (paratypes); *T. macrotrichopterus* Barbosa & Costa, 2010: UFRJ 5776, 2 (paratypes); *T. mimonha* Costa, 1992: UFRJ 0644, 1 paratype; *T. mirissumba* Costa, 1992: UFRJ 4730, 5; *T. novalimensis* Barbosa & Costa, 2010: MZUSP 37145, 5 (paratypes); *T. pirabitira* Barbosa & Azevedo, 2012: UFRJ 8264, 3 (paratypes); UFRJ 5829, 3 (paratypes); *T. potschi* Barbosa & Costa 2003: UFRJ 4728, 5; *T. rubiginosus* Barbosa & Costa, 2010: MZUSP 37168, 5 (paratypes); *T. vermiculatus* (Eigenmann, 1918): UFRJ 5206, 5; **subgenus *Psammocambeva*:** ***T. pradensis* group:** *T. melanopygius* Reis, dos Santos, Britto, Volpi & de Pinna, 2020: UFRJ 557, 1; *T. pradensis* Sarmiento-Soares, Martins-Pinheiro, Aranda & Chamon, 2005: UFRJ 10920, 1; UFRJ 10921, 2; *T. tete* Barbosa & Costa, 2011: UFRJ 7774, 3 (paratypes); *Trichomycterus* sp.: UFRJ 12260, 3; ***T. travassosi* group:** *T. cf. T. alternatus* (Eigenmann, 1917): UFRJ 9900, 4; UFRJ 556, 2; UFRJ 5673, 1; UFRJ 11913, 3; *T. aurogutattus* Costa, 1992: UFRJ 12476, 3; UFRJ 4558, 2; UFRJ 4562, 3; *T. caudofasciatus* Alencar & Costa, 2004: UFRJ 5656, 5 paratypes; *T. gasparinii* Barbosa, 2013: UFRJ 8158, 7 paratypes; *T. jacupiranga* Wosiacki & Oyakawa, 2005: UFRJ 4549, 5; UFRJ 5639, 2; *T. longibarbatulus* Costa, 1992: UFRJ 5674, 3; UFRJ 1141, 1; *T. macrophthalmus* Barbosa & Costa, 2012: UFRJ 5675, 3 paratypes; *T. mimosensis* Barbosa, 2013: UFRJ 7792, 5 paratypes; *T. pantherinus* Alencar & Costa, 2004: UFRJ 5660, 6 (paratypes); *T. puriventris* Barbosa & Costa, 2012: UFRJ 5677, 1 (paratype); UFRJ 5405, 6 (paratypes); UFRJ 8432, 5 (paratypes); *T. travassosi* (Miranda-Ribeiro 1949): UFRJ 4554, 3; UFRJ 4563, 1; *Trichomycterus* sp.: UFRJ 5434, 2; *Trichomycterus* sp.: UFRJ 5833, 2.

