

A New Species of *Hypomasticus* from Eastern Brazil Based on Morphological and Molecular Data (Characiformes, Anostomidae)

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A new species of *Hypomasticus* is described from the Rio de Contas, a coastal drainage of eastern Brazil, and its phylogenetic position is proposed based on molecular data. The new species is diagnosed among Anostomidae by possessing a downturned mouth with compressed teeth arranged side by side, 37 or 38 lateral-line scales, three scale series between the dorsal-fin origin and the lateral line and between the lateral line and the pelvic-fin origin, and 12 scale rows around the caudal peduncle. Mitochondrial DNA sequences provide evidence that the new species is closely related to *H. mormyrops*, *H. thayeri*, *H. copelandii*, and *H. steindachneri*, a clade of species endemic to coastal drainages of eastern Brazil. These results combined with a previous phylogeny support the reallocation of the latter two species from *Leporinus* to *Hypomasticus*. The new species is herein considered to be Endangered under the IUCN criteria, due to a small Extent of Occurrence, severely fragmented population, and continued decline of area, extent, and quality of habitat.

SYSTEMATICS is central to exploring and understanding biodiversity, and recent advances in molecular biology have a profound impact in Neotropical fish systematics (Birindelli and Sidlauskas, 2018). They provide additional tools for testing and proposing new hypotheses, being especially useful in species delimitation (e.g., Melo et al., 2016; Mateussi et al., 2019; Serrano et al., 2019), and making species descriptions (Melo et al., 2011; Andrade et al., 2017) and taxonomic revisions (Benine et al., 2015; Silva et al., 2016; Burns et al., 2017) more rigorous and complete. Likewise, hypotheses on species phylogenetic relationships have greatly increased clade supports and sample/species scope when based exclusively on molecular data (Oliveira et al., 2011; Betancur-R. et al., 2019) or using molecular data combined with morphological characters (Arce-H. et al., 2016; Mirande, 2019).

The rate of species descriptions in Characiformes is one of the highest among fish groups, together with other ostariophysan orders Cypriniformes and Siluriformes (Fricke et al., 2019). Yet we are far from understanding the real diversity of those freshwater fishes (Birindelli and Sidlauskas, 2018), especially because there are still many regions in the Neotropics that remain unexplored. The Anostomidae is the second largest family of Characiformes, a megadiverse order of freshwater fishes distributed in both Africa and Central and South America (Sidlauskas and Birindelli, 2017). With 15 genera and around 150 species, our understanding of the phylogenetic relationships among anostomid species and genera have been expanded recently (Sidlauskas and Vari, 2008; Ramirez et al., 2017). However, several clades still need systematic and taxonomic attention, as is the case for species of *Leporinus* and *Hypomasticus* from eastern Brazil.

During recent expeditions in the Rio de Contas, a coastal drainage of Bahia, eastern Brazil, a new medium-sized species

of Anostomidae was discovered. The aim of the present contribution is to describe it, diagnosing it among species of the family, and discuss its phylogenetic relationships using both morphological and molecular data. Furthermore, the conservation status of the new species was evaluated based on the IUCN criteria (IUCN, 2019).

MATERIALS AND METHODS

Material.—Specimens were obtained from scientific collections or collected in the wild. Collecting of fish was authorized by SISBIO (ICMBio) under the process 37910-1 dated 26/02/2013 to JLOB and the samples were conducted in accordance with the policies of the Ethical Conduct Committee on Animal Use (CEUA, process 3014.2018.86) as administered by the Universidade Estadual de Londrina, Brazil. The specimens were euthanized with an anaesthetic overdose of benzocaine, as per Resolution 1000/2012 of the Federal Council of Veterinary Medicine, Brazil. Tissue samples were taken from anesthetized specimens and preserved in 95% ethanol for molecular studies. Specimens were then fixed in 10% formalin for morphological studies and transferred to 70% ethanol in the laboratory for preservation in collection.

All examined specimens are alcohol preserved, except when indicated by CS, which were cleared and stained according to Taylor and Van Dyke (1985), or SK, dry skeleton prepared mostly following methods outlined by Bemis et al. (2004). Institutional abbreviations follow Sabaj (2016).

Procedures and terminology for morphological analyses.—Counts and measurements were taken according to Britski and Garavello (1978) and Winterbottom (1980). Standard length (SL) is expressed in mm, and all other measurements are expressed as percents of SL, except subunits of head,

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Submitted: 3 December 2019. Accepted: 18 March 2020. Associate Editor: R. E. Reis.

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which are expressed as percents of head length (HL). Meristic data are given in description, with frequency of each count provided in parentheses after respective count, and an asterisk indicating counts of holotype. Lateral-line scale counts included pored scales extending onto base of median caudal-fin rays; counts of longitudinal scale rows above lateral line exclude the lateral-line scale row and the middorsal scale row; and counts of longitudinal scale rows below lateral line exclude the lateral-line scale row. Weberian-apparatus vertebrae were counted as four elements, included in the vertebral counts, and the fused PU1+U1 of the caudal region were counted as a single element. *Leporinus copelandii* and *L. steindachneri* are herein transferred to *Hypomasticus* as a result of the phylogenetic hypothesis and treated below as *H. copelandii* and *H. steindachneri* (see Discussion for more details).

Molecular data and phylogenetic analyses.—Genomic DNA from eight specimens of the new species of *Hypomasticus* was extracted following the manufacturer's instructions of Wizard Genomic DNA Purification Kit (Promega). Partial sequences of the mitochondrial *cytochrome oxidase c subunit 1* (*COI*) gene was obtained using the primers FishF1 and FishR2 (Ward et al., 2005). PCR reactions included 15 μ l total volume, with 11.2 μ l of double-distilled water, 1.25 μ l 5X buffer, 0.5 μ l MgCl₂ (50 mM), 0.5 μ l dNTP mix, 0.25 μ l of each primer at 10 μ M, 0.2 μ l PHT Taq DNA polymerase enzyme (5 units/ μ l; Phoeutria, <https://phoeutria.com.br>) and 1.0 μ l genomic DNA (10–50 ng). The PCR program involved 3 min at 95°C (denaturation) followed by 30 cycles of 30 s at 95°C (denaturation), 45 s at 52°C (primer hybridization), and 60 s at 68°C (nucleotide extension), followed by a 10 min at 68°C (final extension). After visualization of fragments/confirmation of amplification using 1% agarose gel, the sequencing reaction was performed using dye terminators (BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems; <https://www.appliedbiosystems.com>) and the products purified through ethanol precipitation. Then, the PCR products tagged by the terminators were loaded onto an automatic sequencer ABI 3130-Genetic Analyzer (Applied Biosystems) in LBP/UNESP, Botucatu, Brazil.

