

A new miniature species of *Priocharax* (Characiformes: Characidae) from the upper rio Ipixuna, Purus drainage, Brazil



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A new species of miniature fish of the characid genus *Priocharax* is described from a small lake near the rio Ipixuna, rio Purus drainage, Amazonas State, Brazil. It is distinguished from all congeners except *P. pygmaeus* by the lower number of teeth on the maxilla and dentary. It differs from *P. pygmaeus* by the presence of two postcleithra and 22–27 branched anal–fin rays (*vs* absence and 19–22). The new species is further distinguished from other species of *Priocharax* by a combination of characters involving the number of pelvic–fin rays and branched anal–fin rays, the number of postcleithra, the shape of postcleithrum 3, and the absence of the claustrum. Molecular evidence based on COI sequences of all valid species of *Priocharax* also corroborates the validity of this new species.

Keywords: Biodiversity, Miniaturization, Ontogenetic truncation, Osteology, Taxonomy.

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Uma nova espécie de peixe miniatura do gênero de caracídeo *Priocharax* é descrita de um pequeno lago próximo ao rio Ipixuna, drenagem do rio Purus, estados do Amazonas, Brasil. Ela difere de todas as congêneres, exceto *P. pygmaeus* pelo menor número de dentes no maxilar e dentário. Distingue-se de *P. pygmaeus* pela presença de dois pós-cleitros e 22–27 raios ramificados na nadadeira anal (*vs* ausência e 19–22). A nova espécie também difere de outras congêneres por uma combinação de caracteres como número de raios das nadadeiras pélvica e anal, número de pós-cleitros e formato do pós-cleitro 3, e ausência de claustrum. Evidências moleculares com base em sequências do gene COI de todas as espécies válidas também corrobora a validade da nova espécie.

Palavras-chave: Biodiversidade, Miniaturização, Osteologia, Taxonomia, Truncamento ontogenético.

INTRODUCTION

Priocharax Weitzman & Vari, 1987 is a genus of characid fishes from the Amazon and Orinoco basins that currently includes four species, all of them miniature. *Priocharax ariel* Weitzman & Vari, 1987 and *P. pygmaeus* Weitzman & Vari, 1987 were both described with the genus from the upper reaches of the ríos Orinoco and Negro in Venezuela and from the upper rio Amazonas in Letícia, Colombia, respectively (Weitzman, Vari, 1987). Almost 30 years later, *Priocharax nanus* Toledo-Piza, Mattox & Britz, 2014 was described from the surroundings of Santa Isabel do Rio Negro in the middle rio Negro, Brazil. Recently, *Priocharax varii* Mattox, Souza, Toledo-Piza, Britz & Oliveira, 2020 was described from the rio Jamari, a tributary of the Madeira system, also in Brazil.

All four species share the conspicuous larval form of the pectoral fin in adults (*i.e.*, a rayless fin with a soft flap of cartilage without endoskeletal ossifications) which is interpreted as a terminal ontogenetic truncation in *Priocharax* (Mattox *et al.*, 2016). In addition, the four species also share other characters useful to distinguish *Priocharax* from most characids such as small conical teeth along the premaxilla, maxilla (which is fully toothed) and dentary, a triangular pseudotympanum anterior to the rib of the fifth vertebra, a diminutive body size, a translucent colour pattern and the presence of 5–6 branched pelvic-fin rays. In a recent expedition to the rio Ipixuna, an affluent of the Purus drainage, specimens of a new species were sampled which is described herein.

MATERIAL AND METHODS

Morphological analysis. Counts and measurements follow Fink, Weitzman (1974), Weitzman, Vari (1987), and Menezes, Weitzman (1990) and were taken on the left side of each specimen whenever possible. All measurements other than standard length (SL) are expressed as percentages of SL, except for subunits of the head which are expressed as percentages of head length (HL). Caudal-peduncle depth is also expressed as a percentage of caudal-peduncle length (Weitzman, Vari, 1987) and snout length is also

expressed as a percentage of orbital diameter. Measurements were taken point to point with a precision of 0.1 mm from digital photographs of specimens taken under a Zeiss Discovery V20 stereomicroscope. In text and Tabs., SD is used for standard deviation. Counts of vertebrae, supraneurals, teeth, gill-rakers, procurrent caudal-fin rays, and information about osteological characters were obtained from six specimens cleared and double stained for cartilage and bone following the protocol of Taylor, Van Dyke (1985). Total vertebral number includes the four vertebrae of the Weberian apparatus as separate elements. The compound ural centrum was counted as a single vertebra. The gill raker at the junction of the ceratobranchial and epibranchial is considered as the posteriormost gill raker on the lower part of the gill arch. Information on meristic and morphometric data of *Priocharax ariel*, *P. pygmaeus*, *P. nanus*, and *P. varii* were taken from Weitzman, Vari (1987), Toledo-Piza *et al.* (2014), and Mattox *et al.* (2020), except for percentage of snout length in relation to orbital diameter which were not available directly from those studies.

Photographs were made with a Zeiss Discovery V20 stereomicroscope using a Zeiss Axiocam digital camera attached. Osteological terminology follows Weitzman (1962) with updates summarized in Mattox *et al.* (2014). In the description, the frequency of each count is provided in parentheses after the respective count, with the count of the holotype indicated by an asterisk. Specimens examined are deposited in the Coleção de Peixes da Universidade Federal de Rondônia (UFRO-I), Instituto Nacional de Pesquisas da Amazônia (INPA), Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista, Botucatu (LBP), Museu de Zoologia da Universidade de São Paulo (MZUSP), and United States National Museum of Natural History – Smithsonian (USNM). Sampling for this study was authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) through permit number SISBIO/MMA 45429 and it is in accordance with the National Council for the Control of Animal Experimentation (CONCEA) approved by UNESP Ethics Committee on Use of Animals (CEUA), protocol number 1058.

Taxon sampling for molecular analysis. Specimens for the molecular study are deposited and preserved in 95% ethanol in the collection of the LBP. Twenty sequences of specimens of *Priocharax* were used (from Mattox *et al.*, 2020) plus one sequence of the outgroup taxon *Galeocharax humeralis* (Valenciennes, 1834) deposited in the GenBank database by Díaz *et al.* (2016). For this study, five sequences of the new species were generated. Voucher data are summarized in Tab. 1.

DNA extraction and sequencing. DNA extraction followed Ivanova *et al.* (2006), and partial sequences of the cytochrome c oxidase subunit I (COI gene) were amplified by polymerase chain reaction (PCR) with primers FishF1, FishR1 and FishF2, FishR2 (Ward *et al.*, 2005) or L6252-Asn, H7271-COXI (Melo *et al.*, 2011). Amplifications were performed in a total volume of 12.5 µl with 1.25 µl of 10X buffer, 0.25 µl of MgCl₂ (50 mM), 0.2 µl dNTPs (2 mM), 0.5 µl of each primer (5 mM), 0.1 µl of PHT Taq DNA polymerase (*Phonotria*), 1.0 µl of genomic DNA (200 ng), and 8.7 µl ddH₂O. The thermocycling were processed in a thermocycler (Applied Biosystems, Veriti), with initial denaturation (94 °C for 5 min), followed by 30 cycles of chain denaturation (94 °C for 40s), primer hybridization (FishF1 – FishR1 = 52 °C (30s); FishF2 – FishR2 =

TABLE 1 | Species, lots, vouchers, basins, locality information, and GenBank accession numbers of samples used in this study. In the locality column, Brazilian states are abbreviated as follows: AM = Amazonas, RO = Roraima.

