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Relationships and Description of a New Species of *Trichomycterus* (Siluriformes: Trichomycteridae) from the Rio Paraíba do Sul basin, South-eastern Brazil

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A phylogenetic analysis using fragments of two nuclear and two mitochondrial genes strongly supported sister group relationships between a new species and *Trichomycterus albinotatus*, corroborated by unique colour patterns of adult specimens and juveniles, and morphology of the autopalatine bone. The new species is distinguished from its closest congener, *T. albinotatus*, by details of the colouration and number of branchiostegal rays. Both the new species and *T. albinotatus* are endemic to the Rio Paraíba do Sul basin, in the Atlantic Forest of south-eastern Brazil, but occur in distant and disjunct areas. The new species, herein described, is endemic to the upper section of the Rio Grande drainage, a right tributary of the lower Rio Paraíba do Sul, an area situated in the Órgãos Mountain Range. *T. albinotatus* is endemic to an area about 200 km distant, in the upper section of the Rio Preto that drains the Itatiaia Massif. However, both species are only known from localities above 1100 m asl, suggesting that they are not able of surviving in ecological conditions present in lower altitudes. This study indicates that efforts are necessary to provide more accurate data on species diversity and distribution of *Trichomycterus* in the biologically diverse and endangered Atlantic Forest of south-eastern Brazil.

Key words: Atlantic Forest, Biodiversity, Catfish, Molecular phylogeny, Neotropics, Systematics.

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BACKGROUND

The Atlantic Forest is one of the largest and most biodiverse phytogeographical provinces in the Americas, with about 150 million ha (Ribeiro et al. 2009), despite a long-term exploration and deforestation reducing its original cover to less than 12.9 % (Morellato and Haddad 2000). Over 270 fish species, belonging to 89 genera and 21 families (Abilhoa et al. 2011; Miranda 2012), are found in this province, which is considered one of the most diverse and endangered biodiversity hotspots in the world (Myers et al. 2000; Miranda 2012). Among fish taxa occurring in this province, catfishes of the Trichomycteridae is one of the most interesting and species-rich freshwater groups. It includes small to medium size fishes, never surpassing 300 mm of standard length (SL), with members easily identified by a modified opercular system with integumentary teeth in the interopercle and opercle bones (Baskin 1973; de Pinna 1998). The mouth is typically subterminal to fully ventral, but in parasitic forms the lips can form a ventral sucking disk (Baskin 1973). The high diversity in trichomycterids comprises about 326 species in 42 valid genera (Fricke et al. 2020) distributed along all the major river drainages between southern Central America, in Costa Rica, and southern South America, in Patagonia (Baskin 1973; de Pinna 1998). In the Atlantic Forest, *Trichomycterus* Valenciennes, 1832 is the most diversified trichomycterid genus and recently has been restricted to about 50 valid species occurring in an area encompassing eastern and north-eastern Brazil (Katz et al. 2018; Costa et al. 2020). They usually are endemic to a single river basin or small areas of mountain rivers and have the ability of climbing waterfalls (Eigenmann 1918) with the support of the interopercular and opercular odontodes, in an “elbowing” behaviour (Adriaens et al. 2010).

Most species of *Trichomycterus* were described after 1992, but many new species are still waiting for formal descriptions. For example, the species here described was first collected by one of us (WJEMC) in 1983 and 1984, but due to the scarcity of adult specimens then found, a formal description was not previously concluded. During more recent collections in the remnants of the Atlantic Forest of south-eastern Brazil, some new samples of this new species were collected, allowing the present study. The objectives are to search phylogenetic relationships of the new taxon through a molecular analysis using fragments of two nuclear and two mitochondrial genes for the new species and 19 other congeners representing all genetic lineages and to provide a formal description of the new taxon.

MATERIALS AND METHODS

Specimens

Most specimens were captured using small dip nets (40 × 30 cm) during daylight, but some were captured at night, using traps consisting of 2 l plastic bottles with small pieces of bacon inside, placed on the river bottom; early in the morning the traps were checked and specimens removed. Collections were made using permits provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade; permit number: 38553-7, 50247-5) and INEA (Instituto Estadual do Ambiente; permit number 037/2018). Specimens were euthanized just after collection by sub-merging them in a buffered solution of ethyl-3-amino-benzoat-methansulfonat (MS-222) at a concentration of 250 mg/l, for a period of 10 minutes or more, until completely ceasing opercular movements, following the methods for euthanasia approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit number: 065/18). Molecular data were obtained from specimens fixed and preserved in absolute ethanol. Specimens used for morphological comparisons were fixed in buffered formalin for a period of 14 days and then transferred to 70% ethanol. In lists of material, the abbreviations C&S indicates specimens prepared for osteological analysis and preserved in glycerine (see below), and DNA indicates specimens fixed and preserved in 98% ethanol. Specimens were deposited in the fish collection of the Institute of Biology, Federal University of Rio de Janeiro (UFRJ) and in the Ichthyological Collection of the Center for Agrarian and Environmental Sciences, Federal University of Maranhão (CICCAA). This study also includes specimens previously deposited in UFRJ fish collection.

Morphology

Measurements and counts follow Costa (1992), with addition of the distance between pelvic-fin bases. Measurements are presented as percentages of standard length (SL) except for subunits of head, which are presented as percentages of head length (HL). All measurements and counts were made on the left side of specimens whenever possible. Counts of procurrent caudal-fin rays, vertebrae, branchiostegal rays and odontodes were made only in cleared and stained (C&S) specimens prepared according to Taylor and Van Dyke (1985). Anatomical illustrations were prepared from sketches taken in stereomicroscope with camera lucida. Terminology for osteological nomenclature followed Adriaens et al. (2010) and Datovo and Bockmann (2010), with the addition of using ‘accessory element of ceratobranchial 4’ for the structure previously identified as ‘epibranchial 5’, following Carvalho et al. (2013). Osteological structures included in the description are those with informative variability in congeners (Costa et al. 2020). Terminology for the cephalic laterosensory system followed Arratia and Huaquin (1995) for nomenclature of supraorbital and infraorbital pores, and Northcutt (1989) for canal nomenclature. A list of comparative material appears in Costa et al. (2020) and Katz et al. (2018).