Other Trichomycterinae

Bullockia maldonadoi (Eigenmann, 1928): MZUSP 36958, 1; *Cambeva balios* (Ferrer & Malabarba 2013): UFRJ 11862, 1; *Cambeva brachykechenos* (Ferrer & Malabarba, 2013): UFRJ 10586, 1; *Cambeva castroi* (de Pinna, 1992): UFRJ 10670, 2; *Cambeva concolor* Costa, 1992: MZUSP 422206, 1 (paratype); *Cambeva crassicaudata* (Wosiacki & de Pinna, 2008): UFRJ 11851, 1; *Cambeva cubataonis* (Bizerril, 1994): UFRJ 8871, 5; *Cambeva davisii* (Haseman, 1911): UFRJ 10713, 8; *Cambeva diatropoporos* Ferrer & Malabarba, 2013: UFRJ 6913, 1; *Cambeva flavopicta* Costa, Feltrin & Katz, 2020: UFRJ 12663, 3 (paratypes); UFRJ 12664, 5 (paratypes); *Cambeva guareiensis* Katz & Costa, 2020: UFRJ 12170, 6 (paratypes); *Cambeva naipi* (Wosiacki & Garavello, 2004): UFRJ 10842, 2; UFRJ 10843, 1; *Cambeva stawianski* (Miranda-Ribeiro, 1968): UFRJ 11850, 1; *Cambeva toroba* (Wosiacki & Garavello, 2004): UFRJ 9752, 5; *Cambeva tropeira* Ferrer & Malabarba, 2011: UFRJ 6935, 2; *Cambeva variegata* (Costa, 1992): UFRJ 9346, 2; UFRJ 8385, 2; *Cambeva zonata* (Eigenmann, 1918): UFRJ 11900, 1; *Cambeva* sp1: UFRJ 6938, 4; *Cambeva* sp2: UFRJ 6912, 3; *Cambeva* sp3: UFRJ 6021, 3; *Cambeva* sp4: UFRJ 6923, 3; *Eremophilus mutisii* Humboldt, 1805: MZUSP 35409, 1; *Hatcheria macraei* (Girard, 1855): MZUSP 35687, 1; *Ituglanis amazonicus* (Steindachner, 1882): USNM 317738, 1; UFRJ 5866, 7; *Ituglanis boitata* Ferrer, Donin & Malabarba, 2015: UFRJ 10583, 1; *Ituglanis eichorniarum* (Miranda-Ribeiro, 1912): UFRJ 5608, 1; *Ituglanis gracilior* (Eigenmann, 1912): USNM 272288, 1; *Ituglanis parahybae* (Eigenmann, 1918): UFRJ 704, 1; UFRJ 0761, 1; *Ituglanis paraguassuensis* Campos-Paiva & Costa, 2007: UFRJ 7282, 1 (paratype); USNM 301016, 2 (paratypes); *Ituglanis props* (Miranda-Ribeiro 1908): UFRJ 8730, 1; *Scleronema guapa* Ferrer & Malabarba, 2020: UFRJ 10645, 2; *Scleronema macunada* Ferrer & Malabarba, 2020: UFRJ 11856, 1; *Scleronema milonga* Ferrer & Malabarba, 2020: UFRJ 5826, 2; *Scleronema minutum* (Boulenger, 1891): UFRJ 11855, 1; *Scleronema* sp.: UFRJ 6942, 3; "*Trichomycterus*" *areolatus* Valenciennes, 1846: MZUSP 36959, 1; "*Trichomycterus*" *punctulatus* Valenciennes, 1846: MUSM 1702, 1; "*Trichomycterus*" *rivulatus* Valenciennes, 1846: MUSM 3115, 1.

Microcambevinae

Listrura tetraradiata Landim & Costa, 2002: UFRJ 4588, 6 paratypes; *Microcambeva ribeirae* Costa, Lima & Bizerril, 2004: UFRJ uncat., 2.

Copionodontinae

Copionodon pecten de Pinna, 1992: MZUSP 48962, 1.

Trichogeninae

Trichogenes longipinnis Britski & Ortega 1983: UFRJ 682, 2.

Nematogenidae

Nematogenys inermis Guichenot, 1848: MZUSP 36957, 2.

Appendix B**Table A1.** Terminal taxa for molecular phylogeny and respective GenBank accession numbers.

Species	COI	CYTB	MYH6	RAG2
<i>Nematogenys inermis</i>	KY857952	————	KY858107	KY858182
<i>Copionodon pecten</i>	KY857929	————	KY858084	KY858169
<i>Trichogenes longipinnis</i>	MK123682	MK123704	MF431104	MF431117
<i>Microcambeva ribeirae</i>	MN385807	————	MN385819	MN385832
<i>Listrura tetraradiata</i>	JQ231083	JQ231088	MN385814	MN385826
<i>Eremophilus mutisii</i>	KY857931	————	KY858086	KY858171
<i>‘Trichomycterus’ punctulatus</i>	KY857983	————	KY858134	KY858203
<i>Bullockia maldonadoi</i>	KY857926	FJ772237	KY858081	KY858166
<i>Hatcheria areolata</i>	KY857964	FJ772214	————	KY858188
<i>Ituglanis boitata</i>	MK123684	MK123706	MF431105	MK123758
<i>Scleronema minutum</i>	MK123685	MK123707	MK123735	MK123759
<i>Scleronema cf. guapa</i>	MK123687	MK123709	MF431107	MF431118
<i>Scleronema cf. ibirapuita</i>	MK123688	MK123710	MK123737	MK123761
<i>Scleronema macanuda</i>	MK123686	MK123708	MK123736	MK123760
<i>Cambeva variegata</i>	KY857991	KY858060	————	KY858211
<i>Cambeva brachykechenos</i>	MN995669	MK123711	MF431108	————
<i>Cambeva diatropoporos</i>	KY857996	KY858065	KY858147	KY858213
<i>Cambeva cubataonis</i>	MK123689	MK123713	MK123740	MN385820
<i>Cambeva davisi</i>	KR140345	MK123714	MK123741	MK123762
<i>Cambeva castroi</i>	————	MK123712	MK123739	————
<i>Cambeva zonata</i>	KY857986	KY858053	KY858137	————
<i>Trichomycterus itatiayae</i>	KY857977	KY858046	KY858128	KY858198
<i>Trichomycterus septemradiatus</i>	MK123700	MK123729	MK123755	MW196781
<i>Trichomycterus pauciradiatus</i>	MT941796	MT941833	MW196769	MW196782
<i>Trichomycterus piratymbara</i>	KY857970	KY858040	KY858121	KY858194
<i>Trichomycterus reinhardti</i>	MK123698	MK123727	MF431106	MF431119
<i>Trichomycterus anaisae</i>	MT941782	MT941820	————	————
<i>Trichomycterus sainthilairei</i>	MT941815	MT941853	————	————