Forward and reverse sequences were assembled and edited to generate a single consensus for each individual in Geneious v11.1.2 (Kearse et al., 2012). We also used *COI* sequences of other *Hypomasticus* from eastern Brazil and of many genera of Anostomidae available in GenBank (<https://www.ncbi.nlm.nih.gov>) or BOLD (<https://www.boldsystems.org>) mostly from previous studies that used well-identified specimens (Ramirez et al., 2016, 2017). We used one sample of *Caenotropus mestomorgmatos* (Chilodontidae) to root all the trees. All sequences were aligned using Muscle v3.8.4 (Edgar, 2004) in Geneious v11.1.2 with default parameters, and had trimmed ends to minimize missing data. Vouchers, locality information, and GenBank accession numbers are summarized in Table S1 (supplementary material; see Data Accessibility; new sequences have been assigned GenBank accession numbers MT427905–MT427935).

Substitution saturation was calculated in DAMBE v5.3.38 (Xia, 2013) and genetic distances in MEGA v7.0 (Kumar et al., 2016). Sequences of species of *Hypomasticus* (including *H. copelandii* and *H. steindachneri*) and closely related species of *Leporinus* (*L. granti* and *L. nijssenii*) were binned into species

groups to calculate the overall mean distance (distance among all specimens), the intraspecific distances (among specimens of each species), and interspecific distances (among species groups) with 1,000 bootstrap replicates. A total of ten groups were identified based on preliminary topologies (neighbor-joining and maximum likelihood analyses). The level of support for each node of the neighbor-joining (NJ) tree was inferred by 1,000 bootstrap pseudo-replicates in MEGA v7.0. The best maximum likelihood (ML) tree was obtained in RAxML-HPC2 on XSEDE implemented in RAxML v8.019 (Stamatakis, 2014) on CIPRES webserver (Miller et al., 2011) using the GTRGAMMA+I model (Stamatakis et al., 2008) and 1,000 bootstrap pseudoreplicates.

***Hypomasticus santanai* Birindelli and Melo, new species**

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Figures 1, 2; Table 1

Holotype.—MZUEL 18604, 208.2 mm SL, Brazil, Bahia, Dario Meira, Rio Gongogi, tributary of Rio de Contas, approximately 2 km above city, 14°27'44.2"S, 39°55'01.4"W, J. L. Birindelli, F. Jerrep, E. Santana, and R. Nascimento, 2 July 2016.

Paratypes.—All from Brazil. LBP 25152, 3, 197.0–237.0 mm SL (tissues 90176–90179); LBP 28094, 7, 150.3–210.4 mm SL (tissues 96847–96850); MZUEL 20020, 1, 159.4 mm SL, Bahia, Iguai, Rio Gongogi, tributary of Rio de Contas, Fazenda Ipanema, 14°45'23"S, 40°05'21"W, L. R. Ribeiro-Silva and E. Sobrinho, 8 January 2018; MZUEL 18605, 1, 179.6 mm SL, Bahia, Gongogi, Rio Gongogi, tributary of Rio de Contas, at Zebrinha, 14°16'36.8"S, 39°37'27.8"W, J. L. Birindelli, F. Jerrep, E. Santana, and R. Nascimento, 1 July 2016.

Diagnosis.—*Hypomasticus santanai* is distinguished from all other Anostomidae, except congeners and some species of *Leporellus*, *Leporinus*, and *Megaleporinus* by having a downturned mouth (vs. terminal or upturned mouth; Fig. 2). *Hypomasticus santanai* is distinguished from species of *Leporellus* by having caudal-fin lobes naked (vs. lobes covered by scales), three dark midlateral blotches on the lateral surface of the body (vs. midlateral or vertical stripes on the body or absence of pigmentation), and 12 scale rows around caudal peduncle (vs. 16). *Hypomasticus santanai* is distinguished from species of *Megaleporinus* by having four premaxillary teeth (vs. three). *Hypomasticus santanai* is distinguished from congeners and species of *Leporinus* by having 12 scale rows around caudal peduncle (vs. 16 in all species except *H. copelandii*, *H. mormyrops*, *H. pachycheilus*, *H. steindachneri*, *H. thayeri*, *Leporinus amae*, *L. amblyrhynchus*, *L. bahiensis*, *L. bistriatus*, *L. boelckeii*, *L. britskii*, *L. gomesi*, *L. marcgravi*, *L. melanopleura*, *L. melanopleurodes*, *L. microphthalmus*, *L. multimaculatus*, *L. tristriatus*, *L. nijssenii*, *L. octomaculatus*, *L. reticulatus*, *L. sexstriatus*, and *L. trimaculatus*), and three scale rows between dorsal-fin origin and lateral line and between lateral line and pelvic-fin origin (vs. four or more scale rows in all species except *L. amae*, *L. bistriatus*, *L. melanopleura*, *L. melanopleurodes*, *L. marcgravi*, *L. microphthalmus*). *Hypomasticus santanai* is distinguished from *L. amae*, *L. bistriatus*, *L. melanopleura*, and *L. melanopleurodes*



Fig. 1. *Hypomasticus santanai*, holotype, MZUEL 18604, 208.2 mm SL, Brazil, Bahia, Rio Gongogi, tributary of Rio de Contas, preserved specimen (A) and live specimen (B). Scale bars equal 10 mm.

by having three dark midlateral blotches (vs. dark midlateral stripe), and from *L. marcgavii* and *L. microphthalmus* by having three dark midlateral blotches (vs. a series of seven to nine dark midlateral blotches).

Description.—Morphometric data summarized in Table 1. Medium size relative to congeners. Largest examined specimen 237.7 mm SL. Head and body elongate and moderately compressed. Dorsal profile distinctly convex from snout tip to vertical through anterior nares, gently convex from latter point to dorsal-fin origin, more or less straight along dorsal-fin base, straight from dorsal-fin terminus to adipose-fin origin, and slightly concave from adipose-fin origin to anteriormost dorsal caudal-fin procurrent ray. Ventral profile concave from tip of lower jaw to posterior margin of opercle, gently convex from latter point to anal-fin origin, distinctly convex along anal-fin base, and distinctly concave from base of last anal-fin ray to anteriormost ventral caudal-fin procurrent ray. Greatest body depth at dorsal-fin origin.