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from muscle tissues of the right side of the caudal peduncle using the DNeasy Blood & Tissue Kit (Qiagen), according to the manufacturer's protocol. The analyses included a set of partial sequences of two nuclear genes: myosin heavy chain 6 (MYH6) and recombination activating 2 (RAG2); and partial sequences of two mitochondrial encoded genes: cytochrome *b* (CYTB) and cytochrome c oxidase subunit I (*COI*). Amplification of the target DNA fragments was made through the polymerase chain reaction (PCR) method, using the following primers: Cytb Siluri F, Cytb Siluri R, L5698-ASN and H721-COI (Villa-Verde et al. 2012), myh6_F459, myh6_F507, myh6_R1322 and myh6_R1325 (Li et al. 2007), MHRAG2-F1 and MHRAG2-R1 (Hardman and Page 2003), RAG2-MCF and RAG2-MCR (Cramer et al. 2011), Fish-F1 and Fish-R1 (Ward et al. 2005), RAG2 TRICHO F, RAG2 TRICHO R, MYH6 TRICHO F and MYH6 TRICHO R (Costa et al. 2019). Double-stranded PCR amplifications were performed in 60 μl reactions with reagents at the following concentrations: 5× GreenGoTaq Reaction Buffer (Promega), 3.2 mm $MgCl_2$, 1 μm of each primer, 75 ng of total genomic DNA, 0.2 mm of each dNTP and 1 U of standard Taq polymerase or Promega GoTaq Hot Start polymerase. The thermocycling profile was as follows: initial denaturation for 2 min at 94–95°C; 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min–90 s at 48.0–64.0°C and extension for 1–2 min at 72°C; and terminal extension for 4 min at 72°C. Negative controls were used to check on contaminations. The PCR products were then purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were made using the BigDye Terminator Cycle Sequencing Mix (Applied Biosystems). Cycle sequencing reactions were performed in 20 μl reaction volumes containing 4 μl BigDye, 2 μl sequencing buffer 5× (Applied Biosystems), 2 μl of the amplified products (10–40 ng), 2 μl primer and 10 μl deionized water. The thermocycling profile was: (1) 35 cycles of 10 seconds at 96°C, 5 seconds at 54°C and 4 minutes at 60°C.

Phylogenetic analysis

Taxon sampling included the new species, 19 species of *Trichomycterus* representing all generic lineages and *Cambeva davisi* Hasemann, 1911, a member of the clade sister to *Trichomycterus* (Katz et al. 2018), in which analyses were rooted. A list of specimens and their respective GenBank accession numbers is provided in table S1. Sequences were aligned and edited in Mega X software (Kumar et al. 2018) using the ClustalW algorithm (Chenna et al. 2003). The molecular dataset was analyzed in PartitionFinder2 (Lanfear et al. 2016) to determine the best

partitioning scheme and nucleotide substitution models. The optimal partition strategy is shown in table S2. Bayesian Inference (BI) analysis was conducted using the software Mrbayes 3.2 (Ronquist et al. 2012). The BI analysis was conducted with the following parameters: two independent Markov chain Monte Carlo (MCMC) runs of two chains each for 10 million generations, with a tree sampling frequency of every 1000 generations. The convergence of the MCMC chains and the proper burn-in value were assessed by evaluating the stationary phase of the chains using tracer v. 1.6 (Rambaut et al. 2014). The BI final consensus tree and its Bayesian posterior probabilities were generated with the remaining tree samples after removing the first 25% samples as burn-in.

RESULTS

Phylogenetic relationships

The concatenated matrix comprised 3062 bp after alignment (1088pb for CYTB, 522 pb for *COI*, 909 pb for RAG2 and 543 pb for MYH6). The best log-likelihood score for the BI analysis was -lnl 9093.368. Both analyses generated the same tree topology (Fig. 1), highly supporting the new species as sister to *T. albinotatus* Costa, 1992. The clade comprising the new species and *T. albinotatus* is corroborated by three morphological character states not occurring in other congeners, comprising a colour pattern of adult specimens (presence of a vertical row of dark brown to black spots on the caudal peduncle end, often coalesced to form a precaudal bar), a colour pattern of juveniles (presence of two horizontal rows of white spots on the flank), and an osteological character state (the medial margin of the autopalatine being nearly straight to slightly convex; see diagnosis below).

