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Article

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# A new species of cave catfish from Brazil, *Trichomycterus rubbioli* sp.n., from Serra do Ramalho karstic area, São Francisco River basin, Bahia State (Silurifomes: Trichomycteridae)

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Abstract

*Trichomycterus rubbioli*, herein described, is a new species from the limestone caves of the Água Clara system from the Serra do Ramalho karstic area, southwest of Bahia State, north-eastern Brazil. The new species is diagnosed by the combination of the following non-exclusive characters: highly reduced skin pigmentation; reduced eyes, externally visible as small black or reddish-brown spots; long barbels, especially the nasal (86.9–127.1% of head length) and maxillary (98.1–129.3% of head length); and pectoral-fin ray count reaching I, 9. The troglobitic status is suggested by the presence of troglomorphisms on an advanced degree, especially the reduction of skin pigmentation and the regression of the eyes, besides the absence of individuals in superficial drainages of the region. Population densities, recorded on two occasions, were extremely low. *Trichomycterus rubbioli* occurs in water bodies formed by infiltration in the rock, and, because of this, it must be considered fragile and as a priority in conservation programs of Brazilian karstic areas.

Key words: New cavefish, troglobite, subterranean ichthyofauna, north-eastern Brazil, taxonomy

#### Introduction

Brazil has one of the most remarkable subterranean ichthyofaunas in the world, comparable to just a few other countries or geographically comparable karst areas, such as Mexico, China and southeastern Asia (Bichuette & Trajano, 2008; Trajano *et al.*, 2009; Trajano & Bichuette, 2010). Brazilian cavefishes have worldwide relevance not only in terms of species richness, but also in view of the high diversity of their ecological and evolutionary patterns (Trajano & Bichuette, 2010). Up to now, 25 troglobitic species (exclusively subterranean, usually troglomorphic, i.e., with reduction of eyes and pigmentation, among other characters) have been reported; many still await formal description (Trajano & Bichuette, 2010). Most are siluriforms (Callichthyidae, Loricariidae, Heptapteridae and Trichomycteridae), and trichomycterids are so far the richest family, with 12 reportedly troglobitic species (Trajano & Bichuette, 2010; Rizzato *et al.*, 2011).

The Trichomycteridae, widely distributed in freshwaters from Central and South America, include more than 250 valid species (Nelson, 2006; Castellanos-Morales, 2008; Datovo & Bockmann, 2010, Eschmeyer & Fong, 2012). Their monophyly is well corroborated and their most conspicuous synapomorphies are based on its highly specialized opercular-interopercular apparatus (de Pinna, 1998; Datovo & Bockmann, 2010). Trichomycterid catfishes are among the most successful colonizers in subterranean habitats (Castellanos-Morales, 2008), including 15 nominal troglobitic species in four genera, *Silvinichthys* (Argentina), *Glaphyropoma* (Brazil), *Ituglanis* (Brazil), and *Trichomycterus* (Proudlove, 2010), besides several troglophilic populations (populations of species that are able to live and complete their life cycle both on the surface and in subterranean habitats) throughout Brazil (Mattox *et al.*, 2008; E. Trajano & M.E. Bichuette, unpubl. data).

With more than 150 species already described and many others waiting for description, *Trichomycterus* is the most speciose trichomycterid genus (Alencar & Costa, 2004; Wosiacki, 2005; Wosiacki & de Pinna, 2008; Fernández & Vari, 2009), showing a great potential to colonize extreme habitats, including subterranean habitats (Fernández &

Miranda, 2007). In South America, eight hypogean species have been described: *T. chaberti* Durand, from Bolivia, *T. spelaeus* DoNascimiento, Villarreal & Provenzano, from Venezuela, *T. sandovali* Ardila-Rodriguez, *T. santanderensis* Castellanos-Morales, *T. uisae* Castellanos-Morales and *T. sketi*, from Colombia, *T. itacarambiensis* Trajano & de Pinna, from eastern Brazil and *T. dali* Rizzato, Costa-Jr, Trajano & Bichuette, from southwestern Brazil.

We describe herein a new subterranean, troglomorphic *Trichomycterus* species from peripherical habitats in the Água Clara ("clear water") cave system, where these catfishes are isolated (therefore a troglobite).

#### Study area

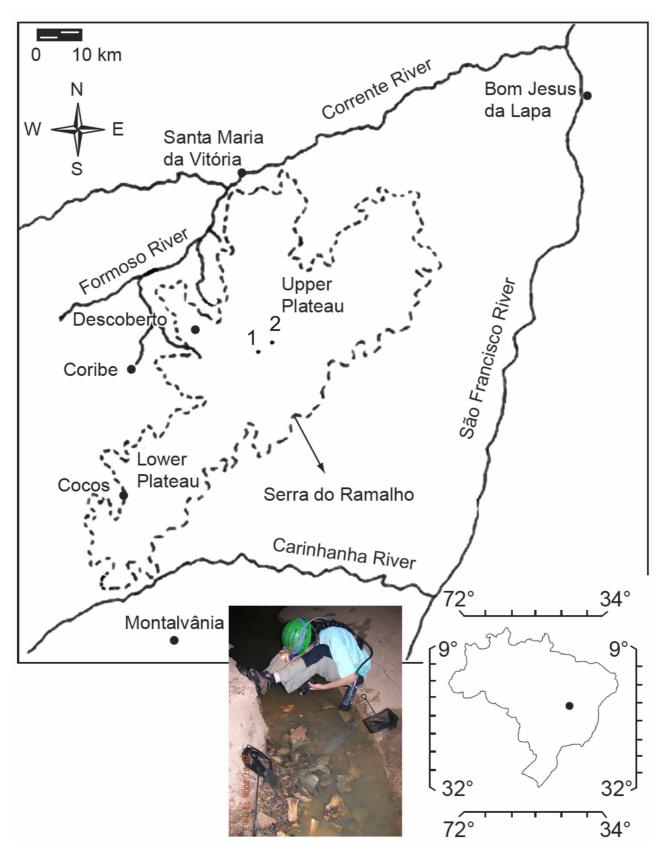
The Serra do Ramalho karstic area, in the southwestern State of Bahia (BA), is a part of the middle São Francisco River basin (Fig. 1), with several important caves reaching more than 30 km in extension. According to the Köeppen (1948) classification, the climate is defined as tropical dry (= semiarid), "Aw" type, characterized by a dry winter (March to October) and an annual precipitation around 640 mm (Instituto Nacional de Meteorologia/INMET). The native regional vegetation consists of Caatinga (mesophytic and xeromorphic forests), interspersed with Cerrado (savannah-like). Serra do Ramalho is dominated by a plateau formed by carbonatic (limestone) rocks of the Bambuí Group (Auler *et al.*, 2001). This plateau extends for kilometers and forms large cave systems in the region, distributed in two sections, the lower plateau, to the south, and the upper plateau, to the north. The caves where *T. rubbioli* is recorded, Gruna da Água Clara (13°48'2.83" S 43°57'4.76" W) and Lapa dos Peixes (13°49'21.78" S 43°57'24.39" W), are located on the lower plateau.

