

Article

Two New Species of Miniature Tetras of the Genus *Priocharax* (Teleostei: Characiformes: Acestrorhamphidae) from the Rio Purus and Solimões Drainages, Amazonas, Brazil [†]

Giovanna Guimarães Silva Lopez ^{1,2} , Camila Silva Souza ³ , Lais Reia ⁴ , Larissa Arruda Mantuaneli ⁴, Bruno Ferezim Morales ⁵ , Flávio Cesar Thadeo Lima ⁶ , Claudio Oliveira ⁴  and George Mendes Taliaferro Mattox ^{2,*}

¹ Instituto de Biociências, Universidade de São Paulo, São Paulo 05508-090, SP, Brazil; giovanna.lopez@usp.br

² Laboratório de Ictiologia de Sorocaba, Universidade Federal de São Carlos, Sorocaba 18052-180, SP, Brazil

³ Marine Institute, Rinvilla, Oranmore, H91 R673 Galway, Ireland; camilasvsouza@gmail.com

⁴ Departamento de Biologia Estrutural e Funcional, Instituto de Biociências, Universidade Estadual Paulista, Campus Botucatu, Botucatu 18618-689, SP, Brazil; laisreia@gmail.com (L.R.); la.mantuaneli@unesp.br (L.A.M.); claudio.oliveira@unesp.br (C.O.)

⁵ Faculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados, Dourados 79825-070, MS, Brazil; brunomoraes@ufgd.edu.br

⁶ Museu de Diversidade Biológica, Universidade Estadual de Campinas, Campinas 13083-862, SP, Brazil; fctlima@gmail.com

* Correspondence: gmattox@ufscar.br

[†] urn:lsid:zoobank.org:pub:E24A674C-B892-4C70-AD34-65B8E9EA8F3B;

urn:lsid:zoobank.org:act:EB7129F3-B6E4-4AEF-BC3A-9D54602602CC;

urn:lsid:zoobank.org:act:8A135983-6EC1-46D1-B6AE-D65A9480B4C8.

Abstract

Two new miniature tetra species of the genus *Priocharax* Weitzman and Vari 1987 are described, raising the known species diversity to twelve. *Priocharax* is characterized by several pedomorphic features such as reductions in the laterosensory system, number of fin rays, ossification of parts of the skull and the presence of a larval rayless pectoral fin in adults. The species described are found in the Rio Purus and Solimões drainages, in the state of Amazonas, Brazil and are diagnosed among themselves and from other species of the genus by the combination of meristic and osteological characters. Furthermore, the two species differ in overall body shape, with one having a deeper body and the other a more streamlined form. Sexual dimorphism was observed in both species. Molecular species delimitation analyses support the distinctiveness of these species. Similarly to *Priocharax britzi* and to *P. conwayi*, the specimens analyzed here were collected within and around protected areas, highlighting the importance of these areas for conservation and biodiversity knowledge.

Keywords: biodiversity; integrative taxonomy; miniaturization; neotropics



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1. Introduction

Priocharax is a genus of miniature fish (sensu Weitzman and Vari [1]) described by Weitzman & Vari in 1987 to allocate two species: *Priocharax ariel*, described from the Rio Orinoco and Rio Negro in Venezuela, and *Priocharax pygmaeus*, from the Rio Amazonas in Leticia, Colombia [2]. Since then, eight more species have been described: *Priocharax nanus* Toledo-Piza, Mattox & Britz 2014 from the Rio Negro [3], *Priocharax varii* Mattox, Souza, Toledo-Piza, Britz & Oliveira 2020 from the Rio Madeira system [4], *Priocharax britzi* Mattox,

Souza, Toledo-Piza & Oliveira 2021 from the Rio Purus system [5], *Priocharax toledopizae* Mattox, Britz, Souza, Casas, Lima & Oliveira 2023 and *Priocharax marupiara* Mattox, Britz, Souza, Casas, Lima & Oliveira 2023, both known from the Rio Juruá drainage [6], *Priocharax conwayi* Mattox, Lima, Britz, Souza & Oliveira 2024 from the Rio Tapajós drainage [7], *Priocharax phasma* Mattox, Lima, Britz, Souza & Oliveira 2024 from the Amazonas drainage [7] and *Priocharax rex* Mattox, Acosta-Santos, Bogotá-Gregory, Agudelo & Lima 2025 from Río Putumayo drainage [8].

This genus is diagnosed by the presence of a larval pectoral fin in adults (i.e., a rayless pectoral-fin), a single series of conical teeth in the premaxilla, maxilla and dentary and five to six branched pelvic-fin rays. Also noted in the genus is a series of reductive anatomical characters associated with miniaturization, such as the loss of the laterosensory canal system, loss of bones in the infraorbital series and the presence of a gap in the Weberian apparatus between neural arches three and four [2,4–7]. Additional diagnostic features of the genus are a posteriorly directed curved process on the cleithrum immediately below the ventral tip of the supracleithrum and the pseudotympanum restricted to the portion anterior to the rib of fifth vertebra [8]. The analysis of material collected during recent expeditions to the Rio Purus and Solimões drainages, Amazonas, Brazil, combined with the examination of specimens already deposited in collections, has revealed the existence of two new *Priocharax* species from the region, which are described here. Moreover, the proximity of both species sampling sites to Brazilian protected areas highlights the importance of these units for understanding biodiversity and conserving fish species, as is the case of *Priocharax britzi* [5] and *P. conwayi*.

2. Materials and Methods

2.1. Morphological Analysis

Counts and measurements follow Fink and Weitzman [9], Weitzman and Vari [2], and Menezes and Weitzman [10] 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 (CPL) [2] and snout length is also expressed as a percentage of orbital diameter (OD) [5]. 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 (Jena, Germany) using the Zen software (Blue Edition v2.7). Counts of vertebrae, supraneurals, teeth, gill-rakers, procurent caudal-fin rays, and information about osteological characters were obtained from specimens cleared and double stained for cartilage and bone following the protocol of Taylor and Van Dyke [11]. Total vertebral number includes the four vertebrae of the Weberian apparatus counted as separate elements and the compound ural centrum as a single vertebra. The gill raker at the junction of ceratobranchials and epibranchials is considered as the posteriormost gill raker on the lower limb of the respective gill arch. Osteological terminology follows Weitzman [12] with updates summarized in Mattox et al. [13]. 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. Information on meristic and morphometric data of *Priocharax ariel*, *P. pygmaeus*, *P. nanus*, *P. varii*, *P. britzi*, *P. marupiara*, *P. toledopizae*, *P. conwayi*, *P. phasma*, and *P. rex* were taken from [2–8]. The identification of infra-orbitals for *Priocharax ariel* is based on Mattox et al. [14,15].

To better understand morphometric data and the putative differences between the two species described below, the 11 measurements taken from 124 specimens were analyzed further with a Principal Component Analysis (PCA) to determine the morphometric variables with the highest amount of variability, followed by a Linear Discriminant Analy-

sis (LDA) to access the level of jackknifed success of correct reclassification of individual specimens according to their original groupings (i.e., hypothesized species) [16]. These two analyses were done in PAST 4.10 [17].

Photographs were made with a Zeiss Discovery V20 stereomicroscope using a Zeiss Axiocam digital camera (Zeiss Inc., Jena, Germany) attached. Specimens examined are deposited in the Laboratório de Biologia e Genética de Peixes (LBP), Museu de Zoologia da USP (MZUSP), Museu de Zoologia da Unicamp (ZUEC), and Instituto Nacional de Pesquisas da Amazônia (INPA).