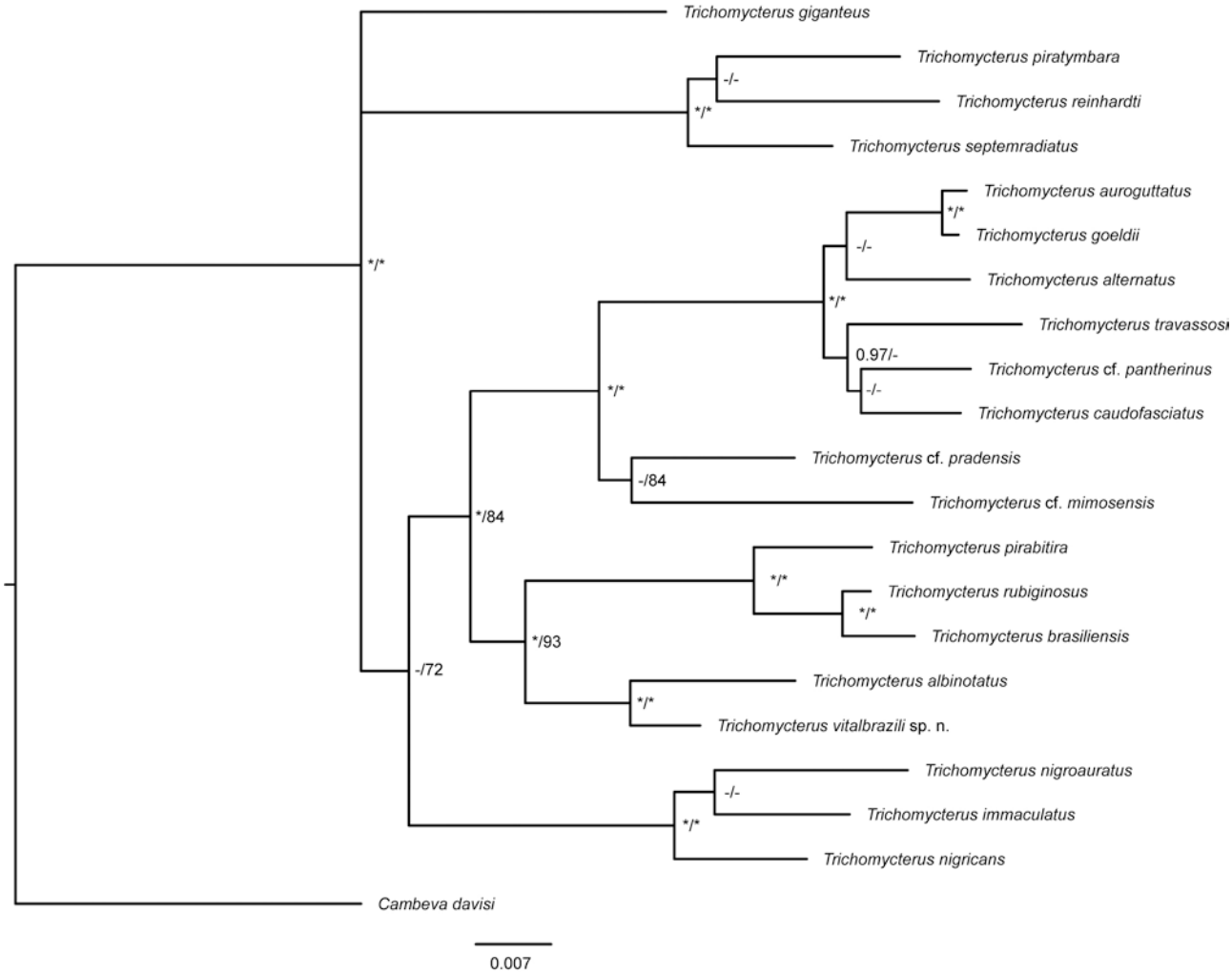

Fig. 1. Phylogenetic relationships among 20 species of *Trichomycterus* inferred by Maximum Likelihood and Bayesian Inference, from the analysis of a multigene data set (3062 bp). Numbers on each node are bootstrap percentages from ML followed by posterior probability from BI; asterisks indicate maximum support value and hyphens, values under 0,95 for BI and 65 for ML.

TAXONOMY

Family Trichomycteridae Bleeker, 1858

Genus *Trichomycterus* Valenciennes, 1832

Trichomycterus vitalbrazili sp. nov. Vilardo, Katz and Costa

(Figs. 2–4,6; Table 1)

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Holotype: UFRJ 12151, male, 88.0 mm SL; Brazil: Rio de Janeiro State: Nova Friburgo Municipality: mountain stream tributary to the Rio Grande drainage, Rio Paraíba do Sul basin, Serra dos Órgãos, inside the advanced campus of the Instituto Vital Brazil, São Lourenço road, 22°20'51"S 42°37'18"W, about 1130 m asl; A. Katz and F. Pereira, 24 Feb. 2016.

Paratypes: All from Brazil: Rio de Janeiro State: Nova Friburgo Municipality: Rio Grande drainage, Rio Paraíba do Sul basin, Serra dos Órgãos. UFRJ 10924, 2, 75.0–75.1 mm SL; UFRJ 10923, 1, 75.4 mm SL; UFRJ 12128, 1, 55.4 mm SL (C&S); collected with holotype. UFRJ 12125, 3, 24.1–29.1 mm SL (DNA); same locality as holotype; A. Katz and P. Vilardo, 20 Jan. 2019. UFRJ 5979, 8, 32.1–50.9 mm SL; UFRJ 12150, 2, 34.4–36.2 mm SL (C&S); stream tributary of Rio Bengala, near km 66 of road RJ-116, 22°21'36"S 42°31'43"W, 1010 m asl; L. Villa Verde, 24 Aug. 2003. UFRJ 7210, 10, 25.0–48.8 mm SL; CICCAA 04083, 3, 31.5–35.2 mm SL; same locality as UFRJ 5979; L. Villa Verde, 11 Sep. 2005.

Diagnosis: *Trichomycterus vitalbrazili* is distinguished from all other congeners except *T. albinotatus* by the presence of a vertical row of dark brown to black spots on the caudal peduncle end, often coalesced to form a precaudal bar in live and preserved specimens (Figs. 2A–B; vs. never a similar colour pattern); two horizontal rows of white spots on the flank in live juveniles (Figs. 3A–C; vs. never a similar colour pattern); and medial margin of the autopalatine nearly straight to slightly convex (Fig. 4A; vs. concave). *Trichomycterus vitalbrazili* is distinguished from *T. albinotatus* (Figs. 5A–B) by possessing diffuse irregularly shaped dark brown blotches on the flank in adult specimens (Figs. 2A–B; vs. well-delimited dark grey to black, sometimes coalesced in larger specimens); fins with dark brown spots in adults specimens (Figs. 2A–B; vs. without spots); and eight branchiostegal rays (vs. seven).

Description: Morphometric data appear in table 1. Body moderately slender, subcylindrical and slightly depressed anteriorly, compressed posteriorly. Greatest body depth at vertical immediately in front pelvic-fin base. Dorsal profile slightly convex between snout and end of dorsal-fin base, straight on caudal peduncle; ventral profile straight to slightly convex between lower jaw and end of anal-fin base, slightly concave on caudal peduncle. Anus and urogenital papilla in vertical through middle of dorsal-fin base, nearer tip of pelvic fin than anal-fin origin. Skin papillae minute. Head trapezoidal in dorsal view. Anterior profile of snout nearly straight to slightly convex in dorsal view. Eye moderate, dorsally positioned in head. Posterior nostril located nearer anterior nostril than anterior orbital rim. Tip of maxillary barbel reaching anterior third of pectoral fin. Tip of rictal barbel reaching behind opercular patch of odontodes. Tip of nasal barbel reaching orbit. Mouth subterminal. Jaw teeth pointed, arranged in irregular rows, three in premaxilla, four in dentary; premaxillary anterior-most row with 12–13 teeth, dentary external row with 14–17. Branchial membrane attached to isthmus only at its anterior point. Branchiostegal rays 7.