The Gruna da Água Clara cave with 13,880 m of mapped passageways, is one of the largest in the lower plateau and one of the longest Brazilian caves (Auler *et al.*, 2001). At the base level, the Água Clara cave system is formed by a relatively large, yet temporary stream, joining the São Francisco River during the peak of the rainy seasons. After leaving the Gruna da Água Clara, upstream, the stream crosses about 4 km in open air (dry during most of the year) before entering the lower conduit of the Lapa dos Peixes, downstream. During heavy rains, fishes from the São Francisco may go upstream to the Água Clara system, becoming trapped into the caves when the water level goes down.

The new species, however, seems to be restricted to upper levels, vadose habitats, such as pools in the Gruna da Água Clara and a small tributary stream in Lapa dos Peixes (Fig. 1), fed by infiltration water. During the peak of the dry season, the Lapa dos Peixes upper stream may be interrupted and reduced to some shallow pools connected by very small watercourses. Therefore, any connection between these populations must occur through subterranean spaces that are inaccessible.

#### **Material and Methods**

The studied specimens were hand-netted in a small stream and in travertine pools at permanently dark zones of the two caves, anesthetized in benzocaine solution until death, preserved in formalin and then transferred to alcohol 70%. All measurements were straight-line, taken on the left side of specimens under stereomicroscope with digital calipers, and expressed to the nearest 0.1 mm. Measurements follow Tchernavin (1944) and de Pinna (1992). One type specimen was cleared and double-stained (C&S) for bone and cartilage by the method of Taylor & Van Dyke (1985). Fin-ray counts were taken on both C&S and non-C&S specimens; with aid of transmitted illumination. Vertebrae, pleural ribs, odontodes, pterygiophores, branchiostegal rays and tooth counts were taken on C&S specimen. Vertebral counts include free vertebrae (those fused in the Weberian complex were not included) plus the compound caudal centrum (PU1 + U1, considered as a single element), as is usual in Trichomycterus species descriptions. Ray counts and abbreviations follow Bockmann et al. (2004): anterior unbranched and unsegmented rays were represented by lower case Roman numerals, unbranched and segmented rays by upper case Roman numerals, and branched and segmented rays by Arabic numerals. Osteological terminology follows de Pinna (1989) and Datovo & Bockmann (2010). Myological terminology follows Datovo & Bockmann (2010) and sensory canal pore terminology follows Bockmann et al. (2004). Anatomical illustrations were sketched using stereomicroscope with camera lucida attachment, and further finalized with observation on C&S specimens. In the drawings, bone is represented by stipple and cartilage by open circles. Abbreviations: MZUSP, Museu de Zoologia da Universidade de São Paulo; SL, standard length; HL, head length; C&S, cleared and stained.



**FIGURE 1.** Map of southwestern Bahia state showing the localities where the collection was made (small numbered dots), main counties (large dots), rivers (continuous lines) and the limits of the Serra do Ramalho karst area (traced line). 1, Lapa dos Peixes cave; 2, Gruna da Água Clara cave. Photograph shows the details of *Trichomycterus rubbioli* habitat in Lapa dos Peixes cave.

# Trichomycterus rubbioli sp.n.

(Fig. 2)

*Trichomycterus* sp. 3—Trajano (2003). *Trichomycterus* undescribed species—Trajano *et al.* (2009). *Trichomycterus* undescribed species 2—Proudlove (2010). *Trichomycterus* sp. 2—Trajano & Bichuette (2004); Mattox *et al.* (2008); Trajano & Bichuette, (2010); Trajano *et al.* (2011).

**Holotype**. MZUSP (110977), 60.3 mm SL, Brazil, south-western State of Bahia, Carinhanha County, Lapa dos Peixes cave (13° 49' 21,78"S 43° 57' 24,39"W), Middle São Francisco River basin, Serra do Ramalho karstic area, 23 April 2000, M. E. Bichuette, E. Trajano & D. Möeller.



**FIGURE 2.** *Trichomycterus rubbioli*, holotype (MZUSP 110977), 60.3 mm SL. Brazil, Bahia State, Serra do Ramalho karstic area, Middle São Francisco river basin, Carinhanha County, Lapa dos Peixes cave. Dorsal view (above) and left lateral view (below).

**Paratypes.** All from Brazil, southwestern State of Bahia, Carinhanha County, Middle São Francisco River basin, Serra do Ramalho karstic area. MZUSP (110978), 1, 56.8 mm SL, Lapa dos Peixes cave, 23 April 2000, M. E. Bichuette, E. Trajano e D. Möeller; MZUSP (110979), 1, 64.1 mm SL, Lapa dos Peixes cave, 23 April 2000, M. E. Bichuette, E. Trajano & D. Möeller; MZUSP (110980), 1, 32.9 mm SL (C&S), Lapa dos Peixes cave, 23 April 2000, M. E. Bichuette, E. Trajano & D. Möeller; MZUSP (110981), 1, 29.7 mm SL, Gruna da Água Clara cave (13° 48' 2.83" S 43° 57' 4.76" W), 11 July 1999, L. Senna-Horta; MZUSP (110982), 1, 29.9 mm SL, Gruna da Água Clara cave, 11 July 1999, L. Senna-Horta; MZUSP (110983), 1, 58.6 mm SL, Gruna da Água Clara cave, 11 July 1999, L. Senna-Horta; MZUSP (110983), 1, 58.6 mm SL, Gruna da Água Clara cave, 11 July 1999, L. Senna-Horta; MZUSP (110983), 1, 58.6 mm SL, Gruna da Água Clara cave, 11 July 1999, L. Senna-Horta; MZUSP (110983), 1, 58.6 mm SL, Gruna da Água Clara cave, 11 July 1999, L. Senna-Horta; MZUSP (110983), 1, 58.6 mm SL, Gruna da Água Clara cave, 11 July 1999, L. Senna-Horta; MZUSP (110986), 1, 21.2 mm SL, Gruna da Água Clara cave, 22 April 2000, M. E. Bichuette & E. Trajano; MZUSP (110986), 1, 16.9 mm SL, Gruna da Água Clara cave, 22 April 2000, M. E. Bichuette & E. Trajano; MZUSP (110986), 1, 16.9 mm SL, Gruna da Água Clara cave, 22 April 2000, M. E. Bichuette & E. Trajano; MZUSP (110986), 1, 16.9 mm SL, Gruna da Água Clara cave, 22 April 2000, M. E. Bichuette & E. Trajano; MZUSP (110986), 1, 16.9 mm SL, Gruna da Água Clara cave, 22 April 2000, M. E. Bichuette & E. Trajano; MZUSP (110986), 1, 16.9 mm SL, Gruna da Água Clara cave, 22 April 2000, M. E. Bichuette & E. Trajano.

**Diagnosis.** *Trichomycterus rubbioli* is readily distinguished from epigean and most hypogean congeners by three distinctive, non-exclusive characters: highly reduced skin pigmentation (except for *T. gorgona* and cave-restricted congeners, *T. chaberti, T. itacarambiensis, T. spelaeus, T. sandovali, T. santanderensis, T. uisae* and *T. dali*) (Fig. 2); reduced eyes, visible externally as small black or reddish-brown spots (except for *T. gorgona* and the hypogean *T. chaberti, T. itacarambiensis, T. santanderensis, T. uisae* and *T. sketi*) (Figs. 2 and 3); and barbels long, especially nasal (86.9–127.1% of HL) and maxillary (98.1–129.3% of HL) (except for *T. longibarbatus* and the hypogean *T. spelaeus, T. santanderensis, T. uisae, T. sketi* and *T. dali*) (Figs. 2 and 3). The characters above, in combination, differentiate new species from all hypogean congeners except for *T. santanderensis* and *T. uisae*. *Trichomycterus rubbioli* can be further diagnosed from almost all congeners, including *T. santanderensis* and *T. uisae* (except for *T. hualco, T. sketi* and *T. dali*) by pectoral-fin ray count reaching I, 9.