2.2. Molecular Analysis

Voucher specimens for the molecular study are deposited and preserved in 95% ethanol in the collection of the LBP (Appendix A). Four sequences were generated in this study, and 49 sequences of *Priocharax* were obtained from Mattox et al. [4–7] plus one sequence of the outgroup taxon *Jupiaba polylepis* (Günther 1864) (GenBank n. FJ749068) from Javonillo et al. [18]. Genomic DNA was extracted using the DNeasy Tissue kit (Qiagen Inc., Germantown, MD, USA) following manufacturer's instructions. 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 [19]. PCR amplifications were performed in a total volume of 10.8 µL, with 1.25 µL of 10×buffer, 0.4 µL of MgCl₂ (50 mM), 0.3 µL dNTPs (2 mM), 0.25 µL of each primer (5 mM), 0.2 µL of PHT Taq DNA polymerase (Phoneutria), 1.5 µL of genomic DNA and 8.15 µL ddH₂O. The thermal cycling conditions consisted of an initial denaturation (1 min at 94 °C), followed by 10 cycles of denaturation (30 s at 94 °C), three stages of primer annealing—10 cycles (30 s at 50 °C), 15 cycles (30 s at 52 °C), and 15 cycles (30 s at 54 °C)—and nucleotide extension (1 min at 68 °C), ending with a final extension (10 min at 68 °C). All PCR products were checked on 1% agarose gels and then purified with ExoSap-IT (USB Corporation—Cleveland, OH, USA) following the manufacturer's instructions. The purified PCR products were submitted to sequencing reactions by the Sanger method using BigDye Terminator v 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems—Thermo Fisher Scientific Inc., Waltham, MA, USA). Sequencing reactions were performed with a final volume of 10 µL, containing 4.5 µL of ultrapure water, 1.5 µL of sequencing buffer 5 ×, 1 µL of primer (10 µM), 1 µL of BigDye and 2 µL of purified PCR products. Sequencing reactions followed of an initial denaturation (2 min at 96 °C), followed by 35 cycles of chain denaturation (30 s at 96 °C), annealing (15 s at 50 °C), and a final extension (4 min at 60 °C). The samples were precipitated in ethanol/EDTA, eluted with 10 µL Formamida Hi-Di and loaded onto an ABI 3130 DNA Analyzer automatic sequencer (Applied Biosystems), in the Instituto de Biotecnologia (IBTEC), UNESP, Botucatu, Brazil.

Raw sequences were assembled to consensus using Bioedit (v.7.7.1) and aligned with CLUSTAL W [20] under default parameters. Substitution saturation was evaluated by the method of Xia et al. [21] in DAMBE v6 [22]. Nucleotide variation and a maximum likelihood (ML) analysis conducted with the General Time Reversible + G model were performed in MEGA (v. 12.0.11). Species delimitation analyses included two approaches: Assemble Species by Automatic Partitioning (ASAP) and Bayesian implementation of the Poisson Tree Process model (bPTP; [23]). ASAP was performed through the web server (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>; accessed on 9 July 2025) using Kimura (K80; 2.0); and bPTP using the best ML tree as input, 100,000 generations, and other parameters at default in the bPTP web server (<http://species.h-its.org/ptp/>; accessed on 9 July 2025). Overall and pairwise genetic distances were estimated based on the Kimura 2-parameter model (K2P) + Gamma using MEGA (v. 12.0.11) and the order of groups was

based on the species delimitation analyses results. ASAP, bPTP and genetic distances were performed excluding the outgroup taxa *Jupiaba polylepis*.

3. Results

3.1. Species Description

3.1.1. *Priocharax robbiei* sp. nov.

Priocharax pygmaeus: -Oliveira et al., 2009: 160–164 [24] [Listed, photo: Catuá-Ipixuna Extractive Reserve, Coari, Amazonas, Brazil].

Zoobank LSD: urn:lsid:zoobank.org:act:EB7129F3-B6E4-4AEF-BC3A-9D54602602CC (accessed on 11 of July of 2025)

Holotype: INPA 61245, 13.2 mm SL, Brazil, Amazonas State, Coari municipality, Rio Solimões, Lago Ipixuna, Igarapé da Água Branca, 3°54'20.7" S 63°52'20.5" W. 14 September 2023. G.M.T. Mattox, F.C.T. Lima and R.O. Duarte.

Paratypes: INPA 61246 (12, 12.0–15.9 mm SL; 5 c&s, 12.3–14.0 mm SL); ZUEC 18492 (3, 12.6–13.2 mm SL); MZUSP 130951 (2, 12.8–13.8 mm SL). All collected with holotype.

Non-types: All from Brazil, Amazonas State, Rio Purus basin: INPA 29181 (45, 11.2–14.8 mm SL; 5 c&s, 12.2–13.5 mm SL), Coari municipality, Lago Uauaçu, Igarapé Mirocaia, 04°13'47" S 62°24'21" W, 19 November 2007, L. Rapp Py-Daniel and C. de Deus; INPA 29286 (44, 11.9–15.3 mm SL; 5 c&s, 12.4–13.3 mm SL), Anori municipality, Lago Ayapuá, Igarapé Ajará, 04°25'07" S 62°15'36" W, 15 November 2007, L. Rapp Py-Daniel and C. de Deus; INPA 32630 (43, 10.2–13.2 mm SL; 6 c&s, 12.2–13.5 mm SL), Anori municipality, Rio Purus, Lago Ayapuá, Igarapé 7, no coordinates, 28 September 2008, E. Ferreira et al.; INPA 60219 (12.7 mm SL), Anori municipality, Rio Purus, Lago Ayapuá, Igarapé 7, no coordinates, 28 September 2008, E. Ferreira et al.

Diagnosis: *Priocharax robbiei* sp. nov. is distinguished from *P. nanus*, *P. toledopizae*, *P. conwayi* and *P. rex* by absence of the claustrum (vs. presence). The presence of two postcleithra distinguishes *P. robbiei* sp. nov. from *P. ariel*, *P. conwayi*, *P. phasma*, *P. piagassu* sp. nov. (vs. one) and *P. pygmaeus* (vs. absent). *Priocharax robbiei* sp. nov. can be partially distinguished from *P. marupiara* by its higher number of branched anal-fin rays (20–28, mode 25 vs. 18–23, mode 20). Furthermore, *Priocharax robbiei* sp. nov. can be distinguished from *P. nanus* and *P. varii* by the presence of five branched pelvic-fin rays (vs. six), from *P. varii* by the absence of an adipose fin (vs. presence), and from *P. rex* by the absence of a round flap of skin between contralateral pelvic-fins bases (vs. presence).

Description: For overall appearance, see Figure 1A,B. Morphometric data are presented in Table 1. Body laterally compressed and elongated, greatest depth at vertical through pelvic-fin in small specimens, at vertical through dorsal-fin origin in larger specimens. Dorsal-fin origin approximately at midbody, at vertical through vent and slightly anterior to anal-fin origin. Pectoral-fin bud at vertical through anterior portion of pseudotympanum. Pelvic-fin origin slightly posterior to vertical at 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; slope less conspicuous from latter point to caudal peduncle. Dorsal profile of caudal peduncle straight 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 anal-fin origin. Ventral profile of body slightly concave and posterodorsally rising along anal-fin base, slightly concave from end of anal-fin base to origin of ventral procurrent rays. Caudal peduncle short. Pseudotympanum large, located anterior to rib of fifth vertebra.