Fig. 2. *Trichomycterus vitalbrazili*. Brazil: Rio de Janeiro State: Nova Friburgo Municipality: Rio Paraíba do Sul basin. (a) UFRJ 12151, 88.0 mm SL (preserved holotype), left lateral view; (b) UFRJ 12151, 88.0 mm SL (live holotype), left lateral view. Caudal-fin margin of holotype is damaged, see Fig.6 for correct information of caudal morphology.

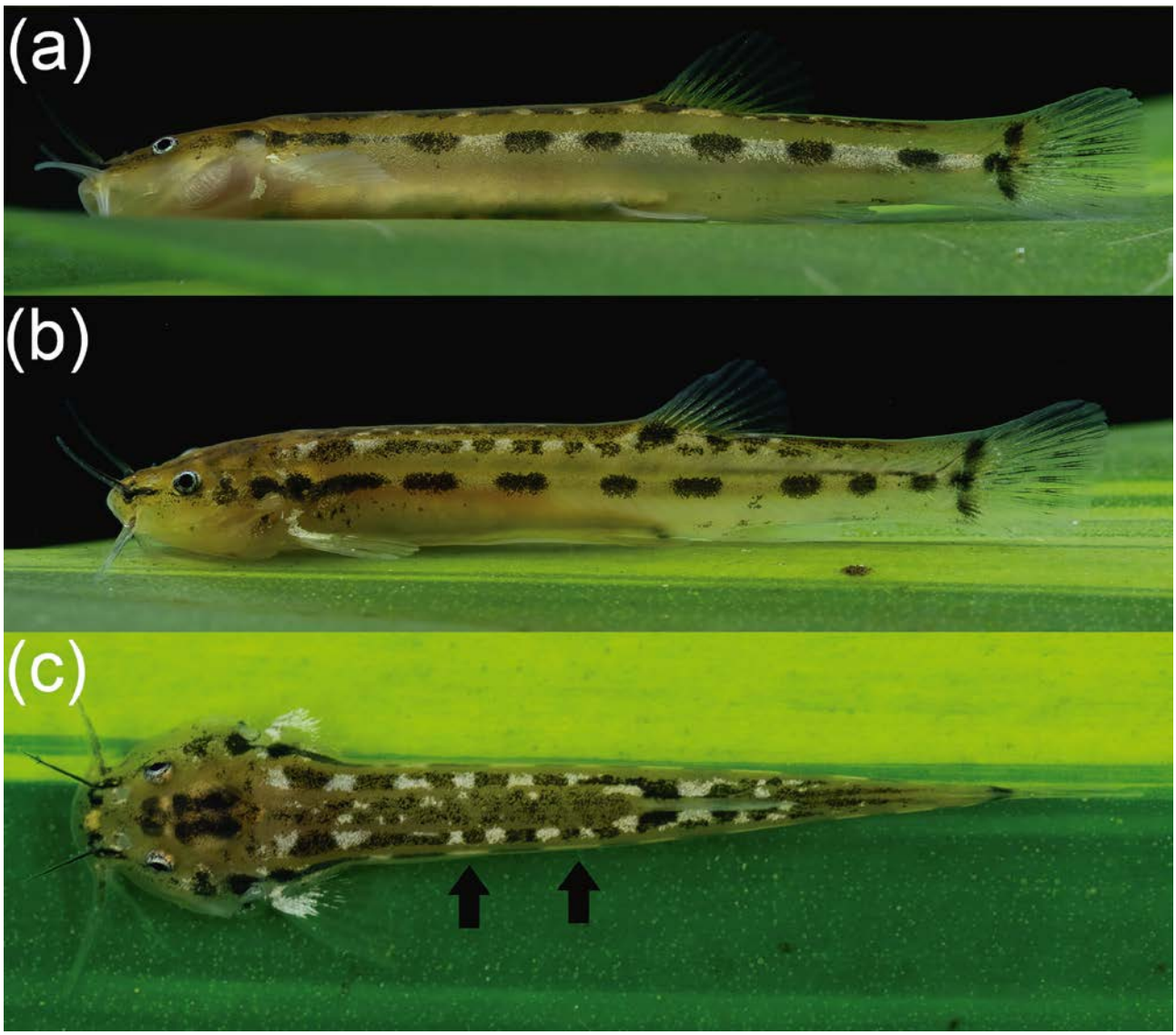

Fig. 3. *Trichomycterus vitalbrazili.* Brazil: Rio de Janeiro State: Nova Friburgo Municipality: Rio Paraíba do Sul basin. (a), UFRJ 12125, 24.1 mm SL (live juvenile paratype), left lateral view; (b) UFRJ 12125, 29.1 mm SL (live juvenile paratype), left lateral view; and (c) UFRJ 12125, 29.1 mm SL (live juvenile paratype), dorsal view. Arrow indicates the midline row with white spots, that is not visible in left lateral view of figure 3b due to light angle.