Mouth subinferior, its cleft longitudinally aligned with ventral margin of opercle in specimens around 200 mm SL. Snout rounded, anteriorly blunt. Premaxillary bone with four teeth arranged side by side and gently decreasing in size from symphyseal tooth (Fig. 2). Dentary bone with four teeth arranged side by side, and gently decreasing in size laterally.

Teeth incisiform, compressed, unicuspid and with blunt margin.

Lateral line complete with 37* (5) or 38 (1) perforated scales, extending from supracleithrum to base of median caudal-fin rays. Horizontal scale rows between dorsal-fin origin and lateral line 3* (6). Horizontal scale rows between lateral line and pelvic-fin origin 3* (6). Horizontal scale rows around caudal peduncle 12* (6). Predorsal scales from dorsal-fin origin to tip of supraoccipital spine 11* (4) or 12 (2).

Dorsal fin ii,10 (5) or i,11* (1). Dorsal-fin origin slightly anterior to middle of standard length and to vertical through pelvic-fin origin; its distal margin straight or gently concave or convex. Adipose fin small, teardrop shaped, its origin approximately at vertical through base of fourth anal-fin branched ray. Pectoral fin i,14* (2), i,15 (3), or i,16 (1); its tip extending more than half distance between origins of pectoral and pelvic fin; its distal margin rounded. Pelvic fin i,8 (6); its tip extending approximately half distance between origins of pelvic and anal fins, with distal margin slightly convex. Anal fin ii,8* (3) or ii,9 (3); its origin approximately at vertical through second scale anterior to adipose-fin origin, when adpressed fin not reaching base of caudal-fin rays; its distal margin straight or slightly concave. Principal caudal-fin rays i,8,9,i* (6). Caudal fin forked, lobes rounded, upper lobe slightly longer than lower lobe.

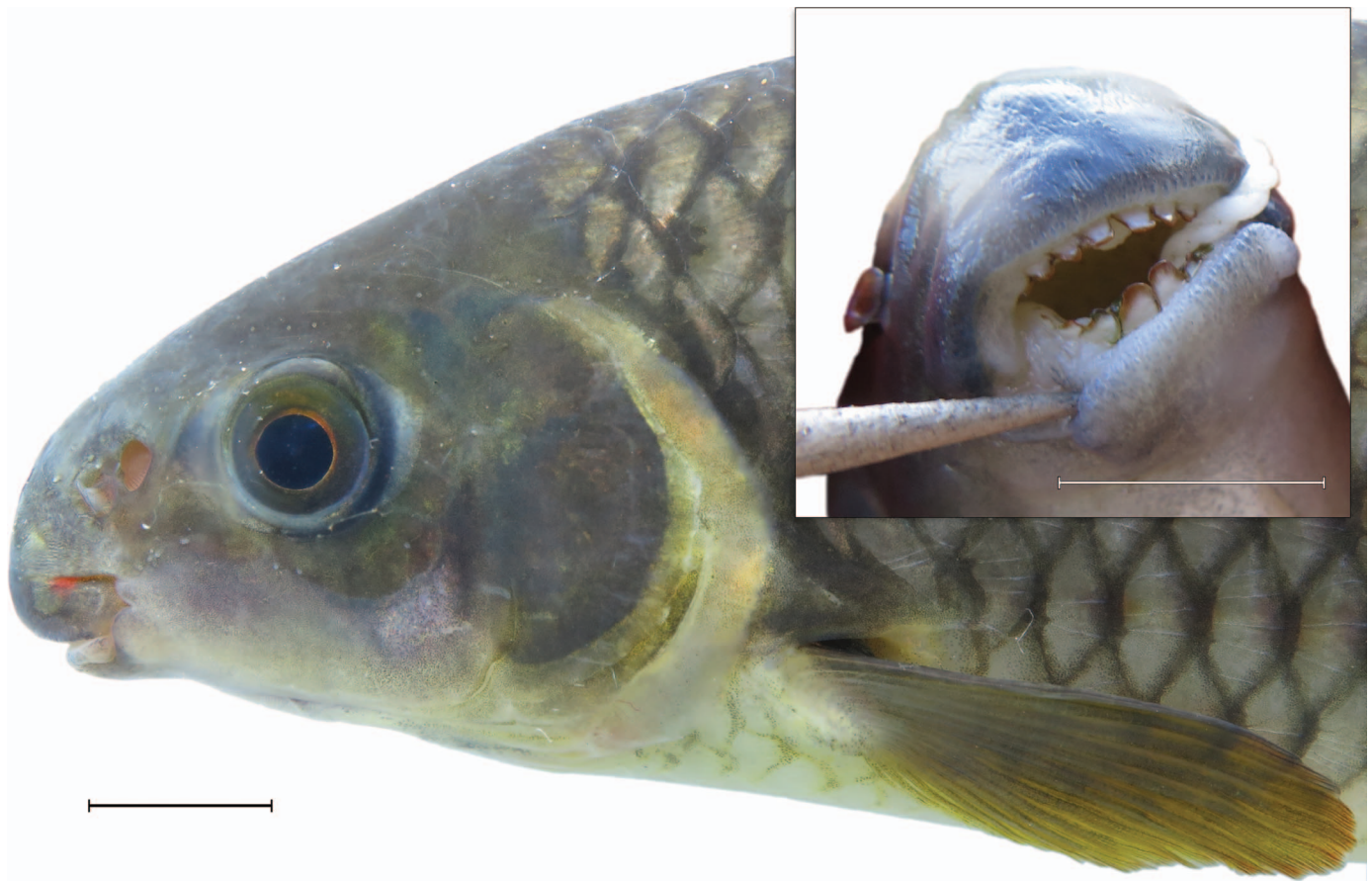


Fig. 2. *Hypomasticus santanai*, holotype, MZUEL 18604, 208.2 mm SL, Brazil, Bahia, Rio Gongogi, tributary of Rio de Contas, preserved specimen, in lateral view of head, and ventral view of mouth in detail with mouth partially opened by forceps showing teeth. Scale bars 10 mm.

Color in alcohol.—Ground color of head and body dark gray and countershaded. Trunk with three dark midlateral blotches, first centered on vertical through base of last third of dorsal-fin base, second centered on vertical through base of anteriormost anal-fin ray, third on posteriormost portion of caudal peduncle. Blotches decreasing in size posteriorly. Ventral surfaces of head and body pale to cream. All fins generally dusky (Fig. 1A).

Color in life.—Live specimens with ground color of lateral face of head slightly green around eyes and opercle; scales of lateral trunk reticulated and each slightly orange on its anteriormost portion; head and trunk completely pale ventrally. Iris slightly orange near pupil; posterior portion of upper lip bearing red spot; dorsal, pectoral, and pelvic fins dark orange; adipose, anal, and caudal fins dark yellow (Fig. 1B).