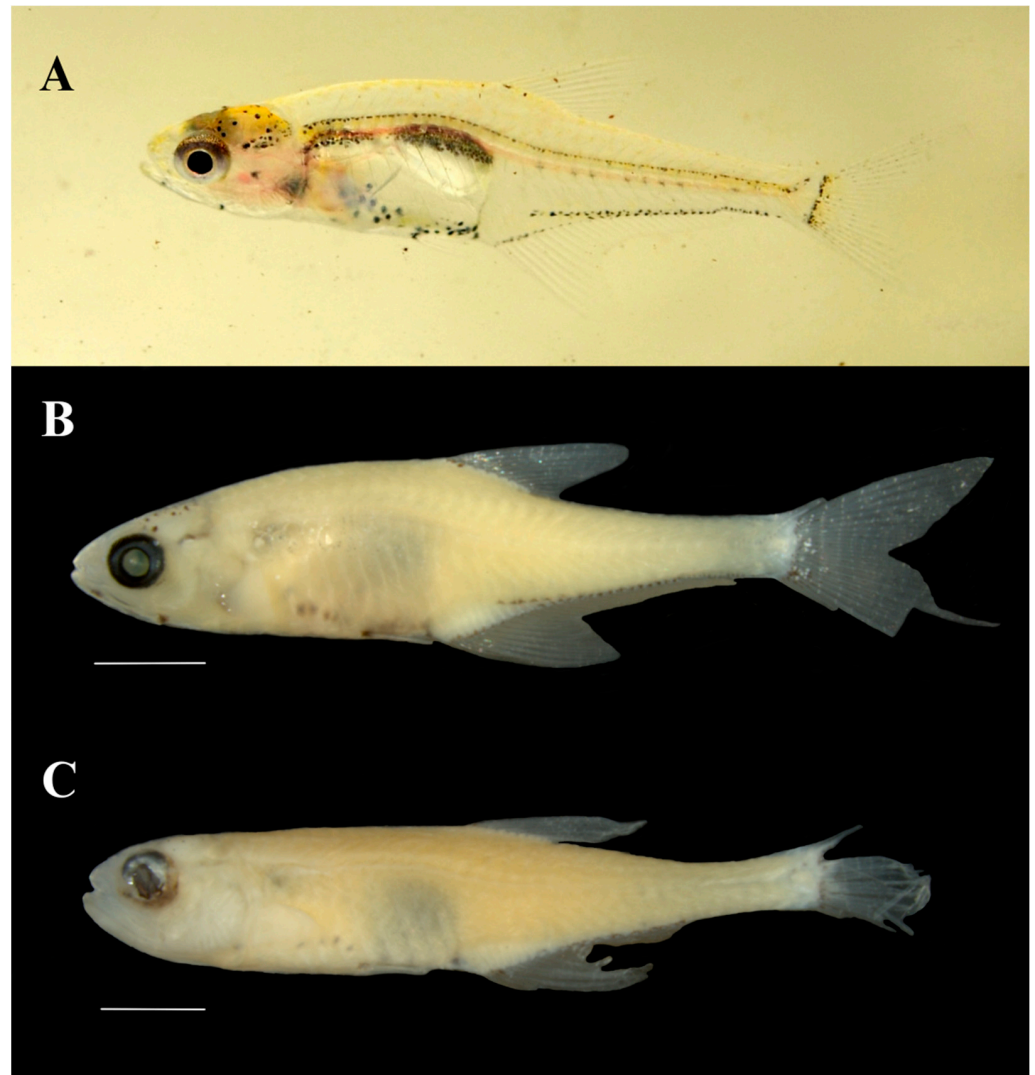


Figure 1. (A) Live paratype of *Priocharax robbiei* sp. nov., INPA 61246, uncertain SL, Brazil, Amazonas State, Coari municipality, Rio Solimões, Lago Ipixuna, Igarapé da Água Branca. (B) Holotype of *Priocharax robbiei* sp. nov., INPA 61245, 13.2 mm SL, Brazil, Amazonas State, Coari municipality, Rio Solimões, Lago Ipixuna, Igarapé da Água Branca. (C) Holotype of *Priocharax piagassu* sp. nov., LBP 36690, 13.8 mm SL, Brazil, Amazonas State, Beruri municipality, Lago Xaviana. Scale bars: 2 mm.

Snout round in lateral view. Eye diameter about one-third of head length. Antorbital and infraorbital 2 present ($n = 16$) (Figure 2A). Infraorbital 1 present in 11 specimens. Infraorbitals 3 to 6 and supraorbital absent ($n = 16$). Mouth terminal. Tip of maxilla elongate, posterior border reaching vertical through midway between middle and posterior border of eye. Premaxillary teeth in single series, premaxilla with 19(1), 20(3), 21(2), 22(3), 23(2), 24(2), 25(1), 26(1) or 28(1) teeth. Maxilla with 22(2), 24(2), 25(2), 26(5), 27(1), 29(1), 32(1), 33(1) or 35(1) teeth. Dentary with 26(2), 28(2), 29(3), 30(3), 31(1), 32(1), 33(2), 34(1) or 36(1) teeth. Dentary teeth in single series, with few anterior teeth slightly displaced from series anteriorly. A conspicuous, elongate foramen at the anterior portion of the dentary. All jaw teeth small, conical, and curved lingually to a moderate extent (Figure 2B).

Table 1. Morphometric data of *Priocharax robbiei* sp. nov. All measurements other than standard length (SL) are expressed as percentages. * Four specimens listed as “non-type” were too damaged to be measured and were, therefore, excluded from this table. Abbreviations: CPL = caudal-peduncle length; HL = head length; N = number of specimens; OD = orbital diameter; SD = standard deviation; SL = standard length. Range includes holotype.

	Holotype	N	Range	Mean	SD
Standard length (SL) (mm)	13.2	146 *	10.2–15.9	12.5	-
Percentages of Standard Length					
Depth at dorsal-fin origin	25.2	138	19–28	23	1.5
Snout to dorsal-fin origin	53.7	140	50–58	54	1.4
Snout to pelvic-fin origin	41.4	139	37–45	41	1.2
Snout to anal-fin origin	53.4	139	50–57	57	1.2
Dorsal-fin length	26.2	107	23–29	26	1.2
Dorsal-fin base	12.1	139	11–15	13	0.6
Pelvic-fin length	8.9	134	8–13	10	0.8
Anal-fin length	23.9	89	19–26	23	1.3
Anal-fin base	37.6	130	30–38	34	1.2
Caudal-peduncle depth	7.9	140	6–10	7	0.6
Caudal-peduncle length	12.1	131	12–19	14	1.2
Percentages of head length (HL)					
Head length	24.1	139	19–29	26	1.2
Orbital diameter	34.4	128	30–38	34	1.6
Interorbital distance	29.8	129	23–33	27	1.9
Snout length	20.9	125	16–26	20	2.0
Upper jaw length	55.4	119	42–57	49	2.8
Percentages of caudal-peduncle length (CPL)					
Caudal-peduncle depth	65.5	134	37–67	50	5.0
Percentages of orbital diameter (OD)					
Snout length	60.7	134	39–87	59	9.3

Dorsal-fin rays ii,7(1), ii,8(2) and ii,9*(133). Endoskeletal part of pectoral fin and some thin exoskeletal bones of the pectoral girdle showing larval structure (Figure 2C). 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, with postcleithrum 3 slender and forming a sinuous arch. Cleithrum with posteriorly directed, curved process immediately below ventral tip of supracleithrum. Pelvic-fin rays i,5*(141). Posterior tip of pelvic fin on vertical through vent in females and extending further posterior slightly beyond anal-fin origin in males. Anal-fin rays ii,20(1), ii,21(2), ii,22(4), ii,23(10), ii,24*(24), ii,25(35), ii,26(16), ii,27(11) or ii,28(6). Anal-fin margin concave with anterior lobe formed by elongated fin rays and posterior section of short rays. Principal caudal-fin rays i,8,7,i(1), i,8,8,i(3), i,9,6,i*(2), i,9,7,i(4) or i,9,8,i(64), dorsal procurrent rays 5(1), 6(1), 7(6), 8(8) or 9(2), ventral procurrent rays 4(1), 5(1), 6(12), 7(2) or 8(1). Caudal fin forked. Adipose fin absent.