Species	Lot	Voucher	Basin	Locality	Coordinates	GenBank n.
<i>Priocharax varii</i>	LBP 28495	96981	Rio Madeira	Rio Preto, affluent of rio Jamari, Candeias do Jamari, RO, Brazil	08°52'53.5"S 63°37'50.8"W	MT754786
<i>Priocharax varii</i>	LBP 28495	96982	Rio Madeira	Rio Preto, affluent of rio Jamari, Candeias do Jamari, RO, Brazil	08°52'53.5"S 63°37'50.8"W	MT754785
<i>Priocharax varii</i>	LBP 28495	96984	Rio Madeira	Rio Preto, affluent of rio Jamari, Candeias do Jamari, RO, Brazil	08°52'53.5"S 63°37'50.8"W	MT754783
<i>Priocharax varii</i>	LBP 28495	96985	Rio Madeira	Rio Preto, affluent of rio Jamari, Candeias do Jamari, RO, Brazil	08°52'53.5"S 63°37'50.8"W	MT754784
<i>Priocharax ariel</i>	LBP 28442	98284	Rio Negro	Igarapé Tibarrá on left side of rio Negro, Santa Isabel do Rio Negro, AM, Brazil	00°26'28.1"S 64°56'57.5"W	MT754780
<i>Priocharax ariel</i>	LBP 28442	98285	Rio Negro	Igarapé Tibarrá on left side of rio Negro, Santa Isabel do Rio Negro, AM, Brazil	00°26'28.1"S 64°56'57.5"W	MT754781
<i>Priocharax ariel</i>	LBP 27704	98286	Rio Negro	Igarapé Tapage, rio Urubaxi, approximately 1 h from mouth of river, S. I. Rio Negro, AM, Brazil	00°30'05.3"S 64°49'11.7"W	MT754778
<i>Priocharax ariel</i>	LBP 27704	98287	Rio Negro	Igarapé Tapage, rio Urubaxi, approximately 1 h from mouth of river, S. I. Rio Negro, AM, Brazil	00°30'05.3"S 64°49'11.7"W	MT754782
<i>Priocharax ariel</i>	LBP 27704	98288	Rio Negro	Igarapé Tapage, rio Urubaxi, approximately 1 h from mouth of river, S. I. Rio Negro, AM, Brazil	00°30'05.3"S 64°49'11.7"W	MT754779
<i>Priocharax ariel</i>	LBP 25858	96383	Rio Negro	Igarapé Uacatuna, São Gabriel da Cachoeira, AM, Brazil	00°03'38.0"S 67°05'45.0"W	MT754777
<i>Priocharax nanus</i>	LBP 28490	98283	Rio Negro	Igarapé Tibarrá on left side of rio Negro, Santa Isabel do Rio Negro, AM, Brazil	00°26'28.1"S 64°56'57.5"W	MT754766
<i>Priocharax pygmaeus</i>	LBP 22464	96986	Rio Amazonas	Quebrada La Ponderosa, Letícia, Colombia	04°08'24.4"S 69°56'53.4"W	MT754771
<i>Priocharax pygmaeus</i>	LBP 22464	96987	Rio Amazonas	Quebrada La Ponderosa, Letícia, Colombia	04°08'24.4"S 69°56'53.4"W	MT754774
<i>Priocharax pygmaeus</i>	LBP 22464	96988	Rio Amazonas	Quebrada La Ponderosa, Letícia, Colombia	04°08'24.4"S 69°56'53.4"W	MT754769
<i>Priocharax pygmaeus</i>	LBP 22464	96998	Rio Amazonas	Quebrada La Ponderosa, Letícia, Colombia	04°08'24.4"S 69°56'53.4"W	MT754768
<i>Priocharax pygmaeus</i>	LBP 22739	96989	Rio Amazonas	Quebrada Pichuna, Letícia, Colombia	04°07'33.8"S 70°00'28.9"W	MT754772
<i>Priocharax pygmaeus</i>	LBP 22739	96990	Rio Amazonas	Quebrada Pichuna, Letícia, Colombia	04°07'33.8"S 70°00'28.9"W	MT754773
<i>Priocharax pygmaeus</i>	LBP 22739	96991	Rio Amazonas	Quebrada Pichuna, Letícia, Colombia	04°07'33.8"S 70°00'28.9"W	MT754776
<i>Priocharax pygmaeus</i>	LBP 22739	96992	Rio Amazonas	Quebrada Pichuna, Letícia, Colombia	04°07'33.8"S 70°00'28.9"W	MT754770
<i>Priocharax pygmaeus</i>	LBP 22739	96993	Rio Amazonas	Quebrada Pichuna, Letícia, Colombia	04°07'33.8"S 70°00'28.9"W	MT754775
<i>Priocharax britzi</i>	LBP 28493	98295	Rio Purus	Marginal lake to rio Ipixuna, Canutama, AM, Brazil	07°31'11.5"S 63°20'59.6"W	MW374298
<i>Priocharax britzi</i>	LBP 28493	98296	Rio Purus	Marginal lake to rio Ipixuna, Canutama, AM, Brazil	07°31'11.5"S 63°20'59.6"W	MW374297
<i>Priocharax britzi</i>	LBP 28493	98297	Rio Purus	Marginal lake to rio Ipixuna, Canutama, AM, Brazil	07°31'11.5"S 63°20'59.6"W	MW374296
<i>Priocharax britzi</i>	LBP 28493	98298	Rio Purus	Marginal lake to rio Ipixuna, Canutama, AM, Brazil	07°31'11.5"S 63°20'59.6"W	MW374300
<i>Priocharax britzi</i>	LBP 28493	98299	Rio Purus	Marginal lake to rio Ipixuna, Canutama, AM, Brazil	07°31'11.5"S 63°20'59.6"W	MW374299
<i>Galeocharax humeralis</i>	MAG ICT 0206	LAR217	Rio Paraná	Entre Rios, Rosario, Argentina	32°51'46.8"S 60°38'27.6"W	KU288918

50°C (30s); L6252-Asn – H7271-COXI = 54°C (30s)) and nucleotide extension (68°C for 1 min), plus a final extension (68°C for 8 min). All PCR products were visually checked on 1% agarose gels and then purified with ExoSap-IT (USB Corporation) following the manufacturer's instructions. The purified PCR products were submitted to sequencing reactions using BigDye Terminator v 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems), purified again through ethanol precipitation, and loaded onto an ABI 3130 DNA Analyzer automatic sequencer (Applied Biosystems). All sequences produced in this study were deposited in GenBank.