Table 1. Morphometric data for *Hypomasticus santanai*, new species. SD means standard deviation.

	<i>n</i>	Mean	Range	SD	Holotype
Standard length (mm)	6	200.14	159.4–237.7	—	208.2
Percentages of standard length					
Predorsal distance	6	49.47	47.06–51.07	1.33	49.47
Dorsal-fin origin to adipose-fin origin	6	39.95	37.78–41.86	1.64	37.78
Prepelvic distance	6	51.36	49.77–52.47	0.95	51.13
Body depth	6	25.91	24.62–26.87	0.81	26.46
Caudal peduncle length	6	10.41	9.85–10.80	0.33	9.85
Caudal peduncle depth	6	11.70	10.70–12.39	0.63	11.71
Anal-fin lobe length	6	13.25	11.56–14.79	1.12	11.56
Head length	6	21.33	20.38–22.34	0.71	21.25
Percentages of head length					
Preopercle length	6	75.86	73.20–78.74	2.06	73.80
Snout length	6	42.49	38.95–45.11	2.34	43.31
Head depth	6	85.11	80.27–87.67	2.68	87.14
Eye diameter	6	20.96	19.07–23.31	1.49	21.18
Bony interorbital	6	44.97	41.42–46.76	1.96	45.93

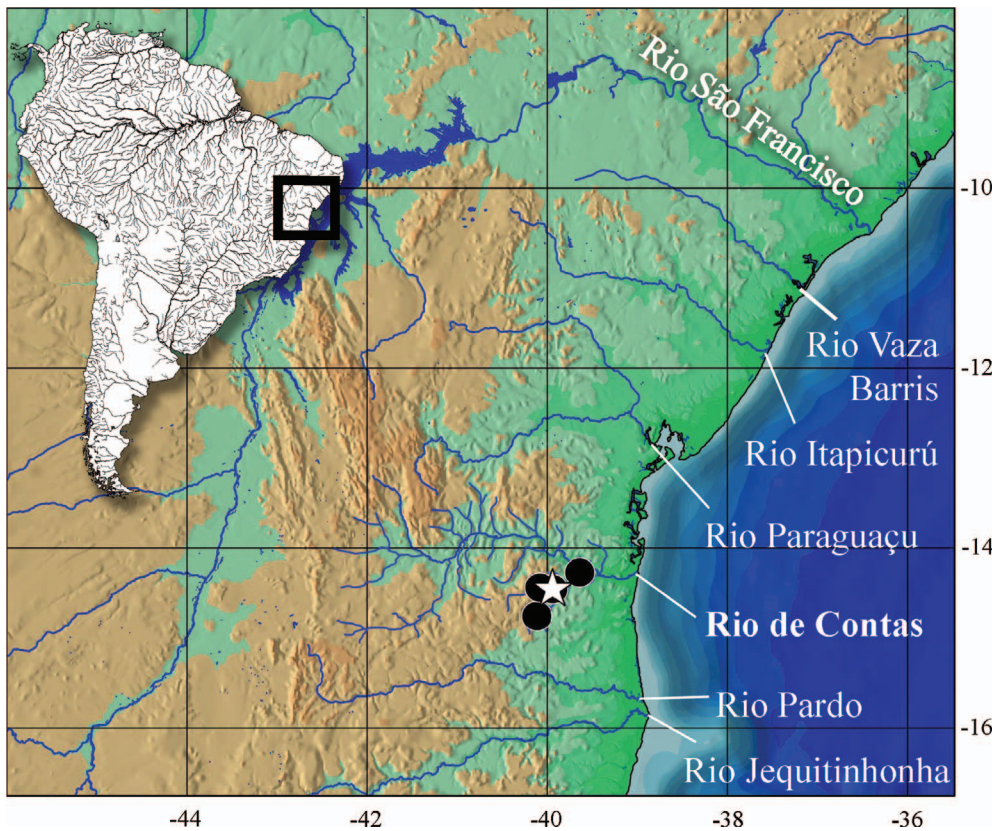


Fig. 3. Geographic distribution of *Hypomasticus santanae* in eastern Brazil. Star represents type locality.

Distribution.—*Hypomasticus santanae* is only known from the Rio Gongogi and so far endemic to the Rio de Contas basin, a coastal drainage in the eastern portion of the state of Bahia, Brazil (Fig. 3).

Ecological notes.—*Hypomasticus santanae* was collected in clear water, in rapids with sandy and rocky bottom, with exposed bedrock covered by Podostemaceae, and marginal vegetation covered by macrophytes (Fig. 4). *Hypomasticus santanae* was collected in sympatry with *Leporinus bahiensis*, *Megaleporinus brinco*, and *M. gaiero* (Birindelli et al., 2020). As morphologically similar congeners, such as *H. mormyrops* and *H. thayeri*, the species is probably restricted to fast flowing stretches of the Rio de Contas and tributaries.

Conservation status.—*Hypomasticus santanae* is apparently endemic to the Rio de Contas basin, including the lower portion of the Rio Gongogi, its largest tributary. The Rio de Contas is approximately 500 km long and drains an area of approximately 50,000 km² that is covered by the Caatinga (=Brazilian xeric shrublands) in the west, and by the Atlantic Forest in the east. The upper portion of the basin, covered by Caatinga, is composed of intermittent rivers. The increasing practice of building dams for water storage in the area is turning rivers more intermittent. As a consequence, river stretches used to sustain health fish populations throughout the year were found completely dry during the winter (pers. obs.). In addition, the Rio de Contas flows through several urban communities, including some of the largest cities of southeastern Bahia (Ipiaú and Jequié), and has a large reservoir of 101 km² at 18 km upstream from Jequié (at 13°52'S, 40°14'W, built in 1978) that has impacted fish migration and water quality. *Hypomasticus santanae* is rare

and probably restricted to fast flowing stretches of the Rio de Contas and tributaries. It is likely that the species was once much more widespread in the Rio de Contas basin, restricted now to a few better-preserved areas in the Rio de Contas drainage, such as the Rio Gongogi. The same situation was observed in *Megaleporinus gaiero* (Birindelli et al., 2020).