**Description.** Morphometric data of holotype and paratypes are given in Table 1.

	Holotype	Range (n=8)	Mean	SD	
Standard length	60.3	14.4–64.1	40.4	19.4	
Percents of standard length					
Total length	119.3	112.3-121.0	117.0	2.7	
Predorsal length	60.9	57.3-60.9	59.3	1.2	
Preanal length	76.7	70.5-78.2	74.3	2.6	
Prepelvic length	65.1	59.3-65.3	62.2	2.3	
Caudal peduncle length	26.2	14.3–26.3	21.7	4.1	
Caudal peduncle depth	16.3	8.1–16.3	13.9	2.8	
Dorsal-fin base length	10.8	8.8-15.0	11.3	1.7	
Anal-fin base length	10.7	7.8–11.4	9.3	1.1	
Pectoral-fin base length	5.1	4.9–6.4	5.5	0.4	
Pelvic-fin base length	3.2	2.5-3.2	2.7	0.2	
Dorsal-fin length	17.4	15.1-22.5	18.3	2.4	
Anal-fin length	14.6	14.2–19.6	16.0	2.1	
Pectoral-fin length	23.3	19.1–23.6	22.4	1.4	
Pelvic-fin length	10.2	8.7–12.4	10.7	1.2	
Head length	22.2	19.8–23.2	21.8	1.3	
Body depth	12.7	11.4–13.3	12.4	0.6	
Body width	19.4	18.1–22.4	19.9	1.4	
Percents of Head length					
Head depth	87.2	83.2–98.3	91.8	5.0	
Head width	57.5	50.4-63.8	57.2	4.5	
Interorbital width	34.1	27.7-34.9	32.2	2.3	
Eye diameter	7.3	4.0–7.3	6.1	1.1	
Snout length	15.4	13.9–24.0	19.1	4.2	
Maxillary barbel length	129.3	98.1–129.3	109.9	9.7	
Rictal barbel length	77.1	50.5-77.1	60.1	9.5	
Nasal barbell length	86.9	86.9–127.1	103.3	15.4	
Mouth width	70.7	28.5-70.7	49.1	16.3	

**TABLE 1.** Morphometric and meristic data of holotype and the eight paratypes of *T. rubbioli* from the Serra do Ramalho karstic area, Bahia State. SD, standard deviation.

Body elongate semi-cylindrical, becoming compressed towards the caudal fin (Fig. 2). Dorsal profile of the body straight, very slim, with a straight slope from the tip of the snout to the anterior portion of trunk; ventral profile of body straight in lateral view. Dorsal profile of caudal peduncle slightly convex to beginning of caudal fin; ventral portion of caudal peduncle straight. Caudal fin spatulate, with distal margin straight. Dorsal-fin origin on posterior half of body, slightly anterior to origin of pelvic fin. Anal-fin origin posterior to distal margin of dorsal

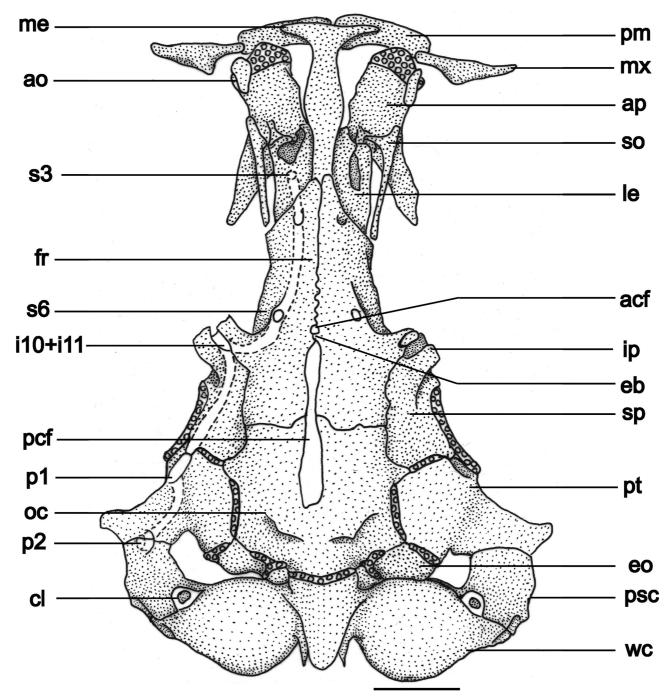
fin. Adipose cutaneous folds present in young and adult specimens along the dorsal profile of the body: anterior to dorsal fin (pre-dorsal) well developed in young, poorly developed to absent in adult specimens, not supported by procurrent rays; posterior to dorsal fin (post-dorsal) well developed in young and relatively reduced in adult specimens, slightly convex in lateral view, supported by procurrent rays only in the posterior end. Urogenital and anal openings on vertical through posterior end of dorsal-fin base, between distal margins of pelvic fins.



**FIGURE 3.** *Trichomycterus rubbioli*, holotype, (MZUSP 110977), 60.3 mm SL, 13.4 mm HL. Brazil, Bahia State, Serra do Ramalho karstic area, Middle São Francisco river basin, Carinhanha County, Lapa dos Peixes cave. Dorsal close-up view of the head.

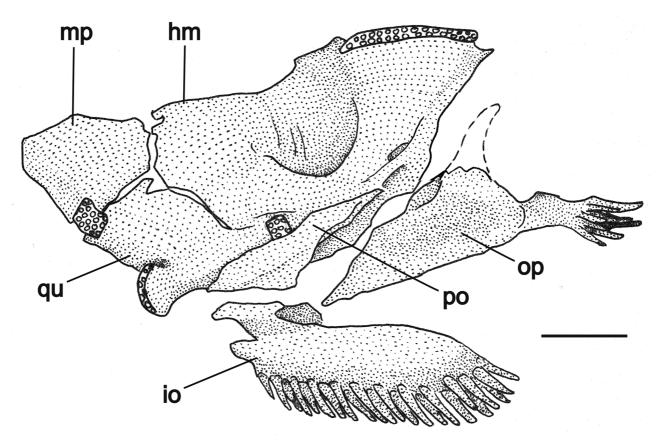
Head relatively wide and depressed, semicircular in adult and trapezoidal in young specimens in dorsal view (Fig. 3). Adductor mandibulae muscles well developed in adults, easily noticeable externally, dorsally delimited by a longitudinal facial groove. Very reduced eyes, visible externally as small black or reddish-brown dots. Eyes more superficial especially in some smaller individuals, covered with a thin layer of skin and with very regular forms (rounded); others, especially larger individuals, with deeper eyes (covered by a thick layer of skin), with irregular form. On at least one individual (MZUSP, 110983), right eye not visible externally; holotype with pale red eyes. Anterior nostril transversally ovoid and slightly smaller than posterior, surrounded laterally by nasal barbels. Posterior nostril rounded, surrounded anteriorly by large flap of integument. Mouth slightly subterminal, convex in dorsal view, rictus laterally directed. Barbels long, especially nasal and maxillary. Maxillary barbel, extending to middle of pectoral fin; nasal barbel extending to origin of pectoral fin, and submaxilary barbel extending to opercle. Opercular patch of odontodes very small and circular. Interopercular odontodes forming a slightly convex patch throughout ventral margin of interopercle. Opercle with 5–13 odontodes, interopercle with 35–38 odontodes.