Molecular data analysis. Sequences were assembled to consensus using Geneious v7.1.9 (Kearse *et al.*, 2012) and aligned using the MUSCLE algorithm (Edgar, 2004) under default parameters. The method of Xia *et al.* (2003) in DAMBE v5.3.38 (Xia, 2013) was used to evaluate the index of substitution saturation (Iss). Nucleotide composition and substitution patterns were estimated in MEGA X (Kumar *et al.*, 2018). A maximum likelihood (ML) analysis was conducted under RAxML HPC-PTHREADS-SSE3 (Stamatakis, 2006) with the GTRGAMMA model using five random parsimony starting trees (Stamatakis *et al.*, 2008) on 2x 40 CPU 128GB Brycon server at LBP/UNESP. Automatic Barcode Gap Discovery analysis (ABGD; Puillandre *et al.*, 2012) and Poisson Tree Processes (PTP; Zhang *et al.*, 2013) were used in the molecular identification of species. ABGD was performed using the webserver (<http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html>), under the default parameters of Pmin = 0.001 to Pmax = 0.1, steps = 10, X (relative gap width) = 1.5, Nb bins (for distance distribution) = 20, and the Kimura (K80) molecular model. PTP were generated using the ML tree and other parameters at default in the PTP webserver (<http://species.h-its.org/ptp/>). ABGD and PTP were performed excluding the outgroup taxa *Galeocharax humeralis*. Overall and pairwise genetic distances were estimated based on the Kimura 2-parameter model (K2P) + Gamma using MEGA X and the order of groups was based on the ABGD and PTP results.

RESULTS

Priocharax britzi, new species

urn:lsid:zoobank.org:act:A2309042-25EE-4849-8488-77B2A5FDE955

(Figs. 1–5; Tab. 2)

Holotype. MZUSP 125822, 13.7 mm SL, Brazil, Amazonas, Canutama, small lake approximately 400 m from the rio Ipixuna, after bridge crossing this river, on the right side of the Transamazônica Road (BR-230) going from Humaitá to Lábrea, rio Ipixuna drainage, upper Purus basin, 07° 31' 11.46" S 63° 20' 59.58" W, 3 Sep 2018, G. M. T. Mattox & S. Souza.

Paratypes. All collected with holotype. LBP 28493, 5, 11.7–12.7 mm SL. MZUSP 125823, 43, 9.3–14.1 mm SL (6 c&s, 11.0–12.9 mm SL). UFRO-ICT 27662, 5, 10.2–12.0 mm SL.

Diagnosis. *Priocharax britzi* is distinguished from all congeners except *P. pygmaeus* by the lower number of teeth in the maxilla (21–30 *vs* 32–58) and dentary (26–29 *vs* 33–55). It differs from *P. pygmaeus* in the presence of two postcleithra (*vs* absence) and by having 22–27 (modes 25 and 26) branched anal-fin rays (*vs* 19–22, mode 19). The new species is further distinguished from *P. nanus* and *P. varii* by having i,5 pelvic-fin rays (*vs* i,6) and a slender and sinuous postcleithrum 3 (*vs* postcleithrum 3 relatively thick and straight), from *P. ariel* by the presence of 22–27 branched anal-fin rays (*vs* 16–22) and the presence of two postcleithra (*vs* absence). *Priocharax britzi* is further distinguished

from *P. varii* by the absence of the adipose fin (*vs* presence) and from *P. nanus* by the absence of the claustrum (*vs* presence). Complementarily, *Priocharax britzi* has a shorter snout relative to the orbital diameter when compared to all congeners except *P. nanus*. This difference is reflected in the range of the proportion of snout length in relation to orbital diameter and its mean which is 45–59 (mean = 53.6; SD = 3.8) in *P. britzi* *vs* 60–76 (mean = 65.5; SD = 4.9) in *P. pygmaeus*, 54–81 (mean = 67.0; SD = 5.1) in *P. varii* and 54–84 (mean = 68.4; SD = 6.3) in *P. ariel*. The molecular analyses provide additional support towards the recognition of the new species.



FIGURE 1 | *Priocharax britzi*, **A.** Holotype, male, MZUSP 125822, 13.7 mm SL; Brazil, Amazonas, Canutama, small lake approximately 400 m from the rio Ipixuna, after bridge crossing this river, on the right side of the Transamazônica Road (BR-230) going from Humaitá to Lábrea, rio Ipixuna drainage. **B.** Live paratype photographed immediately after capture.

TABLE 2 | Morphometric data of *Priocharax britzi*. N = number of specimens; SD = standard deviation. Range includes the holotype. [Note: In the recent description of *Priocharax varii* (Mattox *et al.*, 2020:423, table 2), data of anal-fin length is incorrect. The correct information is as follows: Holotype = 22, range = 20–24, mean = 21.8, SD = 0.9, with n = 48 including the holotype].

	Holotype	N	Range	Mean	SD
Standard length (mm)	13.7	48	9.3–14.1	11.8	-
Percentages of standard length					
Depth at dorsal-fin origin	23	48	20–24	22.1	0.9
Snout to dorsal-fin origin	53	48	52–56	53.9	0.9
Snout to pelvic-fin origin	38	48	38–43	39.9	1.0
Snout to anal-fin origin	52	48	49–55	51.8	1.0
Dorsal-fin length	28	46	23–28	25.4	1.0
Dorsal-fin base	12	48	9–14	11.7	1.0
Pelvic-fin length	12	47	9–12	10.3	0.7
Anal-fin length	22	46	21–25	22.8	1.0
Anal-fin base	35	45	30–37	34.3	1.3
Caudal-peduncle depth	8	47	7–8	7.5	0.4
Caudal-peduncle length	15	45	13–19	15.7	1.2
Head length	25	48	23–27	25.3	0.9
Percentages of head length					
Orbital diameter	35	46	33–39	36.2	1.2
Interorbital distance	40	44	30–42	35.9	2.6
Snout length	19	46	16–22	19.5	1.2
Upper jaw length	50	47	46–58	50.9	2.7
Percentages of caudal peduncle length					
Caudal-peduncle depth	53	45	39–56	48.4	3.6
Percentages of orbital diameter					
Snout length	56	45	45–59	53.6	3.8

Description. For overall appearance, see Fig. 1. Morphometric data are presented in Tab. 2. Body laterally compressed and elongated, greatest depth at vertical through dorsal-fin origin. Dorsal-fin origin approximately at midbody, at vertical slightly anterior to anal-fin origin. Pectoral-fin bud at vertical through anterior portion of pseudotympanum. Pelvic-fin origin approximately midway between posterior margin of opercle and anal-fin origin. Dorsal profile of head and body slightly convex from tip of snout to dorsal-fin origin. Dorsal profile of body along dorsal-fin base nearly straight, gently sloping posteroventrally; sloping more conspicuous from latter point to caudal peduncle. Dorsal profile of caudal peduncle slightly concave to base of dorsal procurrent rays. Ventral profile of head and body slightly convex from symphysis of lower jaw to vertical through pectoral-fin origin; straight to slightly convex from latter point to pelvic-fin origin. Ventral profile of body posteroventrally sloping from pelvic-fin to anal-fin origin; straight and posterodorsally rising along anterior one-half of anal-fin base, gently concave from latter point to base of ventral procurrent rays. Caudal peduncle short. Pseudotympanum located anterior to rib of fifth vertebra.