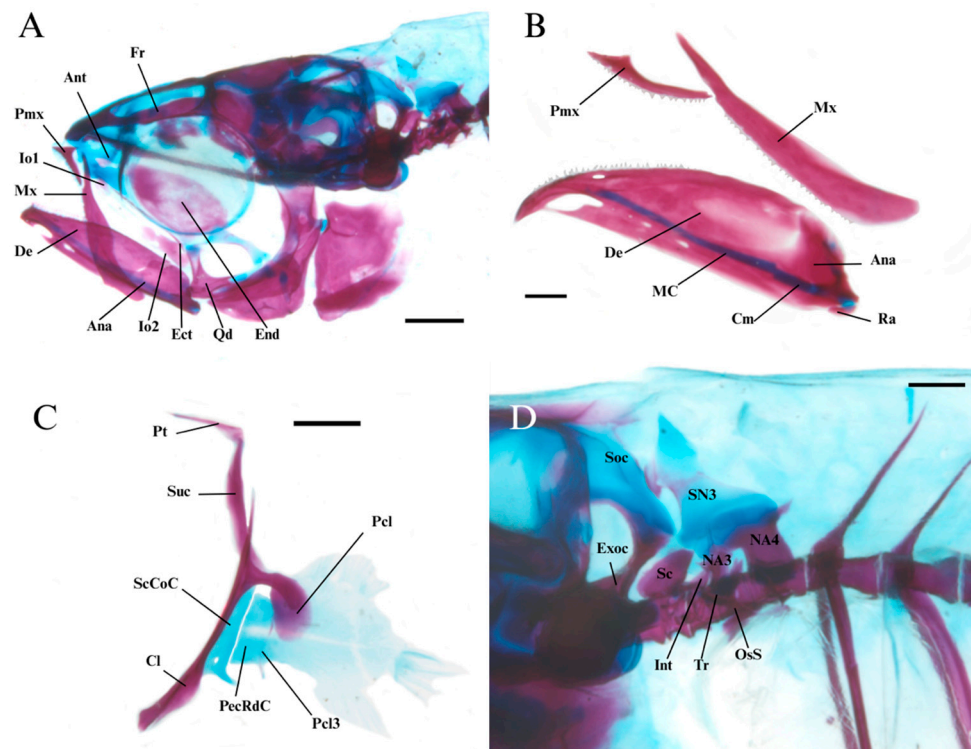


Figure 2. *Priocharax robbiei* sp. nov., INPA 29286, 12.9 mm SL, c&s. (A) Dorsolateral view of head showing infraorbital bones. (B) Left upper jaw in left lateral view, right lower jaw in medial view, flipped. Meckel's cartilage broken near posterior third portion. (C) Left pectoral girdle in lateral view. (D) Weberian apparatus in lateral view. Abbreviations: Ana, anguloarticular; Ant, antorbital; Cl, cleithrum; Cm, coronomeckelian; De, dentary; Ect, ectopterygoid; End, endopterygoid; Exoc, exoccipital; Fr, frontal; Int, intercalarium; Io1–2, infraorbitals 1 and 2; MC, Meckel's cartilage; Mx, maxilla; NA3–4, neural arches 3–4; PecRdC, pectoral-fin radial cartilage; Pel, unidentified postcleithrum; Pcl3, postcleithrum 3; Pmx, premaxilla; Pt, posttemporal; OsS, os suspensorium; Qd, quadrate; Ra, retroarticular; Sc, scaphium; ScCoC, scapulocoracoid cartilage; SN3, supraneural 3; Soc, supraoccipital; Suc, supracleithrum; Tr, tripus. Scale bars: (A) 0.5 mm, (B) 0.2 mm, (C) 0.5 mm, (D) 0.2 mm.

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 22(2), 23*(4), 24(4) or 25(1); no canal-bearing lateral line scales. Scale rows between dorsal-fin origin and pelvic-fin origin 8(5) or 9*(6). Scale rows around caudal peduncle 9(2) or 10*(9). Predorsal scales typically absent but occasionally one or two scales present immediately anterior to dorsal fin. Caudal-fin squamation restricted to base of caudal-fin rays, no scales on caudal-fin lobes.

Total vertebrae 32(8), 33(7) or 34(1) with 14(15) or 15(1) abdominal vertebrae and 18(8) or 19(8) caudal vertebrae. Total number of gill-rakers on first branchial arch 11(4), 12(5) or 13(2), with 2(1) or 3(10) gill-rakers on upper limb, and 8(3), 9(6) or 10(2) gill-rakers on lower limb. Weberian apparatus well-developed, all components ossified except for claustrum, which is absent (Figure 2D). Large gap between neural arches 3 and 4, with gap partially covered by dorsally projecting pointed process from vertebra 3. Inner arm of os suspensorium large, projecting forward to vertical through middle of second centrum. Supraneurals 4(2), 5(16), 6(2) or 7(1).

Color in alcohol: Overall body color pale yellow (Figure 1B). Melanophores scattered on dorsal surface of head in region of brain. Concentration of melanophores present on anterior region of dentary, with additional patch located along posterior margin of lower jaw. Thin horizontal line of melanophores radiating from posterodorsal margin of orbital

cavity. Overall coloration of eye around pupil black with faint silver of guanine. Thin, irregular line of melanophores present along base of dorsal fin, with higher concentration on anterior rays. Patch of melanophores on bases of pelvic fins. Thin short vertical line of melanophores along myoseptum posterior to vent. Thin line of scattered melanophores along entire anal-fin base, and another thicker curved line of melanophores along ventral margin of hypaxial myomeres starting at vertical through fourth to sixth branched anal-fin ray, approaching thin line of anal-fin base posteriorly. Fins mostly hyaline, with only a few irregularly distributed melanophores. Scattered melanophores in small patches at base of caudal-fin rays.

Color in life: Body mostly translucent, with melanophore patterns consistent with those observed in alcohol-preserved specimens (Figure 1A). Additionally, melanophores present along dorsal surface of vertebral column and dorsal surface of swim bladder. Patch of xanthophores on dorsal surface of head, snout, anterior tip of dentary, and along vertebral column. Irregular and faint xanthophores also distributed along dorsal region. Blotches of xanthophores present at bases of caudal-fin lobes, while remainder of caudal fin with scattered melanophores. All other fins predominantly hyaline. Scattered xanthophores along dorsal margin of eye, followed by a concentration of melanophores. Ventral half of eye predominantly silvery.

Sexual dimorphism: Male of *Priocharax robbiei* **sp. nov.** with typical sexual dimorphism of other characiforms (e.g., bony hooks on anal-fin) (Figure 3A,C). Hooks on the anal fin present from the first, longest unbranched ray to the fourth branched ray; hooks oriented posteriorly, with seven to nine hooks per ray, a single hook per segment of each ray ($n = 1$). Male with a horizontal slit along the pelvic-fin musculature separating it from the main hypaxial muscle mass of the body (Figure 4A). In addition, overall size of pelvic girdle in mature male larger than that of females, pelvic bone restricted posteriorly to level of rib of sixth vertebra in females, but reaching further anteriorly in mature male. Basipterygium of male better ossified, more robust, and closer to contralateral part than in females.

Distribution: *Priocharax robbiei* **sp. nov.** is known from Lago Ipixuna in the Rio Solimões basin, as well as from two lakes in the Rio Purus basin: Lago Uauaçu and Lago Ayapua (Figure 5). The sampled sites were situated within the Catuá-Ipixuna Extractive Reserve and the Piagaçu-Purus Sustainable Development Reserve, two protected areas located in the State of Amazonas, Brazil.