Table A1. Cont.

Species	COI	CYTB	MYH6	RAG2
<i>Trichomycterus luetkeni</i>	MT941793	MT941831	KY858148	KY858214
<i>Trichomycterus ingaiensis</i>	MT941790	MT941829	————	————
<i>Trichomycterus funebris</i>	MT941785	MT941823	KY858121	KY858194
<i>Trichomycterus humboldti</i>	MT941787	MT941824	————	————
<i>Trichomycterus giganteus</i>	MK123693	MK123720	MK123746	MT446426
<i>Trichomycterus nigricans</i>	MN813005	MK123723	MK123750	MK123765
<i>Trichomycterus caipora</i>	MN813008	MN813000	MN812996	MN812992
<i>Trichomycterus santaeritae</i>	MN813009	MN813001	MN812997	MN812993
<i>Trichomycterus immaculatus</i>	MK123694	MK144348	MK123747	MF431120
<i>Trichomycterus nigroauratus</i>	MK123696	MK123724	MK123751	MK123766
<i>Trichomycterus maculosus</i>	MN813010	MN813002	MN812998	MN812994
<i>Trichomycterus quintus</i>	MT299917	MN812999	MT305242	————
<i>Trichomycterus mimonha</i>	MW196749	MW196758	MW196770	MW196783
<i>Trichomycterus fuliginosus</i>	MW196750	MW196759	MW196771	MW196784
<i>Trichomycterus brunoi</i>	MW196751	MW196760	MW196772	MW196785
<i>Trichomycterus mirissumba</i>	MW196752	MW196761	MW196773	MW196786
<i>Trichomycterus pirabitira</i>	KY857982	KY858051	KY858133	————
<i>Trichomycterus macrotrichopterus</i>	MW196753	MW196762	MW196774	MW196787
<i>Trichomycterus brasiliensis</i>	MK123691	MK123717	MK123744	MK123763
<i>Trichomycterus candidus</i>	MK123692	MK123718	MK123745	MW196788
<i>Trichomycterus potschi</i>	————	MW196763	MW196775	MW196789
<i>Trichomycterus claudiae</i>	MW196754	MW196764	MW196776	MW196790
<i>Trichomycterus novalimensis</i>	MW196755	MW196765	MW196777	MW196791
<i>Trichomycterus rubiginosus</i>	MK123699	MK123728	MK123754	MK123767
<i>Trichomycterus albinotatus</i>	MN813007	MK123716	MK123743	MN812990
<i>Trichomycterus vitalbrazili</i>	MT435137	MK748279	MK748280	MT446428
<i>Trichomycterus travassosi</i>	MK123701	MK123730	MK123757	————
<i>Trichomycterus caudofasciatus</i>	MN812995	MK123719	MK123719	MK123764
<i>Trichomycterus jacupiranga</i>	MK123702	MK123731	MK123757	————
<i>Trichomycterus pantherinus</i>	MK123697	MK123725	MK123752	MN812989
<i>Trichomycterus auroguttatus</i>	MT435135	MT436452	MT436450	————
<i>Trichomycterus alternatus</i>	MK123690	MK123715	MK123742	MN812991
<i>Trichomycterus melanopygus</i>	KY857976	KY858045	KY858127	KY858197
<i>Trichomycterus pradensis</i>	MN813003	MK123726	MK123753	MN812988
<i>Trichomycterus tete</i>	————	MH620991	————	————
<i>Trichomycterus mimosensis</i>	————	MH621150	————	————
<i>Trichomycterus longibarbatu</i>	————	MH621258	————	————

Appendix C

List of morphological characters.