The Extent of Occurrence (EOO) of *H. santanae* was calculated as 830 km² (using a polygon uniting known sampling sites), satisfying the IUCN criterion B1 (IUCN, 2019). In addition, the population is considered severely fragmented (IUCN sub-criterion a) for two conditions. First, because the tributaries of the Rio de Contas are continually becoming more intermittent due to the increasing practice of building dams for water storage. And second, due to the construction of the Usina de Pedra, a hydroelectric dam that transformed approximately 50 km of lotic habitat into a lentic lake and isolated populations above and below it. It is noteworthy that the species is probably restricted to fast flowing stretches of the Rio de Contas and tributaries. In addition, we inferred a continued decline in area, extent, or quality of habitat (IUCN sub-criterion biii) due to the increase of urban occupation and its effects (as mentioned above). Therefore, *Hypomasticus santanae* is herein considered to be Endangered (EN) under the IUCN criterion (B1abiii).

Phylogenetic relationships.—The molecular matrix of *COI* included 64 terminal taxa with 630 bp with 243 variable sites (38.5%) and without substitution saturation. The overall mean genetic distance among the ten delimited groups of *Hypomasticus* and related taxa (see Methods) was 0.105±0.009. Intraspecific genetic distances varied from 0.000 in *H. copelandii*, *H. lineomaculatus*, *H. mormyrops*, and

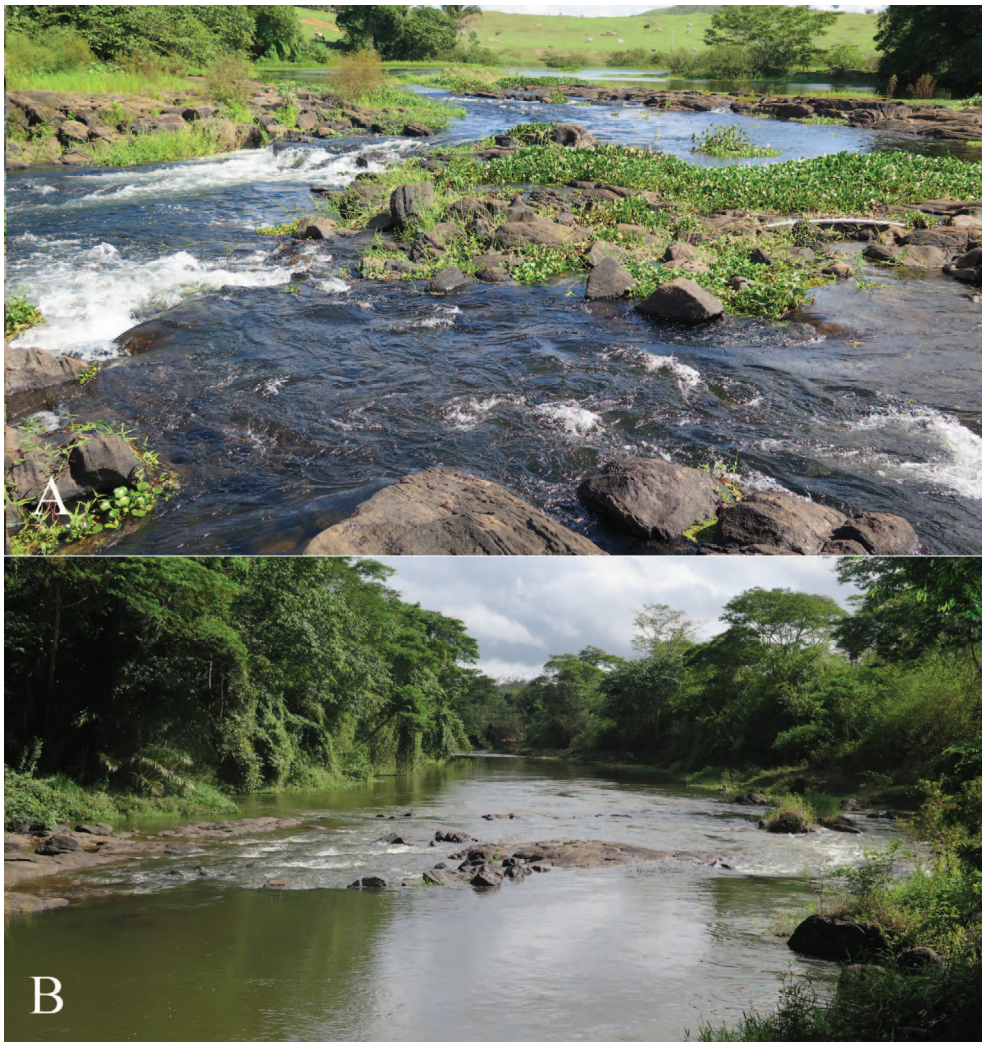


Fig. 4. Sample sites of *Hypomasticus santanai*. (A) Rio Gongogi, tributary of Rio de Contas, approximately 2 km above city of Dario Meira, 14°27'44.2"S, 39°55'01.4"W; (B) Rio Gongogi, tributary of Rio de Contas, at Zebrinha, 14°16'36.8"S, 39°37'27.8"W. All in Brazil, Bahia state.

H. santanai to 0.010 in *H. thayeri*. Interspecific distances ranged from 0.026±0.007 between *H. despaxi* and *H. multi-maculatus* to 0.196±0.023 between *H. pachycheilus* and *H. thayeri*. The lowest values of genetic distance from the new species *H. santanai* are from *H. steindachneri* (0.047±0.009) followed by *H. copelandii* (0.065±0.011) and *H. mormyrops* (0.065±0.011). The highest values compared to *H. santanai* are from *H. pachycheilus* (0.183±0.022) and *H. lineomaculatus* (0.181±0.022; Table 2).

The best maximum likelihood tree (Fig. 5; sum of branch lengths [SBL] = 3.40454700) and the neighbor-joining tree (Fig. S; see Data Accessibility; SBL = 2.04156676) returned topologies that support reciprocal monophyly between the neighbor-joining and likelihood topologies for each species in the clade of *Hypomasticus* from eastern Brazil. Main differences are related to position of major species groups (e.g., *H. despaxi*, *H. lineomaculatus*, and *H. megalepis*, a clade from the Guiana Shield that appears at different places in

Table 2. Pairwise K2P genetic distance among genetic lineages of closely related species of *Hypomasticus* and *Leporinus* (below diagonal) and values of standard error (above diagonal). Numbers in bold represent intraspecific genetic distance. Groups were ordered on the basis of the maximum likelihood analysis.