Ecological notes: *Priocharax robbiei* **sp. nov.** occurs in blackwater systems, including small streams and ponds, and was collected at the margins where riparian vegetation was still present (Figure 6A). At the time of the collection of the type-series of *Priocharax robbiei* **sp. nov.** (September 2023), water levels were exceptionally low due to an El Niño event, so this might have affected the colour of the water in Figure 6A. We tested our data with the framework provided by [25] and it turns out that the locality is indeed a blackwater system, in spite of the name “Água Branca” (White Water, in Portuguese). At the type locality, *Priocharax robbiei* **sp. nov.** was collected at approximately 0.5–1 m in depth and alongside other small fish species, including the tetras *Hemigrammus analis* Durbin 1909, and *Megalampodus bentosi* (Durbin 1908); the acestrorhampid *Tyttobrycon xerui* Géry 1973, the aphyocharacin *Cyanogaster* sp., and the stevardiid *Xenobrycon* sp.; the cyprinodontiform *Fluviophylax simplex* Costa 1996, the pencilfish *Nannostomus digrammus* (Fowler 1913) and the needlefish *Belonion* sp. Notably, *P. robbiei* **sp. nov.** was collected in mixed schools with *Fluviophylax simplex*.

Etymology: The epithet, *robbiei*, honors Roberto “Robbie” Taliaferro Mattox Jr., father of one of the authors (G.M.T.M.) who passed away while G.M.T.M. was in the field with F.C.T.L. collecting the type series. A noun in the genitive case.

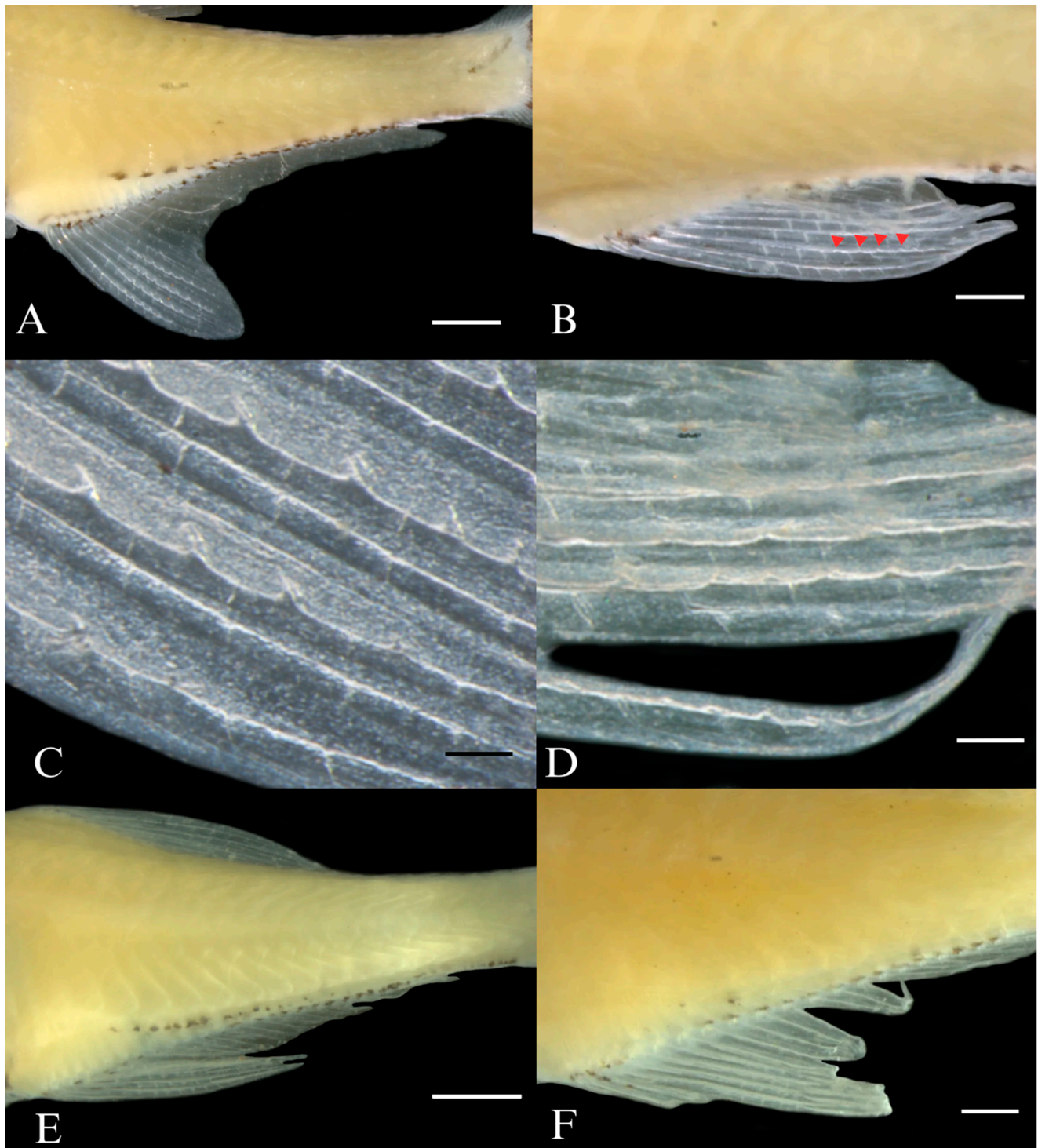


Figure 3. Lateral view of anal fin (A) *Priocharax robbiei* **sp. nov.**, paratype, male, INPA 61246, 15.9 mm SL. (B) *Priocharax piagassu* **sp. nov.**, paratype, male, LBP 31205, 13.9 mm SL. Red arrow heads indicate the small hooks. (C) *Priocharax robbiei* **sp. nov.**, same specimen as in (A), shown at higher magnification to highlight the hooks. (D) *Priocharax piagassu* **sp. nov.**, same specimen as in (B), shown at higher magnification to highlight the hooks. (E) *Priocharax robbiei* **sp. nov.**, paratype, female, INPA 61246, 13.2 mm SL. (F) *Priocharax piagassu* **sp. nov.**, paratype, female, ZUEC 18520, 14.3 mm SL. Scale bars: (A) 1 mm, (B) 0.5 mm, (C,D) 0.1 mm, (E) 1 mm, (F) 0.5 mm.