Jaw suspensorium

1. Autopalatine, dorsal surface, lateral portion, long longitudinal ridge: (0) absent; (1) present.
2. Autopalatine, dorsal surface, latero-middle portion, short longitudinal ridge: (0) absent; (1) present.
3. Autopalatine, postero-lateral process, development: (0) well-developed; (1) rudimentary.
4. Autopalatine, postero-lateral process, direction: (0) postero-lateral; (1) posterior; (2) lateral.
5. Autopalatine, postero-lateral process, length relative to autopalatine longitudinal length excluding postero-lateral process: (0) distinctively shorter; (1) nearly equal or slightly longer.
6. Autopalatine in specimens above 45 mm SL, medial margin, shape: (0) concave, sometimes nearly straight; (1) convex; (2) with deep concavity, posteriorly followed by broad convexity.
7. Autopalatine, articular facet for lateral ethmoid, ventral flap, shape: (0) small or rudimentary; (1) long.
8. Autopalatine, dorsal surface, articular facet for the lateral ethmoid, prominent ridge terminating in process connected to sesamoid supraorbital: (0) absent; (1) present.
9. Autopalatine, dorsal surface, articular facet for the lateral ethmoid, robust comma-shaped support: (0) absent; (1) present.
10. Metapterygoid, posterior portion, expansion: (0) absent; (1) present.
11. Metapterygoid, postero-ventral margin, deep concavity accommodating dorsal expansion of the antero-dorsal process of the quadrate: (0) absent; (1) present.
12. Hyomandibula, anterior-outgrowth, shape: (0) deep; (1) shallow.
13. Hyomandibula, anterior-outgrowth, dorsal margin, shape: (0) slightly curved, sometimes with weak dorsal concavity; (1) nearly straight and horizontal, with deep U-shaped concavity; (2) step anterior and posterior portions converging to pronounced V-shaped concavity reaching central outgrowth; (3) deep concavity reaching ventral outgrowth.
14. Opercle, anterior odontodes, arrangement: (0) transversal; (1) diagonal.
15. Opercle and interopercle, largest and internal-most odontodes, distal portion, shape: (0) straight to slightly curved; (1) strongly curved, its tip forming an angle of about 90° with odontode main axis.
16. Interopercle, anterior portion, distinctive anterior expansion: (0) absent; (1) present.
17. Interopercle, dorsal process, position relative to anterior margin of odontode patch: (0) distant; (1) close.
18. Interopercle, dorsal process, anterior margin, shape: (0) slightly concave; (1) prominent V-shaped notch.
19. Preopercle, ventral flap, extent relative to ventral bone limit: (0) slightly extending beyond it; (1) expanding ventrally beyond it.
20. Maxilla, largest length relative premaxilla largest length: (0) shorter; (1) longer.
21. Maxilla, shape: (0) slightly curved; (1) somewhat folded; (2) nearly straight.
22. Maxilla, postero-middle process, shape: (0) moderate, slightly projecting beyond bone profile; (1) large, forming pronounced expansion.
23. Antorbital, association with anterior segment of the latero-sensory infraorbital canal: (0) associated; (1) separated.
24. Sesamoid supraorbital, distinctive robust lateral process: (0) absent; (1) present.
25. Mesethmoid, lateral margin, ventral fold: (0) absent; (1) present.
26. Lateral ethmoid, articular facet for the autopalatine, folded laminar expansion: (0) absent; (1) present.
27. Lateral ethmoid, antero-dorsal extremity, direction: (0) anterior; (1) lateral; (2) medial.