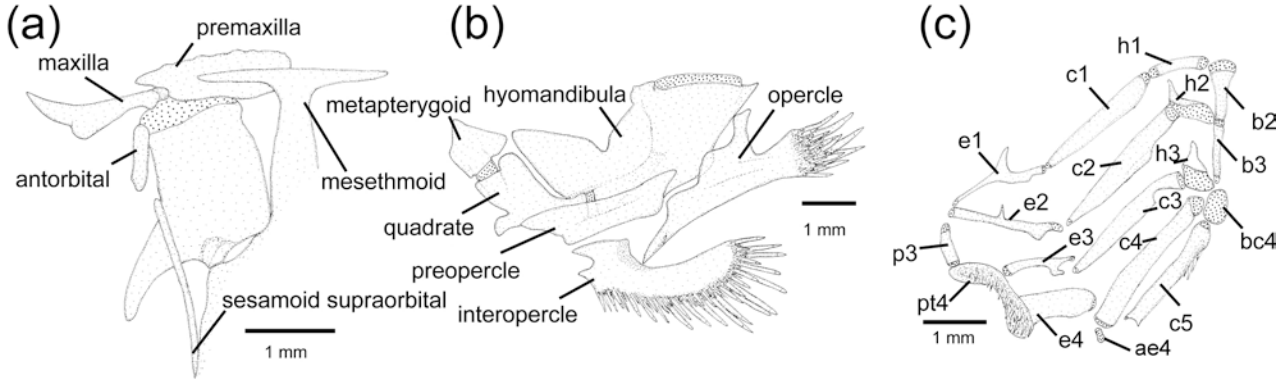

Fig. 4. Osteological features of *Trichomycterus vitalbrazili*. (a), UFRJ 12128, 55.4 mm SL, mesenthmoidal region and adjacent structures, middle and left portion, dorsal view; (b), UFRJ 12150, 36.2 mm SL, left suspensorium and opercular apparatus, lateral view; (c), UFRJ 12128, 55.4 mm SL, middle and left portion of branchial arches, ventral view of dorsal elements on left, dorsal view of ventral elements on right. Larger stippling represents cartilages. Abbreviations: ae4,

accessory element of ceratobranchial 4; b2–3, basibranchials 2–3; bc4, cartilaginous basibranchial 4; c1–5, ceratobranchials 1–5; e1–4, epibranchials 1–4; h1–3, hypobranchials 1–3; p3, pharyngobranchial 3; pt4, pharyngobranchial 4 tooth-plate. Scale bar = 1 mm

Fig. 5. *Trichomycterus albinotatus.* Brazil: Rio de Janeiro State: Itatiaia Municipality: Rio Paraíba do Sul basin. (a) UFRJ 11668, 42.8 mm SL (live adult specimen), left lateral view; and (b) UFRJ 11658, 29.0mm SL (live juvenile specimen), left lateral view.

Table 1. Morphometric data of *Trichomycterus vitalbrazili* sp. nov.

	Holotype	Paratypes (*n* = 23)	Mean	Standard Deviation
Standard length (mm)	88.0	24.1–88.0	37.7	-
Percentage of standard length				
Body depth	14.9	11.2–14.9	12.7	1.3
Caudal peduncle depth	11.2	8.5–11.6	10.1	0.9
Body width	8.9	4.3–8.9	6.9	1.2
Caudal peduncle width	2.6	0.9–2.8	1.5	0.6
Dorsal-fin base length	11.9	7.7–11.9	9.5	1.0
Anal-fin base length	8.0	6.5–8.4	7.4	0.7
Pelvic-fin length	2.6	2.6–10.6	6.7	3.4
Distance between pelvic-fin bases	2.6	0.6–2.6	1.2	0.4
Pectoral-fin length	12.9	12.9–17.6	14.1	1.0
Predorsal length	62.0	55.6–63.8	60.8	2.3
Prepelvic length	56.7	53.8–60.8	57.8	1.8
Head length	18.6	17.4–22.1	19.5	1.3
Percentage of head length				
Head depth	49.5	49.5–60.5	55.4	3.8
Head width	104.0	90.0–112.9	103.2	7.0
Interorbital width	36.3	26.8–42.2	34.0	5.0
Preorbital length	42.8	37.9–51.7	42.9	4.4
Eye diameter	11.4	11.4–22.6	15.6	3.1

Dorsal and anal fins subtriangular, distal border slightly convex; total dorsal-fin rays 11 or 12 (ii–iii + II + 7), total anal-fin rays 10 (iii + II + 5); anal-fin origin in vertical just posterior to dorsal-fin base. Dorsal-fin origin in vertical through centrum of 18th vertebra; anal-fin origin in vertical between centrum of 21st or 22nd vertebrae. Pectoral fin subtriangular in dorsal view, first pectoral-fin ray terminating in short filament reaching about 25–40 % of pectoral-fin length without filament; total pectoral-fin rays 8 (I + 7). Pelvic fin sub-truncate, posterior margin slightly convex; posterior extremity of pelvic fin posteriorly just surpassing urogenital papilla; pelvic-fin bases medially separated by minute interspace; total pelvic-fin rays 5 (I + 4). Caudal fin truncate; total principal caudal-fin rays 13 (I + 6 + 5 + I), total dorsal procurrent rays 13–15 (xiii–xv), total ventral procurrent rays 12–13 (xi–xii + I). Vertebrae 35 or 36. Ribs 13.

Laterosensory system: Supraorbital sensory canal continuous, connected to infraorbital sensory canals posteriorly. Supraorbital sensory canal with 3 pores: s1, adjacent to medial margin of anterior nostril; s3, adjacent to medial margin of posterior nostril; and s6, on middle part of dorsal surface of head, in transverse line through posterior portion of orbit; pores s6 medially in close proximity. Infraorbital sensory canal arranged in 2 segments, each with two pores; anterior segment with pore i1, in transverse line through anterior nostril, and pore i3, in transverse line just anterior to posterior nostril; posterior segment with pore i10, adjacent to ventral margin of orbit, and pore i11, posterior to orbit. Postorbital canal with 2 pores: po1, in vertical line above posterior portion of interopercular patch of odontodes, and po2, in vertical line above posterior portion of opercular patch of odontodes. Lateral line of body short, with 2 pores, posterior-most pore in vertical just posterior to pectoral-fin base.

Mesethmoidal region and adjacent structures (Fig. 4A): Anterior margin of mesethmoid nearly straight, mesethmoid cornu slender, tip rounded to slightly pointed. Antorbital thin, elliptical in dorsal view. Sesamoid supraorbital elongate and narrow, its length about 2.5 times antorbital length. Premaxilla subrectangular in dorsal view, without distinctive processes, sometimes its lateral extremity slightly narrowed. Maxilla boomerang-shaped, slightly shorter than premaxilla. Autopalatine subrectangular in dorsal view when excluding posterolateral process, broad, its width about three fifths of autopalatine length, lateral margin about straight, medial margin straight to slightly convex; latero-posterior process of autopalatine triangular, short, its length about half autopalatine length without process.