		1	2	3	4	5	6	7	8	9	10
1	<i>Hypomasticus pachycheilus</i>	—	0.021	0.019	0.020	0.019	0.021	0.023	0.021	0.021	0.022
2	<i>Hypomasticus lineomaculatus</i>	0.165	0.000	0.007	0.012	0.018	0.021	0.023	0.022	0.022	0.022
3	<i>Hypomasticus despaxi</i>	0.146	0.026	—	0.012	0.018	0.020	0.022	0.021	0.021	0.021
4	<i>Hypomasticus megalepis</i>	0.160	0.076	0.076	0.004	0.019	0.021	0.023	0.021	0.021	0.022
5	<i>Leporinus granti/L. nijsseni</i>	0.150	0.140	0.143	0.144	0.008	0.015	0.017	0.016	0.015	0.016
6	<i>Hypomasticus mormyrops</i>	0.178	0.166	0.162	0.167	0.096	0.000	0.012	0.008	0.010	0.011
7	<i>Hypomasticus thayeri</i>	0.196	0.191	0.185	0.192	0.118	0.062	0.010	0.009	0.012	0.013
8	<i>Hypomasticus copelandii</i>	0.171	0.179	0.168	0.161	0.101	0.034	0.040	0.000	0.009	0.011
9	<i>Hypomasticus steindachneri</i>	0.166	0.186	0.165	0.163	0.103	0.051	0.066	0.049	0.007	0.009
10	<i>Hypomasticus santanai</i>	0.183	0.181	0.167	0.176	0.111	0.065	0.077	0.065	0.047	0.000

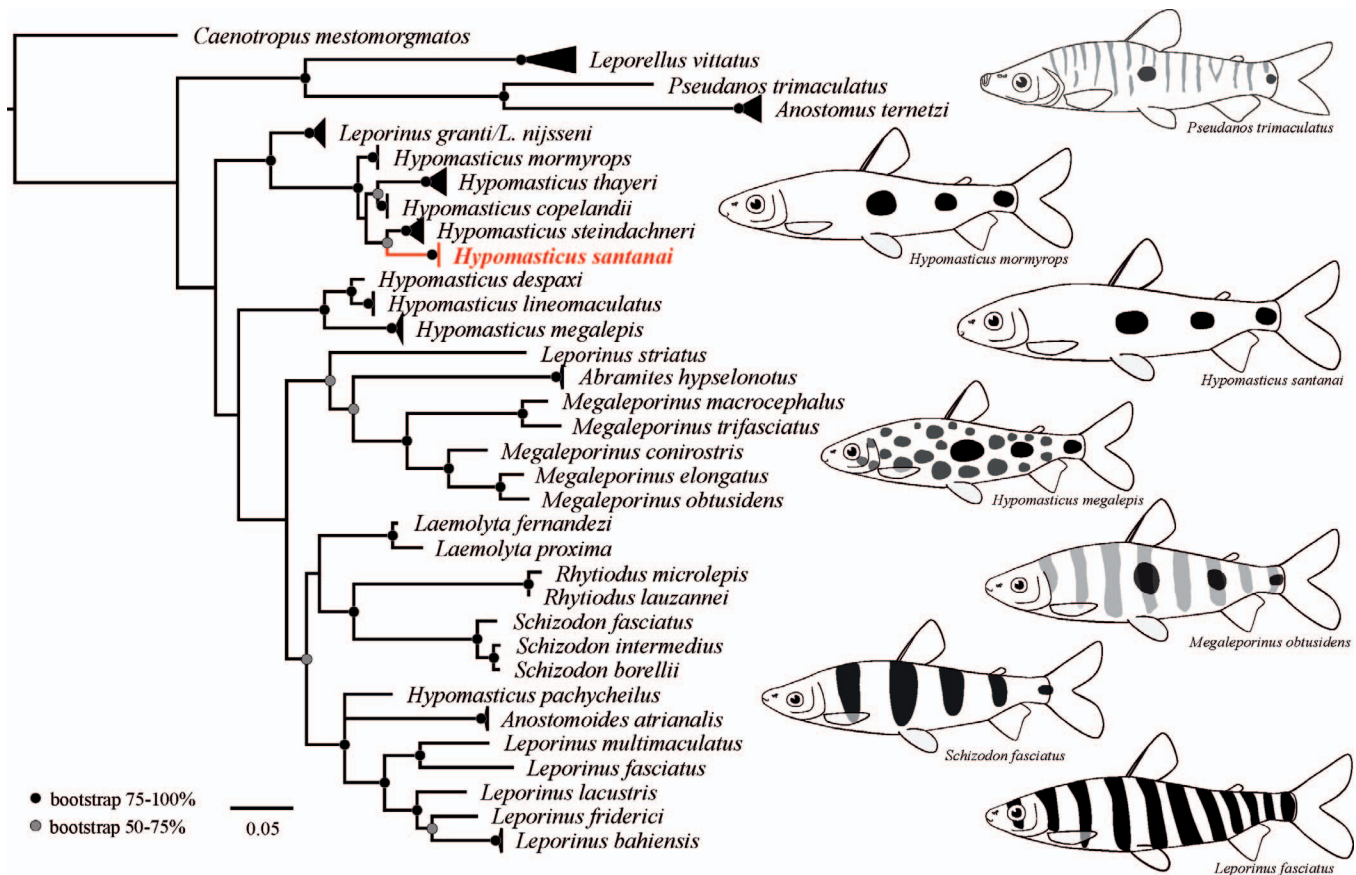


Fig. 5. Maximum likelihood tree of Anostomidae showing the phylogenetic placement of *Hypomasticus santanae*. Bootstrap support is indicated by black (75–100%) and gray dots (50–75%); nodes with support lower than 50% have no dots. See Data Accessibility for tree file.

different analyses); however, relationships among major clades in Anostomidae are out of the scope [of this work]. The phylogenetic analysis placed *H. santanae* as closely related to *H. steindachneri* from Rio Jequitinhonha and Rio Mucuri and with other species from eastern Brazil: *H. mormyrops* from Rio Paraíba do Sul (type species of *Hypomasticus*), *H. copelandii* from Rio Doce and Rio Mucuri, and *H. thayeri* from Rio Paraíba do Sul (Fig. 5). Based on this evidence and on previous phylogenetic hypotheses (Ramirez et al., 2016, 2017), both *H. copelandii* and *H. steindachneri* are herein transferred from *Leporinus* to *Hypomasticus* and the new species is described as a *Hypomasticus*.

Etymology.—The new species is named in honor to Edson Santana, a technician at the MZUEL since 1993, for his help in collecting fishes, preparing vertebrate specimens, and maintaining the collections of the MZUEL. His enthusiasm captivated generations of staff and students of the MZUEL. In addition, Edson Santana was in the expedition that resulted in the discovery of *H. santanae*.