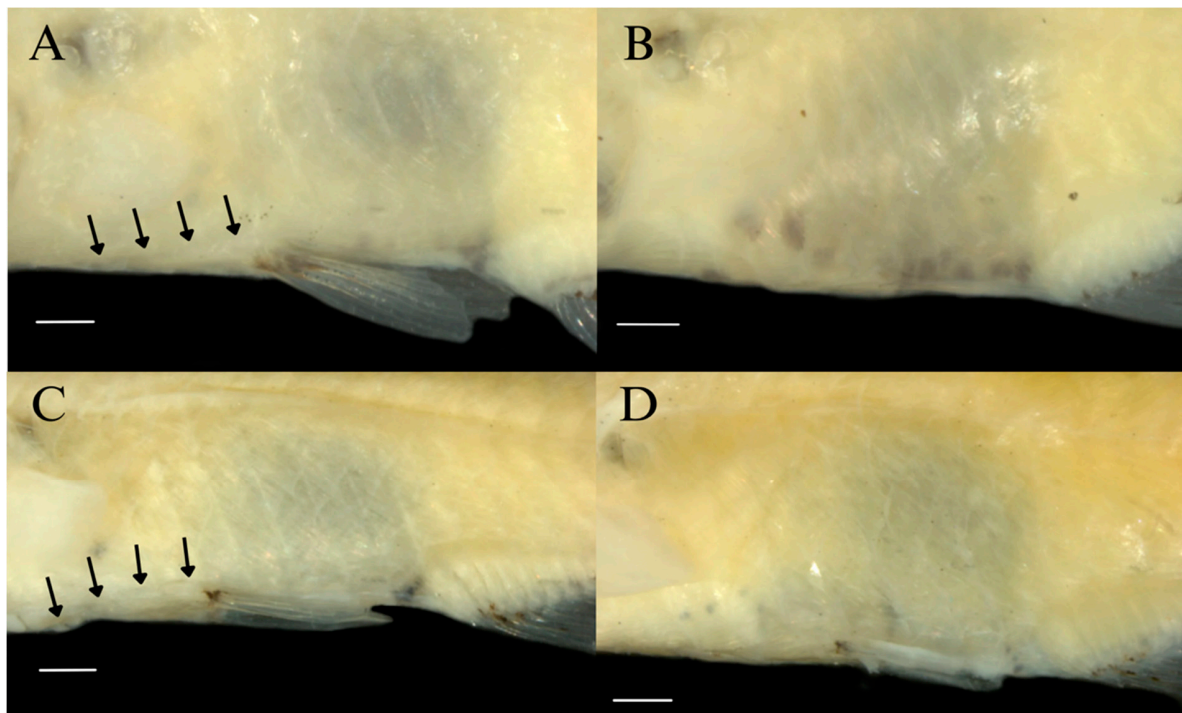


Figure 4. Lateral view of pelvic girdle (A) *Priocharax robbiei* sp. nov., paratype, male, INPA 61246, 15.9 mm SL. (B) *Priocharax robbiei* sp. nov., paratype, female, INPA 61246, 13.2 mm SL. (C) *Priocharax piagassu* sp. nov., paratype, male, LBP 31205, 13.9 mm SL. (D) *Priocharax piagassu* sp. nov., paratype, female, ZUEC 18520, 14.3 mm SL. Arrows point to slit separating body wall from pelvic fin musculature. Scale bars: 0.5 mm.

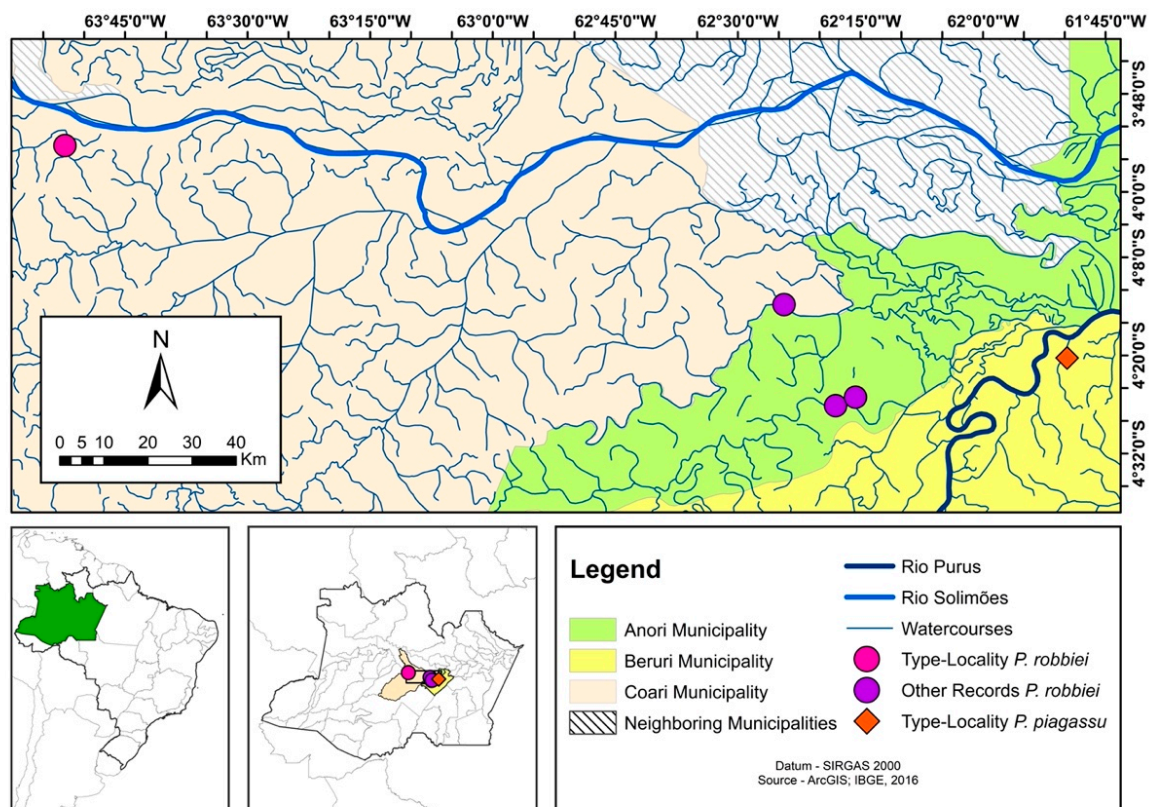


Figure 5. Partial map of Amazonas State, Brazil, showing the sampling sites of *Priocharax robbiei* sp. nov. (in pink and purple) and *Priocharax piagassu* sp. nov. (in orange). The orange diamond marks the

type locality and the only known distribution point of *P. piagassu* **sp. nov.** Dots may represent more than one lot. Due to the large extent of the Catuá-Ipixuna Extractive Reserve and the Piagaçu-Purus Sustainable Development Reserve, these protected areas were not included in the map to maintain visual clarity. State of Amazonas represented in dark green.