Suspensorium and opercular apparatus (Fig. 4B): Metapterygoid subtriangular, deeper than wide. Quadrate robust, dorsoposterior flap continuous to hyomandibula anterior outgrowth. Hyomandibula broad, with well-developed anterior outgrowth flap; middle portion of dorsal margin of hyomandibular anterior outgrowth with shallow concavity; postero-dorsal process of hyomandibula well-developed, pointed. Opercle moderately robust, its smallest depth about one

fourth of its horizontal length measured between point just below articular facet to hyomandibula and posterior limit of odontode patch; dorsal process of opercle short, tip rounded; ventral process of opercle short, its length about two fifths of the opercle horizontal length. Interopercle long, its largest length about three fourths of hyomandibula largest length; dorsal interopercular process with deep anterior concavity. Odontodes conical, numerous, 13–19 on opercle, about 56–70 on interopercle. Preopercle compact, with pronounced ventral flap.

Branchial arches (Fig. 4C): Basibranchial 2 subcylindrical, gradually widening anteriorly, wider and slightly longer than basibranchial 3, basibranchial 3 cylindrical; basibranchial 4 cartilage sub-pentagonal, longer than wide. Hypobranchial 1 subcylindrical, without expansions; hypobranchials 2 and 3 triangular, osseous portion longer than cartilaginous portion. Ceratobranchial 1 slender, proximal portion slightly wider than distal one; ceratobranchial 2 slightly widened in its middle portion, with shallow concavity on posterior margin of proximal portion; ceratobranchial 3 similar to ceratobranchial 2, except by deep concavity on posterior margin of proximal portion; ceratobranchial 4 sub-rectangular, slightly widening distally; ceratobranchial 5 sub-rectangular, slightly curved, moderately wide, its width nearly equal to width of proximal portion of ceratobranchial 4; postero-proximal portion of ceratobranchial 5 bearing patch of small, slightly curved, conical teeth; cartilaginous accessory element of ceratobranchial 4 minute. Epibranchial 1 slender, with well-developed sharp anterior uncinate process and minute posterior process; epibranchial 2 slender, with rudimentary anterior and posterior uncinate processes; epibranchial 3 slender, with well developed, curved posterior uncinate process; epibranchial 4 broad, sub-retangular, proximal portion broader than distal portion. Pharyngobranchial 3 short, subcylindrical; pharyngobranchial 4 long, bearing broad dentigerous plate with curved conical teeth.

Colouration in life: In adult specimens (approximately above 35 mm SL), flank brownish yellow with diffuse irregularly shaped dark brown blotches, more concentrated on dorsal half of flank, often forming interrupted diffuse stripe along lateral midline between pectoral and caudal-fin bases. Posterior end of caudal peduncle with vertical row of coalesced dark brown spots, forming precaudal bar. Dorsum between head and dorsal-fin origin dark brown, with some depigmented small spots; venter brownish yellow, with diffuse pale brown spots. Side and dorsal surface of head dark brown, opercular and interopercular patches of odontodes pale brownish yellow. Barbels dark brown to black. Iris brown, with narrow yellow line around pupil bluish silver iridescence. Fins hyaline with irregular transverse rows of dark brown spots; spots larger and darker in vertical row on caudal-fin base; intense concentration of dark brown pigmentation on dorsal-fin base. In juveniles, flank light yellowish brown, with two horizontal rows of white spots, upper row on dorsal part of flank, lower row on flank midline, upper row with 8–10 small rounded white spots,

alternated with brown spots, lower row with 6 horizontally elongated white spots, often inconspicuous, alternated with similar dark brown to black spots. Vertical black bar on posterior end of caudal peduncle. Head side pale yellowish brown, with two post-orbital dark brown spots. Nasal region and barbel black. Maxillary and rictal barbels hyaline, with grey pigmentation on basal portion. Iris bluish silver, with melanophores on ventral margin. Dark brown blotches on centre of dorsal surface of head. Fins hyaline with transverse rows of small faint grey spots; unpaired fins with yellowish pigmentation concentrated on basal portion of rays; bright white pigmentation over basal portion of pectoral fin. The ontogenetic variation of colour pattern is presented in figure 6.