General morphology of cranium. Cranial fontanels divided by an epiphyseal bar. Anterior fontanel small and rounded, almost at middle length of the frontal bones; posterior fontanel extending from the middle of parieto-supraoccipital to the posterior region of the frontal bones, with a conspicuous constriction at intersection of parieto-supraoccipital and frontal bones (Fig. 4). Parieto-supraoccipital with two posterodorsal processes as insertions to the epiaxialis and supracranialis anterior muscles. Lateral process of pterotic pointing diagonally outwards, in direction to opercular patch of odontodes. Anterior process of sphenotic and posterolateral process of frontal conspicuous, hollow horn-like structure pointing diagonally forward, inside of which emerge infraorbital sensory branches.



**FIGURE 4.** Neurocranium of *Trichomycterus rubbioli*, (MZUSP 110980), 32.9 mm SL, paratype. Dorsal view. Abbreviations: **acf**, anterior cranial fontanel; **ap**, autopalatine; **ao**, antorbital; **cl**, cleithrum; **eb**, epiphyseal bar; **eo**, epioccipital; **fr**, frontal; **i10+i11**, infraorbital sensory branches 10 and 11; **ip**, infraorbital process; **le**, lateral ethmoid; **me**, mesethmoid; **mx**, maxilla; **oc**, parieto-supraoccipital; **p1-2**, postotic sensory branches 1 and 2; **pcf**, posterior cranial fontanel; **pm**, pre-maxilla; **psc**, posttemporo-supracleithrum; **pt**, pterotic; **s3**, supraorbital sensory branch 3; **s6**, supraorbital sensory branch 6; **so**, sesamoid-supraorbital; **sp**, sphenotic-prootic-pterosphenoid complex bone; **wc**, weberian complex and capsule. Scale bar = 2 mm.

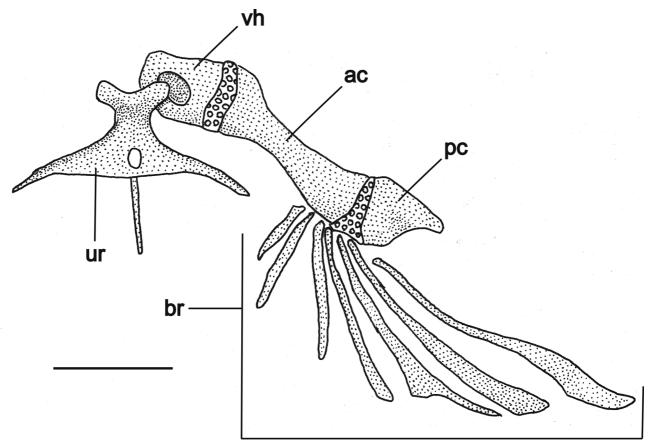
Sesamoid-supraorbital long and cylindrical, with internal, anterolateral projection, pointing to mesethmoid. This projection may be long and sharp or small and slightly convex. Autopalatine with straight anterior margin covered with cartilage, almost rectangular medial region and long posterior process that becomes sharp on distal region and totally covers the internal half of metapterygoid. Distal profile of mesethmoid straight in dorsal view, main body axis with enlarged medial region, cornua reaching half of premaxillary length. Vomer arrow-shaped with long posterior process wider at middle length. Parasphenoid almost circular at medial region, with long and narrow posterior process reaching Weberian complex and two anterior processes surrounding laterally the posterior process of vomer. Three to five irregular premaxillary rows of conic teeth curved backwards. Maxilla boomerang shaped, rounded proximal margin of maxilla joining premaxilla, anterior to antorbital. Lower jaw with conical teeth, curved backwards; three to four rows with larger teeth at middle and one row externally, close to upper process of dentary, with teeth gradually becoming smaller. Hyomandibular with a conspicuous semicircular depression, joined tightly to metapterygoid and quadrate (Fig. 5). Metapterygoid and quadrate united together by anterior block of cartilage. Opercle with dorsal process on which inserts levator operculi and dilatator operculi muscles, with reduced patch of odontodes. Preopercle with sharp posterior region, articulating to dorsal process of interopercle by straight ventral margin, not covered by cartilage. Interopercle long and wide, with dorsal, T-shaped process articulating to preopercle; anterior process without odontodes, bordered ventrally and posteriorly by long, sharp odontodes.



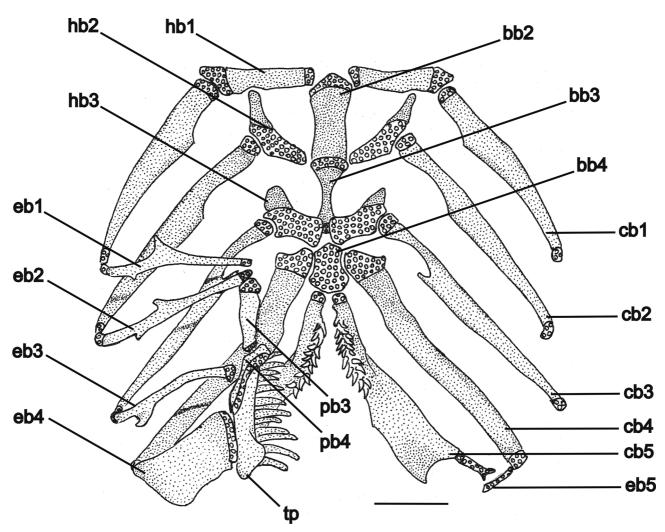
**FIGURE 5.** Left suspensorium and opercular series of *Trichomycterus rubbioli*, (MZUSP 110980), 32.9 mm SL, paratype. Lateral view. Abbreviations: **hm**, hyomandibular; **io**, interopercle; **mp**, metapterygoid; **op**, opercle; **po**, preopercle; **qu**, quadrate. Scale bar = 2 mm.