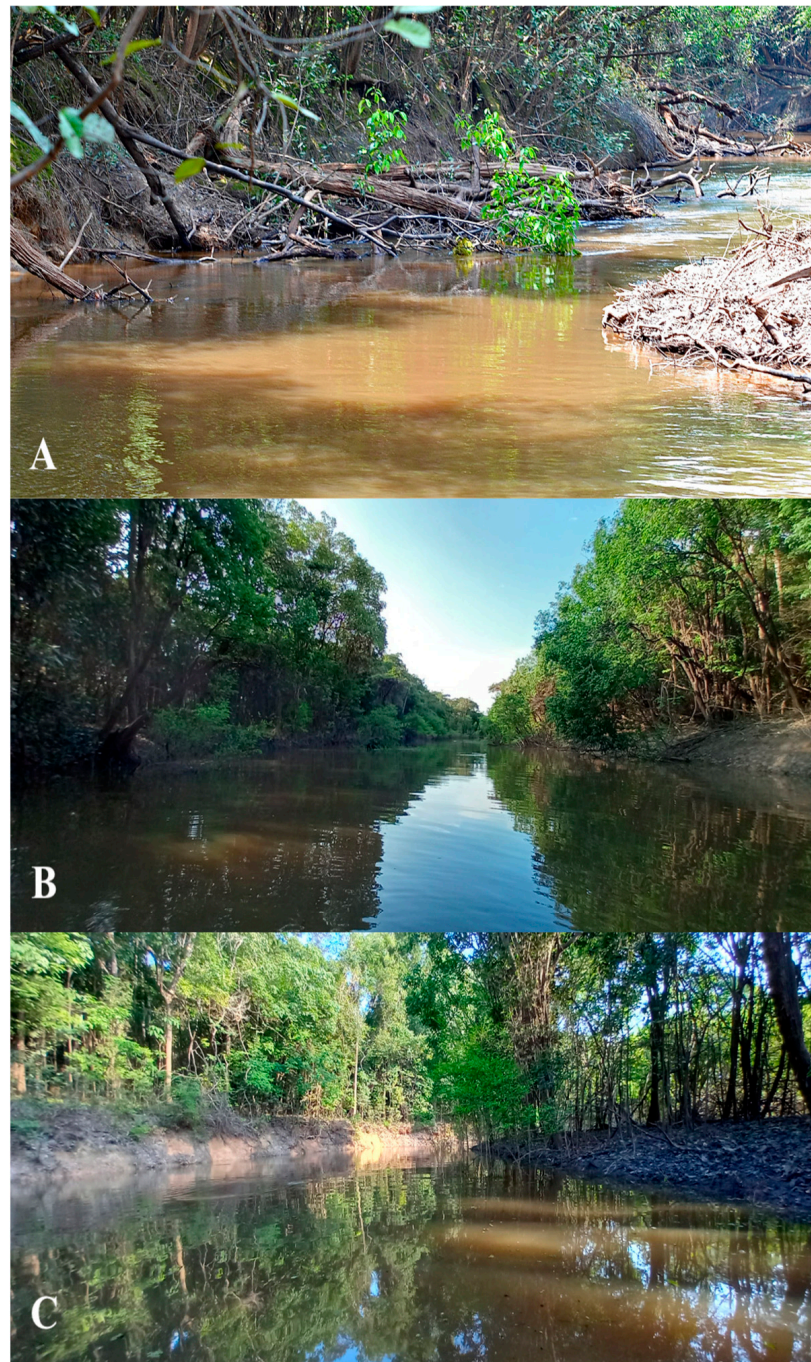


Figure 6. General view of type localities of *Priocharax robbiei* **sp. nov.** (A) and *P. piagassu* **sp. nov.** (B,C). (A) Igarapé da Água Branca, Lago Ipixuna, Rio Solimões, Coari municipality, Amazonas State, Brazil. (B,C) Lago Xaviana, Beruri municipality, Amazonas State, Brazil.

3.1.2. *Priocharax piagassu* **sp. nov.**

Zoobank LSD: [urn:lsid:zoobank.org:act:8A135983-6EC1-46D1-B6AE-D65A9480B4C8](https://zoobank.org/act:8A135983-6EC1-46D1-B6AE-D65A9480B4C8) (accessed on 11 of July of 2025)

Holotype: LBP 36690, 13.8 mm SL, Brazil, Amazonas State, Beruri municipality, Lago Xaviana, Rio Purus, 04°20'18.70" S 61°49'43.20" W, 12 Oct 2021, B.F. Morales.

Paratypes: LBP 31205 (6, 11.5–16.1 mm SL, 2 c&s, 11.7–13.5 mm SL), INPA 61694 (4, 12.5–15.0 mm SL; 1 c&s, 12.6 mm SL), MZUSP 130955 (4, 12.5–15.4 mm SL; 1 c&s, 12.3 SL), ZUEC 18520 (4, 12.9–14.4 mm SL; 1 c&s, 12.5 mm SL), all collected with holotype.

Diagnosis: *Priocharax piagassu* **sp. nov.** is distinguished from *P. nanus*, *P. toledopizae*, *P. conwayi* and *P. rex* by absence of the claustrum (vs. presence). *Priocharax piagassu* **sp. nov.** is distinguished from all congeners by the presence of infraorbitals 2 + 3 (vs. presence infraorbitals 1 + 2 + 3 in *P. ariel* and *P. conwayi*; presence of infraorbitals 1 + 2 in *P. toledopizae*, *P. marupiara*, *P. phasma* and *P. robbiei* **sp. nov.**; presence of infraorbital 2 in *P. britzi*, *P. marupiara* and *P. robbiei* **sp. nov.**; absence of infraorbitals in *P. pygmaeus*, *P. nanus*, *P. varii* and *P. rex*). It is distinguished from all congeners except *Priocharax ariel*, *P. conwayi* and *P. phasma* by the presence of a single postcleithrum (vs. two postcleithra in most congeners and absent in *P. pygmaeus*). Furthermore, *Priocharax piagassu* **sp. nov.** can be distinguished from *P. nanus* and *P. varii* by the presence of five branched pelvic-fin rays (vs. six), from *P. varii* by the absence of an adipose fin (vs. presence), and from *P. rex* by the absence of a round flap of skin between contralateral pelvic-fins bases (vs. presence).

Description: For overall appearance, see Figure 1C. Morphometric data are presented in Table 2. Body laterally compressed and elongated, fusiform, greatest depth at vertical through pelvic-fin in small specimens, at vertical through dorsal-fin origin in larger specimens. Dorsal-fin origin approximately at midbody, at vertical through vent and slightly anterior to anal-fin origin. Pectoral-fin bud at vertical through anterior portion of pseudotympanum. Pelvic-fin origin slightly posterior to vertical at 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; slope less conspicuous from latter point to caudal peduncle. Dorsal profile of caudal peduncle straight to base of dorsal procurent 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 anal-fin origin. Ventral profile of body slightly concave and posterodorsally rising along anal-fin base, slightly concave from end of anal-fin base to origin of ventral procurent rays. Caudal peduncle long and relatively robust. Pseudotympanum large, located anterior to rib of fifth vertebra.

Snout round in lateral view. Eye diameter about one-third of head length. Antorbital and infraorbitals 2 and 3 present ($n = 5$) (Figure 7A). Infraorbitals 1 and 4 to 6 and supraorbital absent ($n = 5$). Mouth terminal. Tip of maxilla elongate, posterior border reaching vertical through midway between middle and posterior border of eye. Premaxillary teeth in single series, premaxilla with 21(2), 22(1), 23(1) or 25(1) teeth. Maxilla with 22(1), 23(1), 25(1), 26(1) or 27(1) teeth. Dentary with 25(1), 26(1), 30(1) or 31(2) teeth. Dentary teeth in single series, with few anterior teeth slightly displaced from series anteriorly. A conspicuous, elongate foramen at anterior portion of dentary. All jaw teeth small, conical, and curved lingually to a moderate extent (Figure 7B).

Dorsal-fin rays ii,9*(15). Endoskeletal part of pectoral fin and some thin exoskeletal bones of the pectoral girdle showing larval structure (Figure 7C). 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 one post-cleithrum. Cleithrum with posteriorly directed, curved process immediately below ventral tip of

supracleithrum. Pelvic-fin rays i,5*(15). Posterior tip of pelvic fin on vertical through vent in females and extending further posterior slightly beyond anal-fin origin in males. Anal-fin rays ii,18(1), ii,19(3), ii,20(3), ii,21*(2), ii,21(1) or ii,23(1). Anal-fin margin concave with anterior lobe formed by elongated fin rays and posterior section of short rays. Principal caudal-fin rays i,8,8,i(2) or i,9,8,i(7), dorsal procurent rays 7(1) or 8(4), ventral procurent rays 6(2) or 7(3). Caudal fin forked. Adipose fin absent.

Table 2. Morphometric data of *Priocharax piagassu* sp. nov. All measurements other than standard length (SL) are expressed as percentages. * One specimen listed as “paratype” were too damaged to be measured and was, therefore, excluded from this table Abbreviations: CPL = caudal-peduncle length; HL = head length; N = number of specimens; OD = orbital diameter; SD = standard deviation; SL = standard length. Range includes holotype.

	Holotype	N	Range	Mean	SD
Standard length (SL) (mm)	13.8	17	11.5–16.1	13.7	-
Percentages of Standard Length					
Depth at dorsal-fin origin	21.1	15	20–25	22	1.3
Snout to dorsal-fin origin	52.9	15	53–56	54	0.9
Snout to pelvic-fin origin	39.1	15	39–43	41	1.3
Snout to anal-fin origin	56.9	15	54–58	57	1.2
Dorsal-fin length	24.7	8	23–26	24	0.7
Dorsal-fin base	11.3	15	11–13	12	0.8
Pelvic-fin length	12.0	14	11–12	12	0.4
Anal-fin length	-	7	22–25	23	1.0
Anal-fin base	30.0	10	28–32	30	1.4
Caudal-peduncle depth	7.3	15	7–9	8	0.4
Caudal-peduncle length	17.2	10	16–20	18	1.3
Percentages of head length (HL)					
Head length	23.1	15	23–29	25	1.5
Orbital diameter	38.4	10	33–38	35	2.1
Interorbital distance	30.5	11	25–31	28	1.5
Snout length	15.4	12	13–19	16	2.1
Upper jaw length	51.7	12	43–52	48	2.5
Percentages of caudal-peduncle length (CPL)					
Caudal-peduncle depth	42.8	10	41–52	47	3.7
Percentages of orbital diameter (OD)					
Snout length	40.1	9	33–58	46	8.6

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 21(3), 22*(1), 23(2) or 24(1); no canal-bearing lateral line scales. Scale rows between dorsal-fin origin and pelvic-fin origin 7*(4), 8(3) or 9(1). Scale rows around caudal peduncle 9(4) ou 10*(3). Predorsal scales typically absent but occasionally one or two scales present immediately anterior to dorsal fin. Caudal-fin squamation restricted to base of caudal-fin rays, no scales on caudal-fin lobes.