Snout round in lateral view. Eye about one-third of head length. Infraorbitals 2 and 3 present but not fully developed in three specimens, absent in the others. Infraorbitals 1, 4 to 6 and supraorbital absent in all specimens. Antorbital present but poorly developed in five specimens (Fig. 2), absent in one specimen. Mouth terminal with lower jaw slightly shorter than upper jaw. Tip of maxilla elongate, posterior border reaching vertical through posterior border of pupil. Premaxillary teeth in single series, premaxilla with 20(3), 22(1), or 23(2) teeth. Maxilla with 21(1), 22(1), 23(1), 25(1), 26(1), or 30(1) teeth. Dentary with 26(1), 27(3), 28(1), or 29(1) teeth. Dentary teeth in single series, with few anterior teeth slightly displaced anteriorly. A conspicuous elongate foramen at the anterior portion of the dentary. All jaw teeth small, conical and lingually curved to a moderate extent (Fig. 3).

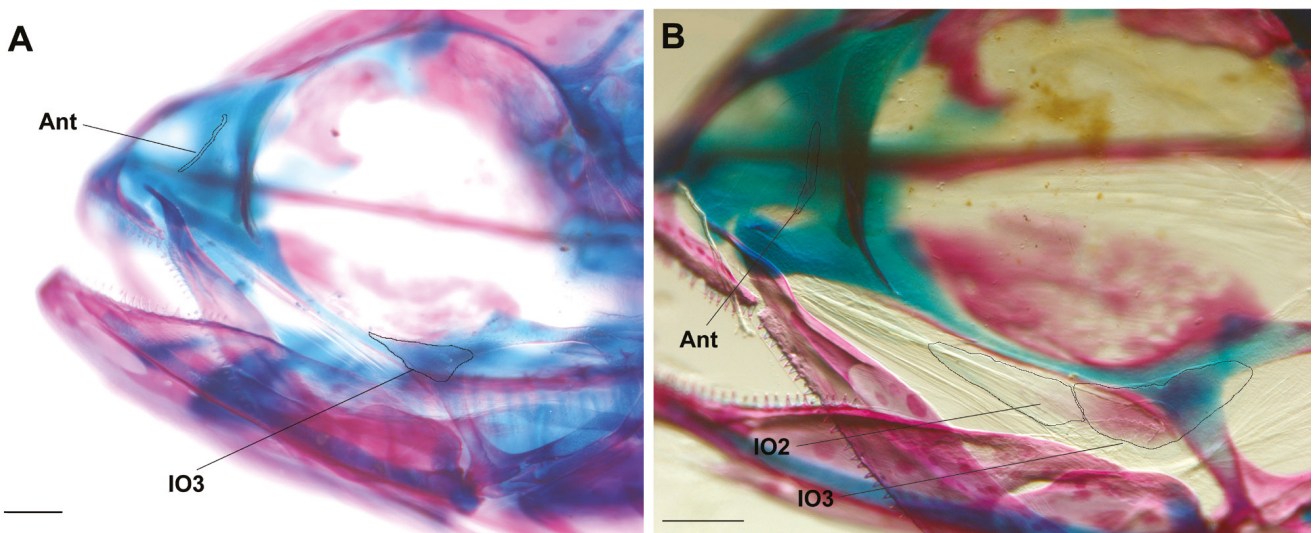


FIGURE 2 | *Priocharax britzi*, paratypes, MZUSP 125823, c&s; anterior region of head in lateral view detailing the orbital region. **A.** 12.5 mm SL. **B.** 12.9 mm SL, image flipped. Antorbital and infraorbitals delimited by black line for clarity. Ant = antorbital; IO2 – 3 = infraorbitals 2 – 3. Scale bar = 0.2 mm.

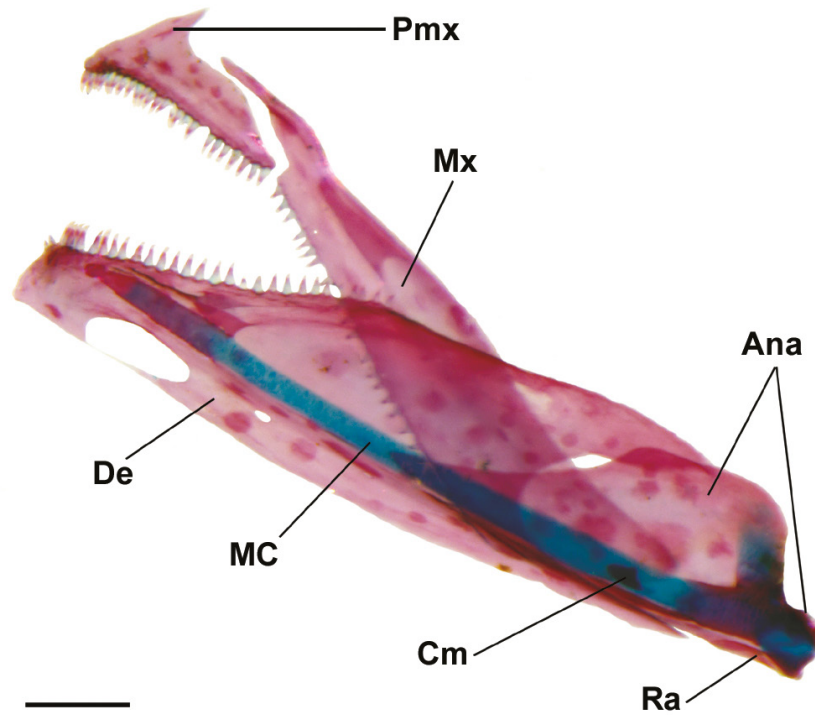


FIGURE 3 | *Priocharax britzi*, paratype, MZUSP 125823, 12.9 mm SL, c&s; jaws in lateral view. Ana = anguloarticular; Cm = coronomeckelian; De = dentary; MC = Meckel's cartilage; Mx = maxilla; Pmx = premaxilla; Ra = retroarticular. Scale bar = 0.2 mm.

Dorsal-fin rays ii,9*(43). Endoskeletal part of pectoral fin and some thin exoskeletal bones showing larval structure (Fig. 4). Cartilaginous pectoral-radial plate with incomplete longitudinal middle fissure leaving upper and lower halves connected at base and tip; base articulating with vertically elongated scapulocoracoid cartilage and round distal margin with larval-like pectoral-fin fold supported solely by actinotrichia. Pectoral-fin rays absent. All bones of endoskeletal pectoral girdle absent, exoskeletal part with posttemporal, supracleithrum, cleithrum and two postcleithra. Postcleithrum 3 poorly developed, slender and sinuous. Cleithrum with posteriorly directed, curved process immediately below ventral tip of supracleithrum. Pelvic-fin rays i,5*(47). Posterior tip of pelvic fin falling short of origin of anal fin but extending slightly beyond vent. Anal-fin rays ii, 22(2), 23(8), 24(7), 25*(13), 26(13), or 27(2). Anal-fin margin concave with anterior elongate lobe formed by elongated fin rays and posterior section of short rays. Caudal-fin rays i,9,7,i (1), or i,9,8,i*(37), dorsal procurrent rays 8(2) or 9(4), ventral procurrent rays 6(3), 7(2), or 8(1). Caudal fin forked. Adipose fin absent.