DISCUSSION

Hypomasticus santanae is one of the seven Anostomidae currently considered to be Endangered, along with *H. thayeri*, *Leporinus guttatus*, *L. pitingai*, *Megaleporinus gaiero* (Birindelli et al., 2020), *M. muyscorum* (Mojica et al., 2012), and *Sartor tucuruensis* (ICMBio, 2018), and the second Endangered species living in the Rio de Contas drainage. In total, four species of Anostomidae occur in the Rio de Contas basin: *H.*

santanae, *L. bahiensis*, *M. brinco*, and *M. gaiero* (Birindelli et al., 2013, 2020). These species are locally known as “piau-bafu,” “piau-preto,” “piau-brinco,” and “piau-gaiero,” respectively. *Hypomasticus santanae* is distinguished from *L. bahiensis*, *Megaleporinus brinco*, and *M. gaiero* by having a downturned mouth with compressed teeth arranged side by side (vs. mouth subterminal with robust teeth arranged in stair steps), and four premaxillary teeth (vs. three). In addition, *H. santanae* differs from *Megaleporinus gaiero* (Birindelli et al., 2020) by having 12 scale rows around caudal peduncle (vs. 16), and body with three dark midlateral blotches (vs. body with three dark midlateral blotches and an irregular dark longitudinal stripe from opercle to second midlateral blotch; and from *M. brinco* by lacking a bright red blotch immediately at dorsal of the pectoral-fin origin in live specimens (vs. presence), and midlateral blotches distinctly decreasing in size posteriorly (vs. blotches increasing in size).

Our phylogenetic analysis based on mitochondrial data showed strong support for a clade that included *Hypomasticus mormyrops*, the type species of *Hypomasticus*, and also *H. thayeri*, *H. santanae*, and two species traditionally placed in *Leporinus* and herein transferred to *Hypomasticus*: *H. copelandii* and *H. steindachneri*. The close relationship between *H. copelandii*, *H. steindachneri*, and *H. mormyrops* was also recovered in a previous molecular phylogeny of Anostomidae based on two mitochondrial and three nuclear genes (Ramirez et al., 2016), and in the analysis of morphological and molecular data of Characidae that included anostomids as outgroup taxa (Mirande, 2019).

Hypomasticus copelandii, *H. mormyrops*, *H. santanai*, *H. steindachneri*, and *H. thayeri* share three main morphological features: 1) color pattern composed of three dark midlateral blotches on body, 2) four teeth on each premaxilla and dentary, and 3) 12 scale rows around caudal peduncle. In addition, these species are endemic to coastal drainages of eastern Brazil. *Hypomasticus copelandii*, *H. mormyrops*, and *H. thayeri* occur in coastal drainages ranging from the Rio Paraíba do Sul to Rio Mucuri, including Rio Doce and Rio Itapemirim, whereas *H. steindachneri* was described based on specimens from the Rio Jequitinhonha and occur in some of the southern drainages, including Rio Paraíba do Sul, Rio Doce, Rio Jururuçu, Rio Mucuri, and Rio São Mateus basins. Among these four species, *H. santanai* share with *H. thayeri* and *H. mormyrops* the possession of a downturned mouth with compressed teeth arranged side by side, whereas *H. copelandii* and *H. steindachneri* have subterminal mouths with robust teeth arranged in stair steps. The taxonomy of *H. copelandii* still needs work as the type series is large and includes at least three distinct species. The distribution of *H. steindachneri* also has to be better evaluated, as its distribution is wider than other species of Anostomidae from the eastern drainages of South America. *Hypomasticus santanai* differs from *H. mormyrops* and *H. thayeri* by having three scale rows between dorsal-fin origin and lateral line and between lateral line and pelvic-fin origin (vs. four or more scale rows). Even though the new species is morphologically similar to *H. mormyrops* and *H. thayeri*, genetic evidence indicates that it is closer to *H. steindachneri* (Fig. 5, Fig. S; see Data Accessibility).

MATERIAL EXAMINED

See also Birindelli et al. (2013, 2020) for lists of comparative material also used in this study.

Hypomasticus copelandii: ANSP 173832, 23, 23.2–196.4 mm SL; ANSP 173834, 2, 70–114 mm SL; ANSP 173836, 5, 34.3–115.5 mm SL; ANSP 173842, 1, 108.4 mm SL; ANSP 173843, 5, 87.0–119.1 mm SL; BMNH 1934.9.30.2-3, 2, 61.84 mm SL; CAS 70573, 1, 107.2 mm SL; CAS 70574, 1, 103.5 mm SL; CAS 70584, 1, 218 mm SL; MCP 20382, 1, 126.6 mm SL; MCP 20386, 1, 131.9 mm SL; MCZ 20361, 6, 131–218 mm SL; MCZ 20363, 1, 183.3 mm SL; MCZ 20365, 1, 135 mm SL; MCZ 20402, 1, 142.7 mm SL; MCZ 20408, 3, 202.6–266.5 mm SL, syntypes; MCZ 20415, 1, 321 mm SL; MCZ 20424, 9, 89–206 mm SL; MCZ 97550, 4, 90.7–167.5 mm SL; MNHN 13-134, 1, 123 mm SL; MNRJ 10524, 1, 159 mm SL; MNRJ 14743, 1, 141.8 mm SL; MNRJ 16918, 1, 119 mm SL; MNRJ 18391, 6, 148 mm SL; MNRJ 28336, 1, 52.5 mm SL; MNRJ 31330, 2, 200 mm SL; MZUSP 1779, 138.9 mm SL; MZUSP 1292, 2, 150.5–180.3 mm SL; MZUSP 3045, 1, 218 mm SL; MZUSP 35814, 1, 267 mm SL; MZUSP 42902, 2, 70.9–116.5 mm SL; MZUSP 47897, 1, 51.4 mm SL; MZUSP 47900, 1, 113.1 mm SL; MZUSP 47901, 1, 117.4 mm SL; MZUSP 48041, 1, 101.2 mm SL; MZUSP 52561, 1, 132.1 mm SL; MZUSP 66177, 2, 90.9–152.8 mm SL; MZUSP 66179, 4, 33.7–122.7 mm SL; MZUSP 69319, 3, 43–53.3 mm SL; MZUSP 73106, 1, 258 mm SL; MZUSP 73128, 2, 267–280 mm SL; MZUSP 73132, 2, 273–300 mm SL; MZUSP 75054, 82.2 mm SL; MZUSP 75076, 2, 93.5–128 mm SL, 1 CS, 101.5 mm SL; MZUSP 75083, 2, 193–214 mm SL; MZUSP 75291, 1, 89.7 mm SL; MZUSP 75293, 1, 90.1 mm SL; MZUSP 75333, 113.4 mm SL; MZUSP 75350, 91.8 mm SL; MZUSP 75391, 1, 107.7