Branchial skeleton and associated structures. Branchiostegal-rays seven. Rays 5, 6 and 7 with enlarged distal extremity. Ray 7 covered by interopercle, reaching ventral margin of opercular patch of odontodes. Urohyal with long, very narrow dorsal process almost reaching cleithrum, broad convex posterior margin, urohyal-foramen slightly ovoid, anterior process T-shaped. Ventral hypohyal with depression on which articulates anterior process of urohyal (Fig. 6). Basibranchials 3, hypobranchials 3, ceratobranchials 5, epibranchials 5, pharyngobranchials 2 (Fig. 7). Basibranchial 1 absent. Basibranchial 2 and 3 connected to each other by their cartilaginous tips, forming long rod. Basibranchial 2 relatively wide with convex anterior margin. Basibranchial 3 with rectangular, rod-like

posterior half, divergently triangular on anterior half, with convex anterior margin. Posterior tip of basibranchial 3 covered by cartilaginous posterior portion of hypobranchials 3. Basibranchial 4 cartilaginous, approximately hexagonally shaped. Anterior margins of basibranchial 4 bordered by cartilaginous posterior portion of hypobranchials 3, lateral and posterior margins bordered by cartilaginous anterior tips of ceratobranchial 4 and ceratobranchial 5, respectively. Hypobranchial 1 with external portion wider than internal, with cartilaginous tips. Hypobranchial 2 boomerang shaped, posterior half cartilaginous, anterior half ossified, forming anterior process that almost reaches external posterior margin of hipobranchial 1. Hypobranchial 3 almost completely cartilaginous, only anterior tip ossified and triangular, closely 88joined to anterior cartilaginous tip of ceratobranchial 3. Hypobranchial 4 absent. Ceratobranchials slightly curved, with cartilaginous extremities. Ceratobranchial 1 with medial tip wider than distal. Ceratobranchial 2 with shallow concavity on its posterior margin, without defined posterior process. Ceratobranchial 3 with pronounced concavity on its posterior margin, limited posteriorly by very small process. Ceratobranchial 5 slightly enlarged, bearing patch of small, narrow conical teeth pointed dorsally on its anterior half, connected to epibranchial 5 only by upper half of posterior margin. Posterior margin of ceratobranchials 3, both margins of ceratobranchials 4 and anterior margin of ceratobranchials 5 bearing one row of conical, very sharp teeth (not shown in fig. 7). Epibranchials 1, 2 and 3 narrow, rod-like, with cartilaginous tips. Epibranchial 1 with long, narrow and sharp anterior process, pointed outwards in acute angle. Epibranchial 2 with two small, acute processes, not uncinate, at the middle of anterior margin and at the external portion of posterior margin. Epibranchial 3 with conspicuous, posteriorly directed, large uncinate process at external portion of its posterior margin. Epibranchial 4 large, curved, with a wide, straight dorsal margin joined to posterior half of tooth plate and covered with cartilage, and with ventral margin very narrow, ovoid and cartilaginous. Epibranchial 5 very small, curved and completely cartilaginous, joining the posterior cartilaginous tip of ceratobranchial 4 with the ventral margin of epibranchial 4. Pharyngobranchials 1 and 2 absent. Pharyngobranchial 3 elongate, rod-like, slightly depressed, with cartilaginous tips. Pharyngobranchial 4 cartilaginous, curved, joined tightly to dorsanterior half of tooth plate. Tooth plate well developed, curved, with two irregular rows of long, conic, internally curved teeth; internal row with thicker, robust teeth.

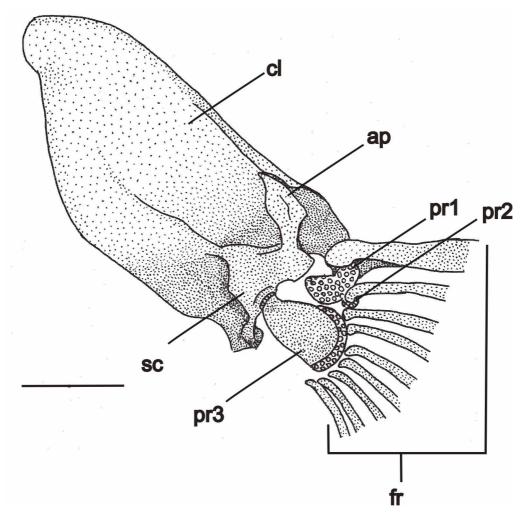


**FIGURE 6.** Left hyoid arch of *Trichomycterus rubbioli*, (MZUSP 110980), 32.9 mm SL, paratype. Ventral view. Abbreviations: **ac**, anterior ceratohyal; **br**, branchiostegal rays; **pc**, posterior ceratohyal; **ur**, urohyal; **vh**, ventral hypohyal. Scale bar = 2 mm.



**FIGURE 7.** Branchial skeleton of *Trichomycterus rubbioli*, (MZUSP 110980), 32.9 mm SL, paratype. Dorsal view. Abbreviations: **bb2–4**, basibranchials 2 to 4; **cb1–5**, ceratobranchials 1 to 5; **eb1–5**, epibranchials 1 to 5; **hb1–3**, hypobranchials 1 to 3; **pb3–4**, pharyngobranchial 3 and 4; **tp**, tooth plate. Scale bar = 2 mm.

Postcranial skeleton. Total vertebrae 31-32, 13-15 pairs of ribs. First rib thicker, posteriormost gradually thinner. Pectoral girdle with wide cleithrum, becoming narrower at anterior half, joined together by very narrow anterolateral margin. Posterior process of pectoral girdle reaching ventral surface of posttemporo-supracleithrum. Scapulocoracoid with conspicuous anterior process projected forward, with narrow base and wide apex reaching external margin of cleithrum. Proximal radial 1 cartilaginous, joined to first and second pectoral-fin rays. Proximal radial 2 cartilaginous, small. Proximal radial 3 large, well ossified, with semicircular cartilaginous posterior margin joined to third to tenth pectoral-fin rays, and small, ovoid anterior cartilaginous margin, joined to scapulocoracoid (Fig. 8). Pelvic girdle with external anterior process narrower than internal anterior process. Short medial process sometimes present, pointing forward (Fig. 9). Epural absent. Neural spine of compound caudal centrum reduced to half or one-fourth of uroneural length, sharp tip projected upward. Hemal spine (n = 2) of penultimate vertebrae divided in a narrow anterior hemal spine and a wide posterior hemal spine with cartilaginous tip bearing one procurrent ray. Parhypural fused to hypural 1+2 into trapezoidal lower hypural plate. Hypural 3 partially fused to hypural 4+5, into triangular upper hypural plate. Uroneural not fused to hypural 4+5, long, reaching distal margin of upper hypural plate, with characteristic shape: proximal half narrow and distal half expanding and sharpening again distally, similar to a scimitar blade (Fig. 10).



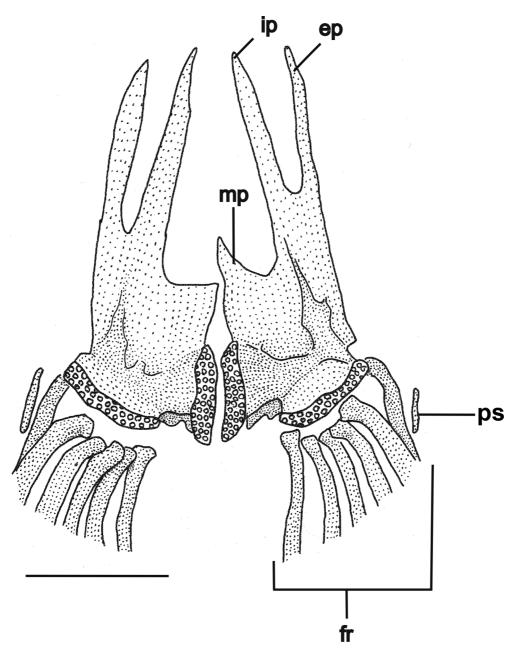
**FIGURE 8.** Left pectoral girdle of *Trichomycterus rubbioli*, (MZUSP 110980), 32.9 mm SL, paratype. Ventral view. Abbreviations: **ap**, anterior process; **cl**, cleithrum; **fr**, fin rays; **pr1–3**, proximal radials 1 to 3; **sc**, scapulocoracoid. Scale bar = 2 mm.