Squamation present in almost all specimens, but scales highly deciduous and easily lost during handling. Scales cycloid, very thin, with no obvious circuli or radii. Scales in midlateral row 23(2), 24(6), 25(12), 26*(17), 27(4), or 28(3); no canal bearing lateral-line scales on body. Scale rows between dorsal-fin origin and pelvic-fin origin 8(1), 9(7), or 10*(2). Scale rows around caudal peduncle 8(7), 9(1), or 10*(1). Predorsal scales typically absent with one or two scales just anterior to dorsal fin in few specimens. Scales restricted to base of caudal-fin rays, not covering caudal-fin lobes.

Total vertebrae 32(1) or 33(5); abdominal vertebrae 14(6); caudal vertebrae 18(1) or 19(5). Total number of gill-rakers on first branchial arch 9(1) or 11(5), upper limb gill-rakers 1(1) or 3(5), lower limb gill-rakers 8(6). Weberian apparatus well-developed, all components ossified except for claustrum (Fig. 5). Large gap between neural arches 3 and 4, with gap partially filled by dorsally projecting pointed process from vertebral centrum 3. Inner arm of suspensorium large, projecting forward to vertical through middle of second centrum. Supraneurals 5(5) or 6(1).

Coloration in alcohol. Overall ground coloration pale yellow (Fig. 1A). Scattered melanophores on dorsal portion of head in a few specimens. Guanine present in eye of most specimens, with melanophores on dorsal surface of eye. Scattered melanophores on bases of posteriormost anal-fin rays forming irregular line along posterior half of fin. Another irregular dark line extending along ventral margin of hypaxial myomeres posteriorly from vertical through seventh branched anal-fin ray. Both lines separated anteriorly but approaching each other posteriorly. A few scattered melanophores on bases of dorsalmost and ventralmost caudal-fin rays. All fins hyaline.

Coloration in life. Body mostly translucent (Fig. 1B). A few melanophores and xanthophores scattered along dorsal surface of vertebral column and on dorsal surface of swim bladder. Xanthophores on dorsal surface of head from vertical through middle of eye to posterior margin of skull. A thin line of chromatophores along anal-fin base, from vertical through 6th branched ray to terminus of anal-fin base. Two faint blotches of xanthophores on bases of caudal-fin lobes, remaining caudal fin and other fins hyaline. Eye silvery, dorsal half predominantly dark.

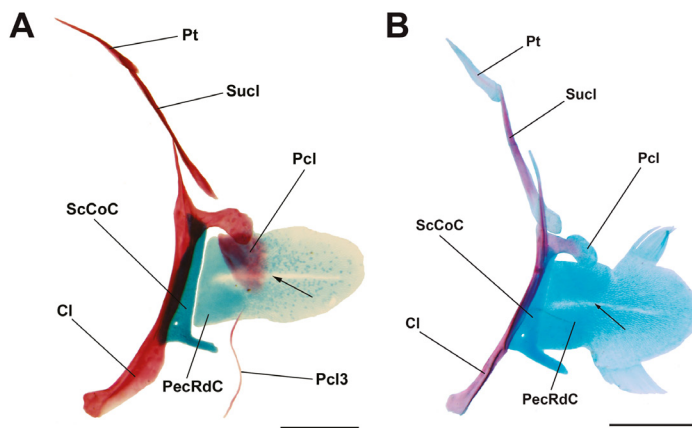


FIGURE 4 | Shoulder girdle in lateral view of **A.** *Priocharax britzi*, paratype, MZUSP 125823, 12.9 mm SL, c&s. Image flipped. **B.** *Priocharax cf. ariel*, UFRO 15521, 11.8 mm SL, c&s. Cl = cleithrum; PecRdC = pectoral-fin radial cartilage; Pcl = unidentified postcleithrum; Pcl3 = postcleithrum 3; Pt = posttemporal; ScCoC = scapulocoracoid cartilage; Sucl = supracleithrum. Arrows point to middle fissure on pectoral-fin radial cartilage. Pcl3 slightly displaced ventrally from original position in A. Scale bars = 0.5 mm.

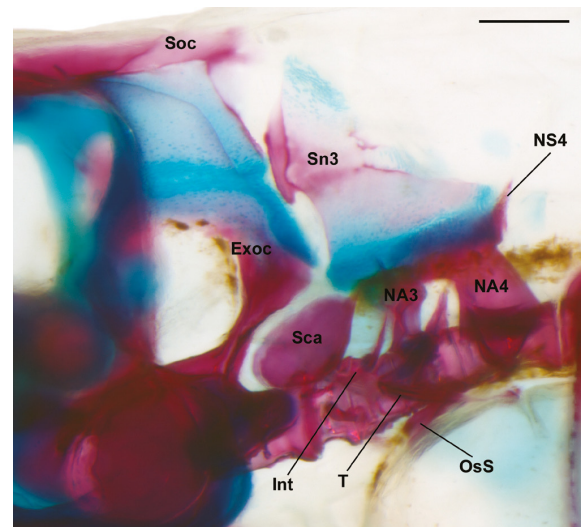


FIGURE 5 | *Priocharax britzi*, paratype, MZUSP 125823, 12.9 mm SL, c&s; Weberian apparatus in lateral view. Exoc = exoccipital; Int = intercalarium; NA3 – 4 = neural arches 3 – 4; NS4 = neural spine 4; OsS = os suspensorium; Sca = scaphium; Sn3 = supraneural 3; Soc = supraoccipital; T = tripus. Scale bar = 0.2 mm.

Sexual dimorphism. Two of 48 analysed specimens with hooks on anal-fin rays (12.4–13.7 mm SL), the larger specimen also with hooks on pelvic-fin rays (Fig. 6). Hooks on anal fin located on posterior margins of posterior unbranched and three anterior most branched rays, typically one hook per segment. Five, seven, seven, and four hooks respectively on each anal-fin ray of larger specimen, two, four, two, one hooks on anal-fin rays of smaller one. Hooks of larger specimen well developed, especially along middle portion of each ray. Hooks of smaller specimen less developed. Hooks on pelvic fin of larger specimen not as developed and resembling bumps along branched rays 1–3 of contralateral fins, more developed on more lateral branched ray. Hooks on pelvic fin always along medial edge of rays. Smaller specimen without hooks on pelvic fin.