28. Lateral ethmoid, medial margin, proximity relative to its paired homologous: (0) in contact; (1) separate.
29. Lateral ethmoid, lateral margin, area close to sesamoid supraorbital, lateral projection: (0) absent; (1) present.
30. Lateral ethmoid, lateral margin, prominent trapezoidal process: (0) absent; (1) present.
31. Lateral ethmoid, lateral margin, widening to form distinct wall limiting fossa aperture: (0) absent; (1) present.
32. Sphenotic-prootic-pterosphenoid complex, lateral process, extent relative to main neurocranium body limit: (0) short, slightly extending beyond it; (1) long, strongly projecting beyond it.
33. Pterotic, lateral process, extremity, shape and extent relative to limit of lateral neighbouring bones: (0) truncate, not projecting beyond; (1) rounded, projecting beyond.
34. Epioccipital, posterior margin, sharp process posteriorly directed: (0) absent; (1) present.
35. Frontal, anterior cranial fontanel, shape: (0) well-developed orifice; (1) minute aperture.
36. First free vertebra, parapophysis, branching: (0) unbranched; (1) branched.
37. Second free vertebra, parapophysis, direction: (0) lateral; (1) posterolateral.
38. Second free vertebra, parapophysis, general shape: (0) robust; (1) slender.
39. Second free vertebra, parapophysis, antero-distal portion, expansion: (0) absent; (1) present.
40. Second free vertebra, parapophysis, posterior margin, small notch: (0) absent; (1) present.
41. Second free vertebra, parapophysis, postero-distal portion, expansion: (0) absent; (1) present.
42. Third free vertebra, parapophysis, direction: (0) postero-lateral; (1) posterior.
43. Third free vertebra, parapophysis, shape: (0) nearly straight; (1) posteriorly curved.
44. Weberian capsule, shape: (0) pear-like, abruptly narrowing laterally; (1) slender, subtly narrowing laterally.
45. Scapulo-coracoid, supra-lateral process, shape: (0) needle-shaped; (1) tree-shaped.
46. Posttemporo-supracleithrum, size and space around it: (0) large, occupying most space among neighbouring bones; (1) small, surrounded by large space separating from neighbouring bones.
47. Pelvic bone, development: (0) well-developed; (1) poorly-developed.
48. Pelvic bone, general shape: (0) compact; (1) elongated.
49. Pelvic bone, posterior process, development: (0) well-developed; (1) rudimentary. Remarks: taxa with poorly developed pelvic bone or lacking a pelvic bone were coded as (?).
50. Pelvic bone, anterior processes, basal region: (0) broad; (1) narrow. Remarks: taxa lacking a pelvic bone were coded as (?).
51. Pelvic bone, anterior processes, number: (0) two; (1) three. Remarks: taxa lacking a pelvic bone were coded as (?).
52. Pelvic bone, postero-medial portion, small process close to cartilage supporting fin rays: (0) absent; (1) present.

Appendix D

Table A2. Matrix of distribution of character states among taxa.

Taxon	Characters 1–52
<i>Nematogenys inermis</i>	00???0?0?0?0?????0?0?00?00000???0?0?00000000?000000
<i>Copionodon pecten</i>	00???0?0?0?0????00?0?00?00000???000000000000?000000
<i>Trichogenes longipinnis</i>	00???0?0?0?0????00?0?00?00000???000000000000?00000?
<i>Microcambeva ribeirae</i>	000000?00????0?0?0100000000000000?0100000000?000000
<i>Listrura tetaradiata</i>	001??0?00????0?0?0100000000000000?0100000000???????
<i>Eremophilus mutisii</i>	000000000000000000000000000000001?0000000000000000???????
<i>Trichomycterus punctulatus</i>	00000000000000000000000000000000?000000000000000000
<i>Bullockia maldonadoi</i>	00001000000000000001100?00000000000000000000000000000
<i>Trichomycterus areolatus</i>	000010000000000000010000000000000000000000000000000000
<i>Ituglanis boitata</i>	00000000000000000000000000000000?00000001000000000
<i>Scleronema minutum</i>	000000000000000100100?0000000000011000010000000000
<i>Scleronema cf guapa</i>	000000000000000100100?0000000000011000010000000000
<i>Scleronema cf ibirapuita</i>	000000000000000100100?0000000000011000010000000000
<i>Scleronema macanuda</i>	000000000000000100100?0000000000011000010000000000
<i>Cambeva variegata</i>	000000000000000100000?0000000000011000010000000000
<i>Cambeva brachychenos</i>	000000000000000100000?0000000000011000010000000000
<i>Cambeva diatropoporos</i>	001?0000000000010000000000000000011000010000000000
<i>Cambeva cubataonis</i>	000000000000000100000?000000000?0011000010000000000
<i>Cambeva davisi</i>	000000000000000100000?000000000?0011000010000000000
<i>Cambeva castroi</i>	000000000000000100000?000000000?0011000010000000000
<i>Cambeva zonata</i>	000000000000000100000?000000000?0011000010000000000
<i>Trichomycterus giganteus</i>	0101000100003010000000000000010000001000000110000010
<i>Trichomycterus itatiayae</i>	001?00000010100010000?00020000000001100000000000001
<i>Trichomycterus septemradiatus</i>	00000010000000001000001000000000000110000000010?000
<i>Trichomycterus pauciradiatus</i>	00000010000000001000001000000000000110000000010?000
<i>Trichomycterus piratymbara</i>	00000010000000001000001000000000000110000000010?000
<i>Trichomycterus reinhardti</i>	00000010000000001000001000000000000110000000010?000
<i>Trichomycterus anaisae</i>	00000010000000001000001000000000000110000000010?000
<i>Trichomycterus funebris</i>	00000010000000001000001000000000000110000000010?000
<i>Trichomycterus humboldti</i>	00000010000000001000001000000000000110000000010?000
<i>Trichomycterus luetkeni</i>	00000010000000001000001000000000000110000000010?000
<i>Trichomycterus ingaiensis</i>	00000010000000001000001000000000000110000000010?000
<i>Trichomycterus saintthilairiei</i>	00000010000000001000001000000000000110000000010?000
<i>Trichomycterus nigricans</i>	0000000001000000000101000010000100001010001000000000
<i>Trichomycterus caipora</i>	0000000001000000000101000010000100001010001000000000
<i>Trichomycterus santaeritae</i>	0000000001000000000101000010000100001000001000000000
<i>Trichomycterus immaculatus</i>	0000000001000000000001000010000100001010001000000000
<i>Trichomycterus nigrauratus</i>	0000000001000000000000000110000100001010001000010000
<i>Trichomycterus maculosus</i>	0000000001000000000000000110000100001010001000010000
<i>Trichomycterus quintus</i>	0000000001000000000000000110000100001010000000010000
<i>Trichomycterus mimonha</i>	10000000000001000000000001001100010001001000001001100