Pectoral fin triangular in dorsal view, with wide base and first ray long, thick, unbranched and filamentous. Pectoral-fin ray count I, 8 or I, 9, even at same individual. Dorsal fin approximately semicircular in lateral view, with nine rays, two first unbranched (II, 7); in three paratyes, including one C&S, I, 7; one or two micro rays anterior to first ray. Pterygiophores with seven or eight narrow basal radials in dorsal fin, slightly curved on basal portion, with spatulate distal region curved backwards and cartilaginous distal tip. First basal radial with narrow cartilaginous distal margin pointing backwards. Last basal radial with laminar posterior expansion on distal fold, joined to two last dorsal fin rays. Each basal radial, except for first and two last, joined to small distal radial, between two basal branches of each ray. First distal radial larger than posterior radials. Anal fin approximately rectangular in lateral view, distal margin rounded, seven rays (II, 5), two micro rays anterior to first ray. On two specimens, I, 6. Pterygiophores with six narrow basal radials on anal fin, morphologically similar to dorsal pterygiophores: spatulate, curved distal region, with cartilaginous tips, last with laminar posterior expansion articulating with the two last rays, each basal radial except first and two last joined to small distal radial ossified between two basal branches of each ray. Pelvic fin rectangular in ventral view, separated from each other, I, 4, one pelvic splint. Caudal fin with 14 principal rays (six in upper lobe, first unbranched; seven in lower lobe, first unbranched; seven in lower lobe, first unbranched), 8–14 dorsal procurrent rays; 5–11 ventral procurrent rays.

**Color in alcohol.** Body generally pale yellow, resembling faded irregular spots. Dorsal fold (anterior and posterior to dorsal fin) lighter than body. Mouth, dorsal region of the head between eyes, and barbels light yellow to white. Eyes, when visible, black or dark brown, with one individual with red eyes. Pectoral, dorsal, pelvic, anal and caudal fins translucent (Fig. 2).

Live color. Body unpigmented. Internal organs can be seen by transparence. When subject to illumination for several hours, as during chronobiological experiments (12:12 light (1000 lux): dark cycles, during seven days;

Trajano *et al.*, 2011), the fish became very dark, due to light-induced synthesis of melanin. After several weeks, specimens of *T. rubbioli* became pale again, and the progressive loss of melanin followed a pattern of large, irregular spots, different from the natural color pattern.



**FIGURE 9.** Pelvic girdle skeleton of *Trichomycterus rubbioli*, (MZUSP 110980), 32.9 mm SL, paratype. Ventral view. Abbreviations: **fr**, fin rays; **ip**, internal anterior process; **ep**, external anterior process; **mp**, medial process; **ps**, pelvic splint. Scale bar = 2 mm.

**Etymology.** The specific name (noun in apposition) is in honor of Ezio Rubbioli, speleologist, the first explorer of Serra do Ramalho caves, who brought this new species to our attention.

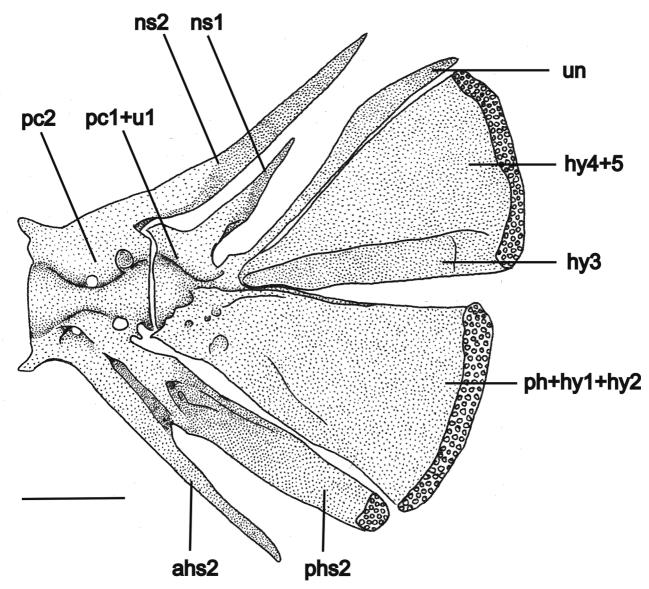
**Distribution.** *Trichomycterus rubbioli* is known exclusively from subterranean waters in at least two caves of the same cave system in the Serra do Ramalho karst area, middle São Francisco River basin: Lapa dos Peixes and Gruna da Água Clara (Fig. 1).

**Notes on habitat and ecology.** The small vadose stream inside Lapa dos Peixes where *T. rubbioli* catfishes were found is about 180 m long (accessible part), with an average width of 0.3 m and maximum depths of 0.5 m. The fishes were concentrated in places with the bottom formed by clay and small blocks of limestone, usually hiding under these blocks and inside crevices in the walls, thus showing a strong cryptobiotic habit. The level of

water in this stream is lower in the dry season, and on two occasions the locality was mostly dry. Non-troglobitic fish, such as tetra characins, *Astyanax* sp., and heptapterids, *Rhamdia* sp. and *Pimelodella* sp., were observed in the locality (Trajano *et al.*, 2009). In April 2000 and September 2003, respectively, 15 and 17 specimens of *T. rubbioli* were counted along the 180 m long stream, but afterwards this number sharply decreased (see Trajano *et al.*, 2009: Table 1), which suggests that the population in the vadose stream inside Lapa dos Peixes is a sink population, i.e., if the source of migrants is cut-off it can become extinct (Fong, 2004).

When not hidden, *T. rubbioli* catfishes are fast swimmers, showing preference for staying on the bottom and on walls (rocky and silt substrate), with occasional incursions into the midwater and surface. The light from spotlights, 1.000 lux (light intensity of a cloudy day) triggers a quick avoidance reaction. In some occasions, the fishes were observed digging the soft bottom, apparently in a foraging behavior. Chronobiological studies indicated a partial regression, not as advanced as in highly troglomorphic species, of the circadian mechanisms of time control (Trajano *et al.*, 2009).

Some adult specimens collected in 2000 were maintained alive until 2008 in captivity, indicating a long lifespan.



**FIGURE 10.** Caudal skeleton of *Trichomycterus rubbioli*, (MZUSP 110979), 64.1 mm SL, paratype. Left lateral view. Abbreviations: **ahs2**, anterior hemal spine of preural centrum 2; **hy3**, hypural 3; **hy4+5**, complex plate formed by co-ossification of hypurals 4 and 5; **ns1–2**, neural spines of preural centrum 1 and 2; **pc1+u1**, complex centrum composed of preural centrum 1 and ural centrum 1; **pc2**, preural centrum 2; **ph+hy1+hy2**, complex plate formed by co-ossification of parhypual, hypurals 1 and 2; **phs2**, posterior hemal spine of preural centrum 2; **un**, uroneural. Scale bar = 2 mm.