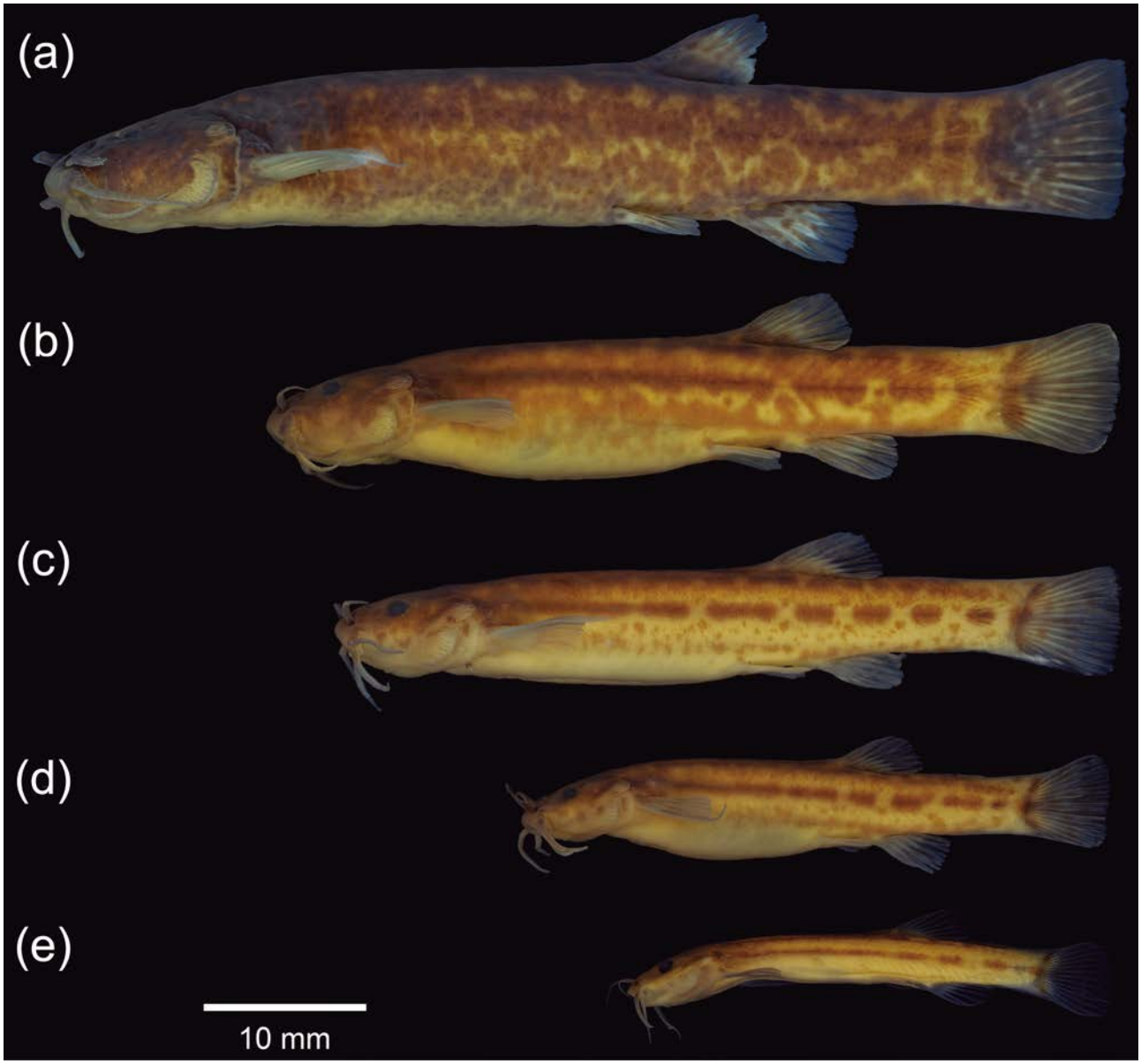

Fig. 6. Ontogenetic variation in *Trichomycterus vitalbrazili*. (a), UFRJ 10924, 75.0 mm SL (preserved paratype), left lateral view; (b), UFRJ 5979, 49.5 mm SL (preserved paratype), left lateral view; (c), UFRJ 5979, 38.2 mm SL (preserved paratype), left lateral view; (d), UFRJ 5979, 32.7 mm SL (preserved paratype), left lateral view; (e), UFRJ 7210, 25.0 mm SL (preserved paratype), left lateral view.

Colouration in alcohol: Similar to colouration in life, but paler, disappearing white marks in preserved juveniles.

Etymology: The name *vitalbrazili* was given in honour of Vital Brazil Mineiro da Campanha (1865–1950), an important Brazilian biomedical scientist, who first discovered the polyvalent anti-ophidic serum, successfully used to treat venomous snake bites, as well as founder of the Vital Brazil Institute where the new species was found.

Distribution, habitat and conservation: *Trichomycterus vitalbrazili* is only known from the upper section of the Rio Grande drainage, Rio Paraíba do Sul basin, south-eastern Brazil (Fig. 7). The type locality is a clearwater mountain stream, about 30–100 cm deep. In this locality, adult specimens (above about 35 mm SL), including the holotype, were only collected using traps during night, in a part of the stream about 1 m wide, within dense forest, bottom comprising rocks and gravel (Fig. 8). Juveniles were collected in gravel and sand on the stream bottom during daytime with small dip nets, just below the other site, where margins were deforested and the stream width was about 2.5 m. In this site speciemens of *Neoplecostomus variipictus* Bizerril, 1995, *Rhamdioglanis transfaciatus* Miranda-Ribeiro (1908), *Characidium interruptum* Pelegrin, 1909 and *Phalloceros harpagos* Lucinda, 2008 were co-occuring with the new species. Both juveniles and small adults about 35 mm SL were collected in 1983 and 1984, but not presently preserved, in the Parque Municipal Juarez Frotté, upper Rio Bengala subdrainage, by one of us (WJEMC). In this site the river was about 5 m wide and about 1.5 m deep in the deepest areas. The bottom was mainly composed of gravel and small rocks. Some juvenile specimens were found during daylight over or buried in the gravel substrate. The region includes well preserved areas indicating that the species is not threatened with extinction.