Table A2. Cont.

Taxon	Characters 1–52
<i>Trichomycterus fuliginosus</i>	10000000000001000000000001001100010001001000001001100
<i>Trichomycterus brunoi</i>	10000000000001000000000001001100010001001000001001100
<i>Trichomycterus mirissumba</i>	10000000000001000000000001001100010001001000001001100
<i>Trichomycterus pirabitiba</i>	10000000000001000000000001001100010001001000001001100
<i>Trichomycterus macrotrichopterus</i>	10000000000001000000000001001100010001001000001001100
<i>Trichomycterus brasiliensis</i>	10000000000001000000000001001100010001001000001001100
<i>Trichomycterus candidus</i>	1000000000000100000000000100110001000100100000100????
<i>Trichomycterus potschi</i>	10000000000001000000000001001100010001001000001001100
<i>Trichomycterus claudiae</i>	10000000000001000000000001001100010001001000001001100
<i>Trichomycterus novalimensis</i>	10000000000001000000000001001100010001001000001001100
<i>Trichomycterus rubiginosus</i>	10000000000001000000000001001100010001001000001001100
<i>Trichomycterus albinotatus</i>	1000010000002100000000001001100001001001000000001000
<i>Trichomycterus vitalbrazili</i>	1000010000002100000000001001100001001001000000001000
<i>Trichomycterus mimosensis</i>	000000000000000000010000000001000101000000000000000
<i>Trichomycterus travassosi</i>	000000000000000000010000000001000101000000000000000
<i>Trichomycterus caudofasciatus</i>	000000000000000000010000000001000101000000000000000
<i>Trichomycterus cf jacupiranga</i>	000000000000000000010001000000100010100000000000000
<i>Trichomycterus pantherinus</i>	000000000000000000010001000000100010100000000000000
<i>Trichomycterus longibarbatulus</i>	0002000000000000001012000000000100000100000000000000
<i>Trichomycterus auroguttatus</i>	000012001000000001011000000000100010100010000000000
<i>Trichomycterus alternatus</i>	0000100000000000001011000000000100010100010000000000
<i>Trichomycterus melanopygus</i>	00000000000100000011000000000000000100000000000000
<i>Trichomycterus pradensis</i>	00000000000100010011000000000000000100000000000000
<i>Trichomycterus tete</i>	00000000000100000011000000000000000100000000000000

Appendix E

Table A3. Best-fitting models.

Partition	Base Pairs	Evolutionary Model
COI 1 st	174	TNe+G4
COI 2 nd	174	F81+F+I+G4
COI 3 rd	174	TIM+F+G4
CYTB 1 st	363	K2P+I+G4
CYTB 2 nd	363	TN+F+I
CYTB 3 rd	362	TIM2+F+I+G4
MYH6 1 st	181	JC+I+G4
MYH6 2 nd	181	HKY+F+I+G4
MYH6 3 rd	181	K2P+G4
RAG2 1 st	274	K3P+I
RAG2 2 nd	274	TVMe+G4
RAG2 3 rd	273	K2P+G4
Osteology	52	JC+I+G4

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