mm SL; MZUSP 75393, 1, 98.4 mm SL; MZUSP 80297, 1, 100.3 mm SL; MZUSP 80995, 1, 88.2 mm SL; MZUSP 81004, 1, 153.8 mm SL; MZUSP 81005, 2, 145–236 mm SL; MZUSP 87886, 1 CS, 101.5 mm SL; MZUSP 108322, 1 SK, 370 mm SL; MZUSP 108463, 1 CS, 113 mm SL; NMW 68391.1, 2, 218.4–357.4 mm SL, syntypes; NMW 68391.2, 2, 144.3–151.2 mm SL, syntypes; NMW 68387, 3, 129.8–159.0 mm SL, syntypes; NMW 38386, 1, 170.4 mm SL; NMW 68384, 1, 107.3 mm SL, syntype; NMW 68393, 1, 148.8 mm SL, syntype; NMW 68394, 3, 38.8–46.4 mm SL; NMW 69592, 2, 47.9–53.5 mm SL, syntype.

Hypomasticus mormyrops: CAS 70613, 1, 135.6 mm SL; MCP 8986, 1, 105.3 mm SL; MCP 27355, 1, 125 mm SL; MCP 34699, 2, 127.5–131 mm SL; MCZ 20369, 126.8 mm SL, syntype; MCZ 20366, 5, 99.1–137.5 mm SL, syntypes; MCZ 20425, 1, 175 mm SL; MCZ 20764, 1, 135.5 mm SL; MNHN 13-132, 1, 105.7 mm SL; MNHN 13-133, 1, 108.3 mm SL; MZUEL 15901, 6, 91.8–181.6 mm SL; MZUSP 1750, 2, 119.8–126.7 mm SL; MZUSP 48502, 1, 207 mm SL; MZUSP 48504, 6, 102–130.8 mm SL; MZUSP 50814, 1, 173.1 mm SL; MZUSP 58485, 1, 126.2 mm SL; MZUSP 63688, 1, 173.5 mm SL; MZUSP 66164, 2, 150.9–152.7 mm SL; MZUSP 66180, 1, 106 mm SL; MZUSP 73109, 2, 94.2–136.7 mm SL; MZUSP 73117, 1, 111 mm SL; 1 CS, 99.8 mm SL; MZUSP 73140, 1, 122 mm SL; MZUSP 75306, 1, 87.9 mm SL; MZUSP 75343, 2, 66.2–102 mm SL; MZUSP 75381, 1, 116.2 mm SL; MZUSP 75383, 1, 149 mm SL; MZUSP 75388, 2, 89.8–135.7 mm SL; MZUSP 80962, 2, 123.3–135 mm SL; MZUSP 81008, 1, 193.8 mm SL; MZUSP 110150, 2 SK, 155–156 mm SL; NMW 68354, 1, 175.1 mm SL, syntype; NMW 68351, 1, 159.2 mm SL; NMW 68352, 2, 136.3–142.6 mm SL, syntypes; NMW 68353, 2, 120.2–131.6 mm SL, syntypes; NMW 68350, 3, 139.5–150.6 mm SL, syntypes; USNM 120420, 1, 127.6 mm SL.

Hypomasticus steindachneri (Jequitinhonha and Pardo basins): MZUEL 16474, 8, 100–207 mm SL, 2 SK, 189–194 mm SL; MZUEL 17996, 9, 99.4–135.1 mm SL; MZUSP 112108, 4 SK, 202 mm SL; NMW 68405, 1, 323 mm SL, holotype.

Hypomasticus cf. *steindachneri* (Paraíba do Sul, Doce, Jururuçu, Mucuri and São Mateus basins): MCZ 50405, 1, 106.8 mm SL; MCZ 20416, 2, 233 mm SL; MCZ 20418, 1, 270 mm SL; MNRJ 13601, 1, 195 mm SL; MZUSP 1557, 1, 167 mm SL; MZUSP 1561, 124.8 mm SL; MZUSP 1680, 1, 96.5 mm SL; MZUSP 1779, 1, 68.3 mm SL; MZUSP 3614, 1, 163.4 mm SL; MZUSP 28984, 2, 183–229 mm SL; MZUSP 36650, 5, 184–204 mm SL; MZUSP 36667, 4, 223–330 mm SL; MZUSP 47896, 1, 226 mm SL; MZUSP 47898, 1, 204.5 mm SL; MZUSP 47899, 1, 265 mm SL; MZUSP 47901, 1, 85 mm SL; MZUSP 48470, 2, 192218 mm SL; MZUSP 58975, 2, 106–257 mm SL; MZUSP 63464, 3, 116.1–175 mm SL; MZUSP 80297, 1, 100.3 mm SL; MZUSP 80999, 1, 196 mm SL.

Hypomasticus thayeri: MCZ 20364, 1, 131.5 mm SL, holotype; MCZ 20368, 1, 187.6 mm SL; MCZ 20414, 1, 192 mm SL; MNRJ 15323, 1, 166.4 mm SL; MNRJ 16895, 1, 206.7 mm SL; MZUEL 10561, 1, 163.9 mm SL; MZUSP 41727, 1, 67.8 mm SL; MZUSP 62747, 2, 198.5–240 mm SL; MZUSP 75388, 2, 63.4–106.3 mm SL; MZUSP 75383, 10, 84.1–117.4 mm SL; MZUSP 87899, 1 CS, 87.7 mm SL; NMW 68233, 1, 131.3 mm SL.

DATA ACCESSIBILITY

Supplemental material is available at <https://www.copeiajournal.org/ci-19-335>.

ACKNOWLEDGMENTS

Thanks are due to B. Sidlauskas for comments that helped to improve the manuscript, to Peter van der Sleen for permitting us to use his fish drawings, and to N. Barreto for helping collect specimens of *Hypomoasticus santanai* in 2016 using his homemade spearfishing equipment and outstanding skills. Authors were financially supported by FAPESP (grant 10/51250-9 to JLOB, grants 16/11313-8 and 18/24040-5 to BFM, grant 14/26508-3 to CO), Fundação Araucária (grant 177/2014, JLOB), and CNPq (grants 420255/2016-8 and 302872/2018-3 to JLOB and grant 404991/2018-1 to BFM).

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