Molecular analysis. The final matrix comprised 26 terminals with 642 bp and 213 variable sites (33.2%). The nucleotide composition was 23.4% adenine, 17.8% guanine, 32.4% thymine, and 26.2% cytosine. The *I*_{ss} values were lower than *I*_{ss.c} values, indicating the absence of saturation. The maximum likelihood (ML) tree showed high bootstrap values supporting each of the analysed species (Fig. 7). The convergence of initial and recursive partitions of the ABGD and ML solution of the PTP analysis delimited five species of *Priocharax*: *P. varii*, *P. ariel*, *P. pygmaeus*, *P. nanus*, and *P. britzi* (Fig. 7; S1 and S2). The overall mean of genetic distances (K2P) among *Priocharax* species was 0.194±0.018. Intraspecific genetic distances ranged from 0.000 within *P. pygmaeus* to 0.002±0.001 within *P. ariel* and *P. varii*. The values of interspecific distances ranged from 0.179±0.021 between *P. varii* and *P. nanus* to 0.249±0.025 between *P. pygmaeus* and *P. ariel* (Tab. 3).

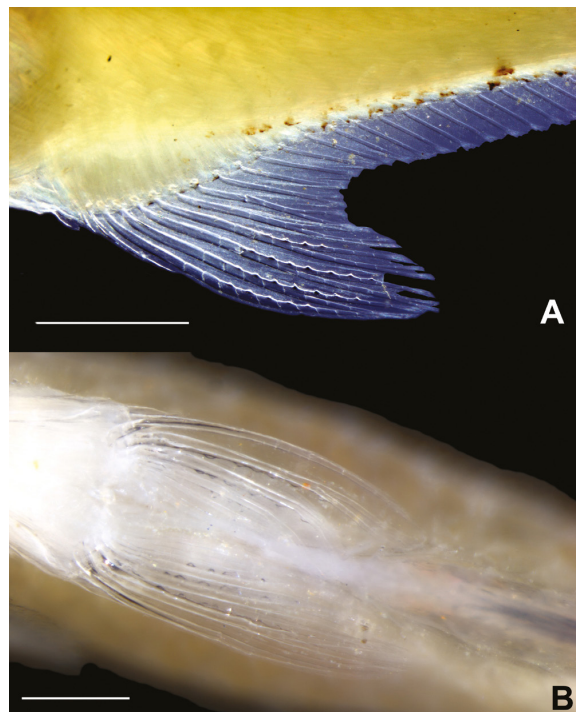


FIGURE 6 | *Priocharax britzi*, holotype, MZUSP 125822, 13.7 mm SL. **A.** Left lateral view of anal fin showing bony hooks. **B.** Ventral view of pelvic fin showing bony hooks. Scale bars = 0.5 mm.

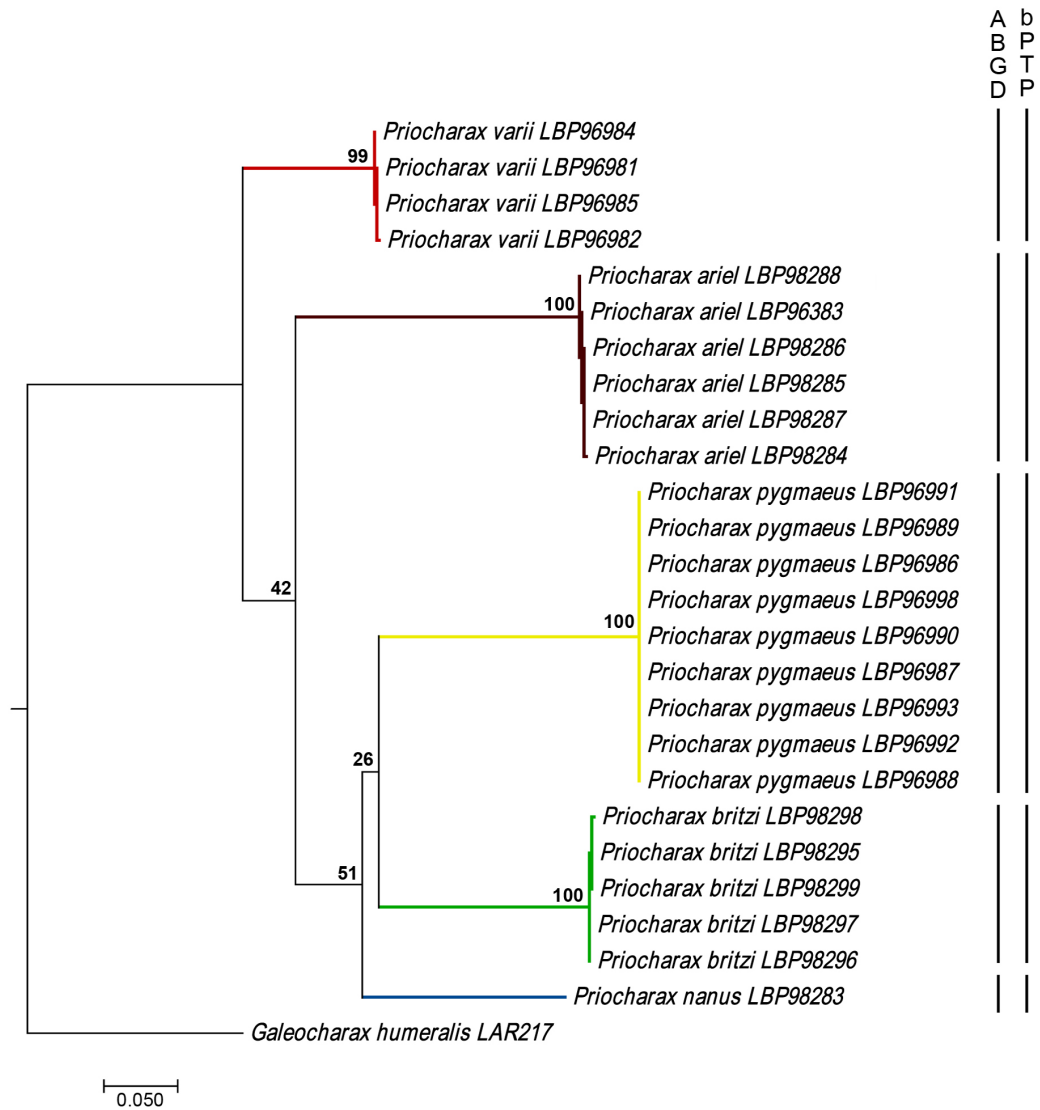


FIGURE 7 | Maximum likelihood tree of five species of *Priocharax*, based on the COI gene (642 bp). Bars represent the number of species obtained by the ABGD and bPTP analyses. Numbers near nodes represent bootstrap values.

TABLE 3 | Pairwise K2P genetic distances (and standard deviation) within and among species of *Priocharax*. Intraspecific genetic distances are highlighted in bold. Values are presented in percentages followed by standard deviation. Number of sequences: *P. ariel* = 6, *P. britzi* = 5, *P. nanus* = 1, *P. pygmaeus* = 9 and *P. variii* = 4.

	1	2	3	4	5
1- <i>P. nanus</i>	-				
2- <i>P. ariel</i>	18.8±2.3	0.2±0.1			
3- <i>P. pygmaeus</i>	18.2±2.1	24.9±2.5	0.0±0.0		
4- <i>P. variii</i>	17.9±2.1	19.3±2.0	20.2±2.1	0.2±0.1	
5- <i>P. britzi</i>	18.6±2.2	21.0±2.1	19.7±2.1	20.4±2.0	0.1±0.1

Geographical distribution. *Priocharax britzi* is known only from its type locality in a marginal lake approximately 400 m from the rio Ipixuna, a tributary of the rio Purus (Fig. 8). Specimens from a close locality in the main channel of the rio Ipixuna (UFRO 15521) were examined morphologically and did not match the diagnostic characters of *P. britzi* (see discussion below). The Fig. 8 also shows the known distribution of all congeners.