## Discussion

**Taxonomy.** *Trichomycterus* is the most species-rich genus within the family, with more than 150 valid species, of which more than 70 occur in Brazil (Eschmeyer & Fong, 2010). In addition, many others are presently awaiting description (Wosiacki & de Pinna, 2008; Fernández & Vari, 2009). Because the phylogenetic history of the genus and the relationships between the species are still obscure (Wosiacki & de Pinna, 2008), many authors have indicated that it is probably not monophyletic (de Pinna, 1998; Wosiacki, 2002; Datovo & Bockmann, 2011). Only species with broadly distinctive features, which can be easily recognized as new taxa, have been recently described (de Pinna, 1998; Wosiacki & de Pinna, 2008). Due to the lack of synapomorphic characters distinguishing *Trichomycterus*, it has accomodated species lacking synapomorphies of other trichomycterine genera: *Bullockia, Eremophilus, Hatcheria, Ituglanis, Rhizosomichthys, Scleronema* or *Silvinichthys*, as proposed by Arratia (1990, 1998), Costa & Bockmann (1993), and Fernández & de Pinna (2005). Because none of the synapomorphies of the other above-cited trichomycterine genera could be recognized for the new species from Serra do Ramalho, its inclusion in *Trichomycterus* seems to be the most reasonable alternative for the moment.

*Trichomycterus rubbioli* is a very distinctive species, especially due to three easily visible character states: (a) reduction of melanic skin pigmentation, (b) reduced eyes, externally visible as small black or reddish brown spots, and (c) very long barbels, especially the nasal and the maxillary. None of these character states are exclusive, but taken together, they can distinguish the new species from almost all congeners. Character (a) distinguishes *T. rubbioli* from all congeners except for the troglobitic *T. chaberti, T. itacarambiensis, T. spelaeus, T. sandovali, T. santanderensis, T. uisae* and *T. dali*, besides the epigean *T. gorgona*; among the troglobitic species, character (b) distinguishes *T. rubbioli* from *T. spelaeus, T. sandovali* and *T. dali* (in which the eyes are not visible externally), and character (c) distinguishes it from *T. gorgona, T. chaberti* and *T. itacarambiensis* (where the nasal and maxillary barbel length are shorter than 90% and 105% of HL, respectively). Only *T. santanderensis* and *T. uisae* share with *T. rubbioli* the three character states together, but they may be distinguished by other characters (a comparison of morphological characters of the nine hypogean *Trichomycterus* species is shown in Table 2).

The above mentioned three character states are clearly related to the subterranean habit of *T. rubbioli*, because reduced eyes and pigmentation and enlarged appendages bearing sensorial structures are broadly recognized as the most common traits characterizing troglobites (Hüppop, 2000; Trajano, 2001), commonly used in descriptions of exclusively hypogean fishes (Renno *et al.*, 2007; see "troglobitic status", below). In fact, the eight hypogean *Trichomycterus* species cited above share these characters with *T. rubbioli*, but each one to a different degree. Thus, as characters (a), (b) and (c) result from its evolutionary history in the subterranean environment, and as neither of them alone, nor all of them together, are exclusive within the genus, other characters, not clearly related to its subterranean habit, are suggested as additional evidence to support the new species.

The first, pectoral-fin ray count reaching I, 9, is a rare condition for the genus, occurring only in other three species: *T. hualco, T sketi* and *T. dali*. Most of *Trichomycterus* species have a pectoral-fin ray count of I, 7, less commonly varying between I, 6 to I, 8, but rarely reaching I, 9. In *T. hualco*, the pectoral-fin varies from I, 7 to I, 9, in *T. sketi* is I, 9 and in *T. dali* and *T. rubbioli*, it varies from I, 8 to I, 9. Other relevant characters for *T. rubbioli* to distinguish it from its congeners are based on internal morphology: posterior cranial fontanel extending from the middle of parieto-supraoccipital to the posterior region of the frontal bones, with a conspicuous constriction on the meeting point of parieto-supraoccipital and the two frontal bones, separated by an epiphyseal bar from the very small anterior fontanel (Fig. 4); hemal spine of penultimate vertebrae divided into a narrow anterior hemal spine and a wide posterior hemal spine (Fig. 10); uroneural with a characteristic shape similar to a scimitar blade (Fig. 10); 35–38 interopercular and 5–13 opercular odontodes. The shape of the posterior cranial fontanel is similar to the condition in *T. dali* (compare Fig. 5 of Rizzato *et al.*, 2011, with our Fig. 4). However, in *T. dali* the anterior cranial fontanel is absent, while in *T. rubbioli* it is present and separated from the posterior cranial fontanel by the epiphyseal bar. Moreover, well-developed pre- and post-dorsal adipose folds in adults, a strong diagnostic character of *T. dali* (Rizzato *et al.*, 2011), are not present in *T. rubbioli*.

**Troglobitic status.** Troglobites are incapable of forming epigean source populations, thus are restricted to subterranean habitats (Trajano, 2012). This category encompasses organisms more specialized to subterranean life, commonly exhibiting a set of character states that can be related to their evolutionary history on this habitat, the so-called troglomorphisms (Barr, 1968; Holsinger & Culver, 1988). Extensive fish collections were carried out in several epigean streams of the Serra do Ramalho karst area and not a single specimen of Trichomycteridae family was found (Mattox *et al.*, 2008). The restriction to cave habitats, associated with advanced troglomorphic traits in *T. rubbioli*, corroborates the hypothesis of a troglobitic status for this species.

TABLE 2. Comparative data on external mor	bhology of Trichomycterus	subterranean species.
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	Eyes	Skin pigmentation	Origin of pelvic fin	Ventral profile of body	Dorsal view of head	Anal fin in lateral view	Profile of caudal fin
T. rubbioli sp.n.	Presence, from visible to imperceptible	Pale yellow with bright white spots in dorsal and dorsolateral region	On vertical through origin of dorsal-fin base	Nearly straight	Semicircular to trapezoidal	Rectangular	Dorsal and ventral profile straight
T. dali	Not visible	Pale yellow to bright white	On vertical through origin of dorsal-fin base	Straight	Trapezoidal	Rectangular, distal margin straight	With a constriction on the origin of rays, dorsal lobe projected upward
T. itacarambiensis	Presence, intraespecific variation	Small, irregular round black spots	Slightly anterior to origin of dorsal fin	Slightly convex or nearly straight	Trapezoidal	Half- ellipsoidal	Dorsal and ventral profile slightly convex
T. spelaeus	Not visible	Bright yellow to light brown	On vertical through origin of dorsal-fin base	Slightly convex	Triangular	Rounded, distal margin semicircular	Dorsal and ventral profile slightly convex
T. chaberti	Presence, variable degree	Variable, moderately pigmented, yellow with brown spots	Anterior to origin of dorsal fin	Slightly concave	Triangular	(no data available)	Dorsal and ventral profile straight
T. sandovali	Not visible	Bright yellow	On vertical through origin of dorsal-fin base	Slightly concave	Triangular	Distal margin rounded	Dorsal and ventral profile slightly convex
T. santanderensis	Presence, from visible to imperceptible	Variable from homogeneous light-red to pale rose with small gray, dorsal round spots	On vertical through origin of dorsal-fin base	Straight	Triangular	Rounded	Dorsal and ventral profile slightly convex
T. uisae	Presence, reduced but well defined	Reduced, light brown without spots, narrow predorsal bluish-gray stripe	Anterior to origin of dorsal fin	Straight	Trapezoidal	Rounded	Dorsal and ventral profile slightly convex
T. sketi	Presence, small, reduced in few specimes	Normally pigmented, light gray to dark gray	Slightly anterior to origin of dorsal fin	Straight	Trapezoidal	Rounded	Dorsal and ventral profile slightly convex

*T. rubbioli* exhibits the two "classical" troglomorphisms: regression of both eyes and melanic pigmentation. The eyes in *T. rubbioli* are reduced ones when compared to the epigean relatives, but there is no record of individuals with eyes not visible externally, differing from some other troglobitic congeners (*T. sandovali*, *T.* 

*spelaeus* and *T. dali*) that lack eyes. The pigmentation is also reduced in *T. rubbioli* when compared to its epigean relatives, but the individuals are still able to synthesize melanin in the presence of light. In view of this, it can be said that *T. rubbioli* exhibits troglomorphism in a less advanced state than other troglobitic species of the genus as, for example, *T. spelaeus* and *T. dali*.