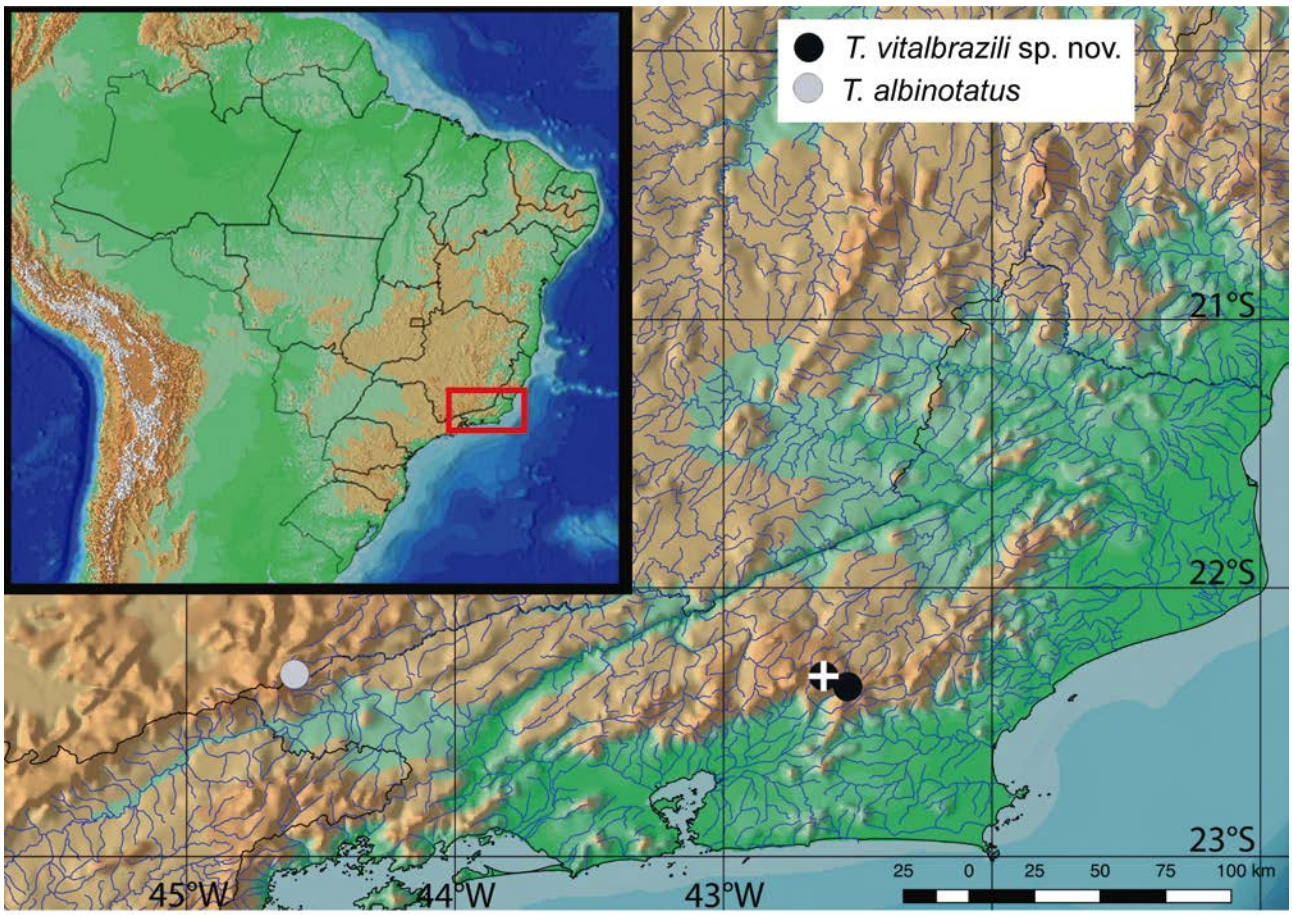

Fig. 7. Geographical distribution of *Trichomycterus vitalbrazili* and *T. albinotatus*. Black circle with a white cross inside indicates type locality.

Fig. 8. Stream tributary to the Grande river drainage, Rio Paraiba do Sul basin, inside the advanced campus of Instituto Vital Brazil in São Lourenço road, the type locality of *Trichomycterus vitalbrazili*.

DISCUSSION

This study highly supported sister group relationships between *Trichomycterus vitalbrazili*, here described, and *T. albinotatus*. Both species are endemic to the Rio Paraíba do Sul basin, but occurring in distinct and disjunct areas (Fig. 7). *Trichomycterus vitalbrazili* is only known from the upper section of the Rio Grande drainage, a right tributary of the lower Rio Paraíba do Sul. This area is situated in the north-eastern part of the Serra dos Órgãos, a mountain range with peaks reaching about 2,275 m asl. No specimen of this species was collected during sporadic field studies between 1990 and 2003 in different parts of the lower Rio Grande drainage (WJEMC, person. observ.), as well as in neighbouring drainages, indicating that this species is geographically restricted to the upper section of the drainage. On the other hand, its sister species *T. albinotatus* is only known from the upper section of the Rio Preto, a tributary of the Rio Paraibuna, which is a left tributary of the middle Rio Paraíba do Sul basin (Costa 1992). The area inhabited by *T. albinotatus*, which is about 200 km from the area inhabited by *T. vitalbrazili*, is part of the Maciço de Itatiaia, a high massif with peaks reaching about 2,900 m asl. During intensive collecting efforts in the middle

and lower section of the Rio Preto drainage and neighbouring drainages, in several expeditions between 1990 and 2019, no specimen of *T. albinotatus* was ever found, supporting a distribution range restricted to the upper portion of the Rio Preto drainage. Interestingly, both *T. albinotatus* and *T. vitalbrazili* are only known from localities above 1100 m asl, suggesting that they are not able to survive in ecological conditions present in lower altitudes.

The present study once again corroborates a high species diversity of *Trichomycterus* concentrated along the river basins draining the Atlantic Forest of south-eastern Brazil (*e.g.*, Costa 1992; Alencar and Costa 2004; Barbosa and Costa 2008; Lima et al. 2008). This biodiversity hotspot is target of heavy anthropogenic pressure and even under conservation policies, the remaining forest fragments are considered small for the persistence of some species (Scarano and Ceotto 2015). Nevertheless, new species of freshwater fishes continue to be described for the region and it is possible that other new species of *Trichomycterus* with restricted distribution ranges can still be found.

CONCLUSIONS

Our molecular analysis first supports a clade comprising two catfish species living in altitudes above to 1100 m, among which one is new and here described. This clade is also supported by three characteristics, one only visible in juveniles. Thus, this study is one of the few showing ontogenetic variation of colour pattern in *Trichomycterus*. The mountains present in the Atlantic Forest of south-eastern Brazil are popularly known for their high species endemism and diversity, which are in great part considered in critically threatened with extinction. Therefore, our study, where a new taxon with restricted geographical distribution is revealed, contributes to a better understanding of this unique biodiversity. More importantly, this study explicitly shows the urgent need to inventory all biotopes of the Atlantic Forest.

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Availability of data and materials: All molecular data and specimens analysed are available in Genbank database and in in the fish collection of Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro (UFRJ).

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Supplementary materials

Table S1. Terminal taxa for molecular phylogeny and respective vouchers and GenBank accession numbers. (download)

Table S2. Substitution models for each gene/partition according to PartitionFinder 2. (download)