Ecological notes. Specimens of *Priocharax britzi* were collected between 3 pm and 5 pm in a small lake approximately 15 m wide and 35 m long, apparently isolated from the main channel of the rio Ipixuna (Fig. 9). The water level was low as sampling occurred during the dry season (September), so it is possible that this lake connects to the main channel in the wet season. The specimens were collected near the shore at depths varying from 0.5–1.0 m. Vegetation around the lake was composed mainly of dead logs and branches. At the time of sampling, the bottom was muddy with patches of leaf litter. *Priocharax britzi* was collected with the characiforms *Carnegiella marthae* Myers, 1927, *Hemigrammus* sp., *Hemigrammus* cf. *gracilis* (Lütken, 1875), *Hyphessobrycon rosaceus* Durbin, 1909, *Iguanodectes spilurus* (Günther, 1864), *Microcharacidium* sp., *Nannostomus digrammus* (Fowler, 1913), *Nannostomus eques* Steindachner, 1867, the catfish *Amblydoras affinis* (Kner, 1855), the knifefishes *Brachyhyppopomus beebei* (Schultz, 1944), *Microsternarchus bilineatus* Fernández-Yépez, 1968, the cichlids *Apistogramma* cf. *pulchra* Kullander, 1980, *Biotocus opercularis* (Steindachner, 1875), and *Crenicichla cyanotus* Cope, 1870.

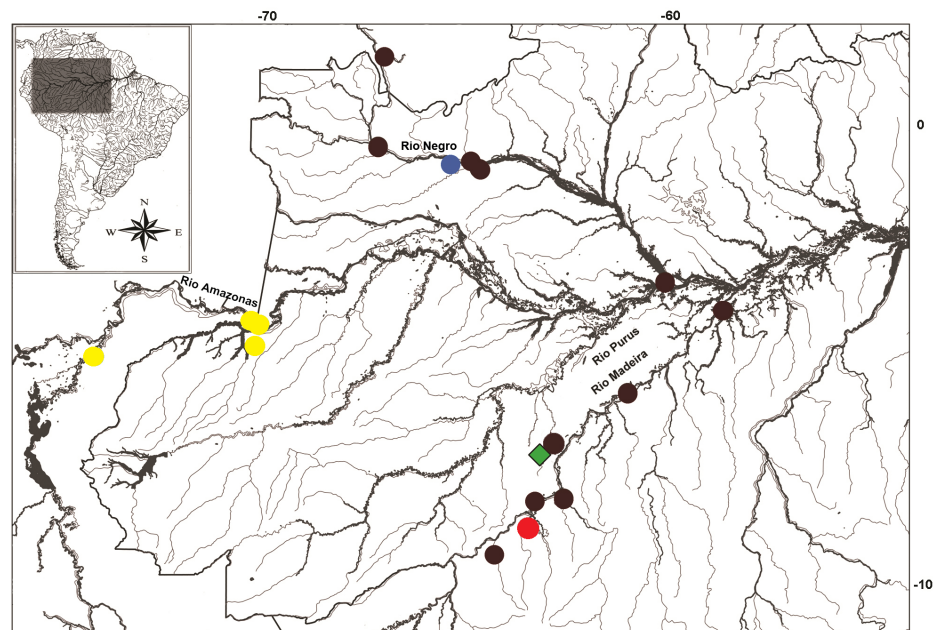


FIGURE 8 | Map of South America with a close-up of the upper and central portions of the Amazon basin, Brazil, illustrating the distribution of the five species of *Priocharax*: *P. britzi* described herein from the rio Purus basin (green losangle), *P. varii* (red dot), *P. ariel* (black dots), *P. nanus* (blue dot), and *P. pygmaeus* (yellow dots). Colors are the same as in the Fig. 7. Some dots may represent more than one lot.



FIGURE 9 | Small lake approximately 400 m from the rio Ipixuna, Canutama, Amazonas, Brazil, the type locality of *Priocharax britzi*.

Etymology. *Priocharax britzi* is named after Dr. Ralf Britz, noteworthy ichthyologist and a dear friend. Dr. Britz has mastered the world of small fishes and has described more than 20 miniature species, including two species of *Priocharax*. A noun in the genitive case.

Conservation status. *Priocharax britzi* was found in a single location, an isolated small lake near the road (Fig. 8). The environmental conditions of the lake seem degraded, with depauperate riparian vegetation and silted substrate. Efforts to find specimens of the new species in the nearby rio Ipixuna and other close locations were not successful. However, the type locality is near a protected area (Floresta Nacional de Balata-Tufari), which was not sampled. Future studies should better investigate the possibility that *P. britzi* occurring in other localities within the protected area and along portions of the rio Ipixuna that may connect to the lake during the wet season. Furthermore, there are no known imminent threats that would put the species at risk of extinction. Hence, we suggest that *P. britzi* should be classified as Least Concern (LC) according to International Union for Conservation of Nature (IUCN) categories and criteria (IUCN Standards and Petitions Subcommittee, 2019) pending further information on its real distribution.

DISCUSSION

Priocharax britzi was sampled in a small lake approximately 400 m from the main channel of the rio Ipixuna. Additional specimens of *Priocharax* (UFRO 15521, n = 11) originating from the main channel of the rio Ipixuna approximately 500 m from the

type locality of *P. britzi* are morphologically more similar to *P. ariel* than to *P. britzi*; they are herein identified as *P. cf. ariel*. Those specimens were collected in 2012 and no tissue samples were available to perform molecular analyses. Several attempts to sample more specimens of *Priocharax cf. ariel* from the main channel of the rio Ipixuna in September 2018 were unsuccessful. Osteologically, *P. britzi* differs clearly from *P. cf. ariel* by the presence of postcleithrum 3 (*vs* absence) (Fig. 4) and in the lower number of supraneurals [5(5) or 6(1) *vs* 7(3) or 8(1) in *P. cf. ariel*]. Furthermore, *P. britzi* has more branched anal-fin rays (22–27; modes 25 and 26 *vs* 19–22; mode=19 in *P. cf. ariel*) with a consequently longer anal-fin base (30–37% of SL, mean = 34.3%, SD = 1.3% *vs* 25–29% of SL, mean = 27.5%, SD = 1.3%).

Meristic characters of specimens of *Priocharax cf. ariel* from the main channel of the rio Ipixuna are within the range of variation of *Priocharax ariel* with the exception of the number of maxillary (25–30) and dentary (27–35) teeth which are considerably lower from the values reported by Weitzman, Vari (1987) for *P. ariel* (38–58 and 38–55, respectively). Though these lower tooth counts suggest that *P. cf. ariel* may represent an additional undescribed species, the lack of molecular data for *P. cf. ariel* from the main channel of the rio Ipixuna currently makes it impossible to test their conspecificity with *P. ariel*.