It is noteworthy that the light induced darkening of the skin does not represent a reacquisition of the epigean phenotype. Evidence from subterranean fishes, including the mosaic distribution of troglomorphic character states (Trajano & Bichuette, 2010), with two types of albinism in totally depigmented fishes (DOPA(+) and DOPA(-); Felice *et al.*, 2008), and different responses to hormones (Grempel, 2011), suggest that the trait "color" encompasses several characters which may regress independently in troglobites: number and size of melanophores; ability to synthesize melanin (lost by at least two different mutations); response of melanin granules to hormones and neurotransmitters; and melanin synthesis induced by light exposure, either as a direct effect of light or as a secondary effect on the hormonal response to light. Therefore, darkened individuals due to light exposition do not necessarily represent a reacquired epigean color phenotype because they may retain the troglobitic melanophore morphology and melanin physiology.

Elongation of the barbels has been observed in troglobitic fishes (Hüppop, 2000, among others), including heptapterids such as *Rhamdia* (Weber, 1996) and several trichomycterids (Fernández & Bichuette, 2002; DoNascimiento *et al.*, 2001; Castellanos-Morales, 2008). Following this trend, *T. rubbioli* exhibits relatively long barbels. Five other species of *Trichomycterus* exhibit barbels as long as or longer than those of *T. rubbioli*: *T. longibarbatus*, *T. spelaeus*, *T. sandovali*, *T. sketi* and *T. dali*. Since *T. rubbioli*, *T. dali*, *T. sketi*, *T. spelaeus* and *T. sandovali* are restricted to subterranean habitats, where barbels constitute an important source of sensory information about the environment in the absence of light, its elongation may be involved in sensory compensation as an adaptation to the permanent darkness of the subterranean habitat. In this case this character may be considered an additional troglomorphism of the new species.

**Conservation.** Few *Trichomycterus* species have been described from river basins draining Bahia State, the majority of which from coastal rivers (Sarmento-Soares *et al.*, 2011). Only two species are reported so far from more internal river basins: *T. payaya* Sarmento-Soares, Zanata & Martins-Pinheiro and *T. tete* Barbosa & Costa. These were also the only known species of the genus inhabiting areas of the Caatinga semi-arid region (Sarmento-Soares *et al.*, 2011; Barbosa & Costa, 2011). The new species herein described must therefore be considered as the third record of the genus for the Caatinga, and the first one of a troglobite. In addition, the new species is the most interior record for the genus in Bahia State, occurring at the Middle São Francisco River basin in the southwestern region of the State.

The Serra do Ramalho represents one of the most extensive karst areas in Brazil. Most of its cave-systems are typical sinkholes, and the aquatic cave fauna is highly dependent of allochthonous items, carried from the surface through floods, mainly in the wet season. The region has been extensively explored by mining and agriculture activities. This fact, allied to the absence of any effective law to grant protection for the caves in Brazil, puts the new species herein described and its habitat under a high level of threat (de Moura *et al.*, 2008). The description of *T. rubbioli* may contribute to the protection of the Água Clara cave system, its habitat, classifying this system as being of maximum relevance in terms of Brazilian conservation laws. However, this action is not sufficient, since the protection is restricted to a small portion of an extensive massif, and the creation of a conservation unit for the whole karst area would probably be the most effective action to ensure the preservation of the biological and speleological heritage of the Serra do Ramalho karst region.

**Comparative material.** *Ituglanis bambui*, MZUSP 79860, holotype, Brazil: Goiás State: São Domingos municipality: Terra Ronca State Park: Angélica Cave. *Ituglanis epikarsticus*, MZUSP 79869, holotype, Brazil: Goiás State: São Domingos municipality: Terra Ronca State Park: São Mateus Cave. *Ituglanis passensis*: MZUSP 80097, 3, Brazil: Goiás State: São Domingos municipality, Passa Três Cave. *Ituglanis proops*: MZUSP 79576, 15, Brazil: Paraná State: Cerro Azul municipality, Ribeirão Bonito. *Ituglanis ramiroi*, MZUSP 79865, holotype, Brazil: Goiás State: São Domingos municipality: Terra Ronca State Park: São Bernardo Cave. *Ituglanis sp.:* MZUSP 53222, 6, Brazil: Goiás State: Minaçú, tributary of Rio Tocantinzinho. *Trichomycterus bahianus*: MZUSP 74655, 10, Brazil: Bahia State: Livramento do Brumado municipality, Rio Brumado. *Trichomycterus bahianus*: MZUSP 106630, holotype, 1, Brazil: Mato Grosso do Sul State: Bonito municipality, Saracura Cave; MZUSP 106631, paratype, 1, Brazil: Mato Grosso do Sul State: Jardim municipality, Buraco das Abelhas Cave; MZUSP 106632, paratype, 1, Brazil: Mato

Grosso do Sul State: Jardim municipality, Buraco das Abelhas Cave; MZUSP 81056, paratype, 1, Brazil: Mato Grosso do Sul State: Jardim municipality, Buraco das Abelhas Cave; MZUSP 106633, paratype, 1, Brazil: Mato Grosso do Sul State: Bodoquena municipality, Morro do Jericó Cave; MZUSP 106634, paratype, 1, Brazil: Mato Grosso do Sul State: Bodoquena municipality, Morro do Jericó Cave; MZUSP 106635, paratype, 1, Brazil: Mato Grosso do Sul State: Bonito, Saracura Cave; MZUSP 109770, paratype, 1, Brazil: Mato Grosso do Sul State: Bonito, Saracura Cave; MZUSP 109770, paratype, 1, Brazil: Mato Grosso do Sul State: Bonito, Saracura Cave; MZUSP 109770, paratype, 1, Brazil: Mato Grosso do Sul State: Jardim municipality, Saracura Cave. *Trichomycterus itacarambiensis*: MZUSP 50548, paratypes, 4, Brazil: Minas Gerais State: Itacarambi municipality, Olhos D'Água Cave; MZUSP 81078, 5, Brazil: Minas Gerais State: Itacarambi municipality, Olhos D'Água Cave; MZUSP 68173, 20, Brazil: São Paulo State: Cajati municipality, Rio do Queimado; MZUSP 23038, 6, Brazil: São Paulo State: Caraguatatuba municipality, Rio d'Ouro. The comparison with other *Trichomycterus* species was based on the literature.

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