Specimens of *Priocharax britzi* smaller than 11.0 mm SL lack the antorbital. In specimens with 11.6 mm SL and larger, the antorbital is present and gradually more developed. Weitzman, Vari (1987) noted a similar pattern in *P. ariel* and *P. pygmaeus* in which the antorbital is only apparent in specimens larger than 13.5 mm SL and 12.2 mm SL, respectively. In those two species the antorbital is the only ossified bone of the infraorbital series, a condition also shared with *Priocharax nanus* and *P. varii* (Toledo-Piza *et al.*, 2014; Mattox *et al.*, 2020). Conversely, in *P. britzi* infraorbital 3 is present at 12.5 mm SL and infraorbital 2 is present at 12.9 mm SL (Fig. 2). Mattox *et al.* (2016) reported the presence of three infraorbitals in 18 specimens of *Priocharax* sp. from the rio Negro, all larger than 12.7 mm SL. The taxonomic significance of the more extensive ossification of the infraorbital series in specimens from that drainage still needs further study.

Two out of 48 specimens of *Priocharax britzi* have hooks on the anal fin, a classic trait of sexual dimorphism in characids usually present only in mature males (Fig. 6) (*e.g.*, Malabarba, Weitzman, 2003; Camelier, Zanata, 2014). Among congeners, hooks on the anal fin were previously reported only for males of *Priocharax ariel* (Weitzman, Vari, 1987:645). More recently, Mattox *et al.* (2016) reported that in addition of hooks on the anal fin, *Priocharax* sp. from the rio Negro also had hooks on the pelvic-fin rays. Of the two specimens of *Priocharax britzi* with hooks reported herein, one has hooks on both the anal and pelvic fins and the other has hooks only on the anal fin. This apparent intraspecific variation may rather reflect different stages of maturity among specimens, as the larger specimen (13.7 mm SL) has more hooks on both fins, with the hooks on the anal fin more developed, while the smaller specimen (12.4 mm SL) has fewer hooks only on the anal fin, with those hooks less developed. Gonads were not checked for maturity because such an examination is a destructive process and specimens of this species are still rare.

Knowledge about the diversity of species of *Priocharax* remained stable for approximately 27 years, from Weitzman, Vari's (1987) proposition of the genus and

description of *P. ariel* and *P. pygmaeus* until 2014, when efforts to collect and study new specimens from the rio Negro basin resulted in the description of *P. nanus* by Toledo-Piza *et al.* (2014). More recent efforts to collect and study specimens from other Amazon subdrainages have resulted in two new species descriptions: *P. varii* (Mattox *et al.*, 2020) and *P. britzi* (herein). Those efforts combined with the examination of material deposited in collections have also shown that the genus is more widespread than previously recorded and revealed that the taxonomy of *Priocharax* is more complex, as exemplified by the specimens collected in the main channel of the rio Ipixuna discussed above. Those detailed studies focusing on *Priocharax* have revealed new aspects about the diversity of this genus and reinforce that there is still much to be discovered regarding the Neotropical freshwater ichthyofauna, especially its miniature species.

Comparative material examined. *Priocharax ariel*: **Brazil, Amazonas, rio Negro basin:** MZUSP 39778, 4, 13.5–14.6 mm SL; MZUSP 55099, 8, 12.4–14.2 mm SL; MZUSP 55097, 4 of 6, 12.2–12.7 mm SL; MZUSP 62230, 2 of 4, 15.1–15.2 mm SL. **Rio Madeira basin:** UFRO-ICT 5761, 1, 14.5 mm SL; UFRO-ICT 19660, 1, 10.9 mm SL; UFRO-ICT 20954, 8 of 10, 10.1–11.8 mm SL. **Rondônia, rio Madeira basin:** UFRO-ICT 2151, 11 of 18, 13.7–16.1 mm SL (4 c&s, 13.7–16.1 mm SL); UFRO-ICT 5744, 3, 12.1–12.5 mm SL; UFRO-ICT 5745, 3, 11.6–12.9 mm SL; UFRO-ICT 8449, 1, 17.1 mm SL; UFRO-ICT 13825, 1, 14.2 mm SL; UFRO-ICT 17412, 2 of 3, 11.6–12.4 mm SL; UFRO-ICT 20075, 1, 17.4 mm SL. **Venezuela, Territorio Federal Amazonas, rio Orinoco basin:** MZUSP 36497, 50, 11.8–15.2 mm SL, paratypes; MZUSP 55142, 12, 12.0–14.7 mm SL (5 c&s, 12.0–14.0 mm SL), paratypes. *Priocharax cf. ariel*: **Brazil, Amazonas, rio Purus basin:** UFRO-ICT 15521, 11 of 17, 10.0–13.6 mm (4 c&s, 11.3–12.7 mm SL). *Priocharax nanus*: **Brazil, Amazonas, rio Negro basin:** INPA 39891, 4, 12.5–13.9 mm SL, paratypes; MZUSP 114014, 13.8 mm SL, holotype; MZUSP 114015, 9, 12.1–15.3 mm SL (3 c&s, 14.1–15.3 mm SL), paratypes; MZUSP 114016, 5, 12.6–14.6 mm SL (2 c&s, 13.4–13.8 mm SL), paratypes; MZUSP 114017, 3, 13.5–14.6 mm SL (1 c&s, 14.6 mm SL), paratypes; MZUSP 114018, 11, 11.1–15.4 mm SL (5 c&s, 12.0–14.0 mm SL), paratypes; USNM 427007, 4, 12.1–13.3 mm SL, paratypes. *Priocharax pygmaeus*: **Colombia, Departamento Amazonas, rio Amazonas basin:** MZUSP 36498, 5, 10.2–10.7 mm SL, paratypes. **Peru, Loreto, rio Ucayali basin:** MZUSP 85644, 1, 16.5 mm SL. **rio Ampiyacu basin:** MZUSP 121212, 10, 11.5–12.7 mm SL. *Priocharax varii*: **Brazil, Rondônia, rio Madeira basin:** LBP 28495, 5, 12.5–12.7 mm SL, paratypes. MZUSP 125786, 12.2 mm SL, holotype. MZUSP 125787, 44, 11.8–14.0 mm SL (5 c&s, 11.8–13.7 mm SL), paratypes. UFRO-ICT 27656, 5, 12.2–13.3 mm SL, paratypes.

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AUTHOR'S CONTRIBUTION

George Mendes Taliaferro Mattox: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing–original draft, Writing–review and editing.

Camila da Silva de Souza: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing–original draft, Writing–review and editing.

Mônica Toledo-Piza: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing–original draft, Writing–review and editing.



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Claudio Oliveira: Data curation, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing-original draft, Writing-review and editing.

ETHICAL STATEMENT

Sampling for this study was authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) through permit number SISBIO/MMA 45429 and it is in accordance with the National Council for the Control of Animal Experimentation (CONCEA) approved by UNESP Ethics Committee on Use of Animals (CEUA), protocol number 1058.

COMPETING INTERESTS

The authors declare no competing interests.

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