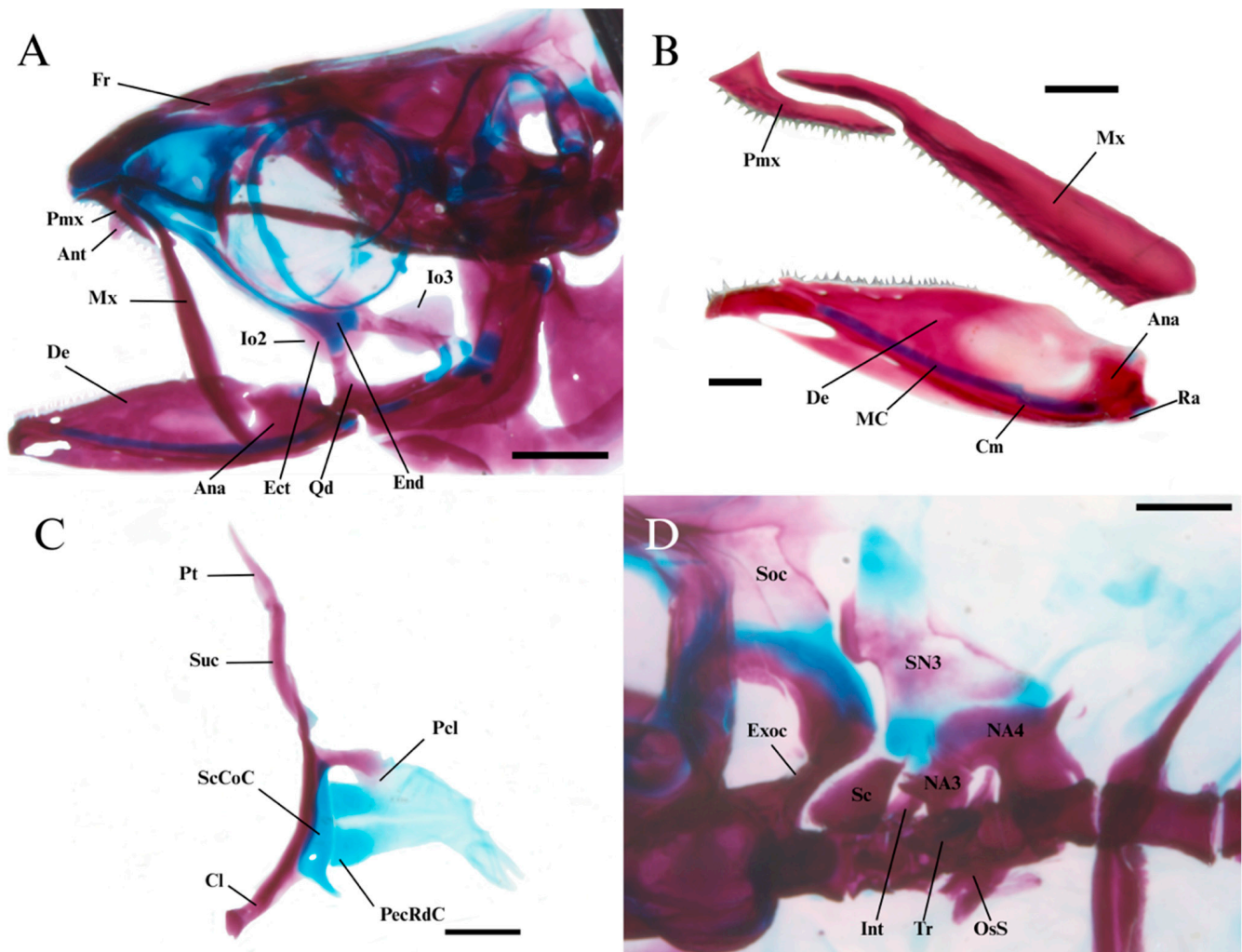


Figure 7. *Priocharax piagassu* sp. nov., paratype, LBP 31205, 13.5 mm SL, c&s. (A) Dorsolateral view of head showing infraorbital bones. (B) Right upper jaw in lateral view, right lower jaw in medial view. Lower jaw slightly damaged in the anterior part of the dentary fenestra. Meckel's cartilage broken near posterior third portion. (C) Left pectoral girdle in lateral view. (D) Weberian apparatus in lateral view. Abbreviations: Ana, anguloarticular; Ant, antorbital; Cl, cleithrum; Cm, coronomeckelian; De, dentary; Ect, ectopterygoid; End, endopterygoid; Exoc, exoccipital; Fr, frontal; Int, intercalarium; Io2–3, infraorbitals 2 and 3; MC, Meckel's cartilage; Mx, maxilla; NA3–4, neural arches 3–4; PecRdC, pectoral-fin radial cartilage; Pcl, unidentified postcleithrum; Pmx, premaxilla; Pt, posttemporal; OsS, os suspensorium; Qd, quadrate; Ra, retroarticular; Sc, scaphium; ScCoC, scapulocoracoid cartilage; SN3, supraneural 3; Soc, supraoccipital; Suc, supracleithrum; Tr, tripus. Scale bars: (A) 0.5 mm, (B) 0.2 mm, (C) 0.5 mm, (D) 0.2 mm.

Total vertebrae 32(3) or 33(2) with 14(5) abdominal vertebrae and 18(3) or 19(2) caudal vertebrae. Total number of gill-rakers on first branchial arch 11(2), 12(2) or 13(1), with 2(3) or 3(2) gill-rakers on upper limb, and 9(3) or 10(2) gill-rakers on lower limb. Weberian apparatus well-developed, all components ossified except for claustrum, which is absent (Figure 7D). Large gap between neural arches 3 and 4, with gap partially covered by dorsally projecting pointed process from vertebra 3. Inner arm of os suspensorium large, projecting forward to vertical through middle of second centrum. Supraneurals 5(3) or 6(2).

Color in alcohol: Overall body coloration pale yellow (Figure 1C). Few melanophores scattered on dorsal surface of head, particularly over brain region. Distinct patch of melanophores present along posterior margin of lower jaw. Some specimens with three to four inconspicuous melanophores radiating horizontally from tip of snout to anterior mar-

gin of orbital cavity. Dorsal portion of eye black, with decreasing density of melanophores ventrally toward center. Eye with overall silvery appearance, due to underlying guanine layer. Thin, irregular line of melanophores present along base of dorsal fin. Patch of melanophores on bases of pelvic fins. Pelvic-fin base with conspicuous dark patches of melanophores. Thin line of scattered melanophores along entire anal-fin base. Fins mostly hyaline, with only few irregularly distributed melanophores. Melanophores arranged in small patches at base of caudal-fin rays.

Sexual dimorphism: Males of *Priocharax piagassu* **sp. nov.** with typical sexual dimorphism of other characiforms (e.g., bony hooks on anal-fins) (Figure 3B,D). Hooks on the anal fin present from the first, longest unbranched ray to the fourth branched ray; hooks oriented posteriorly, with two to seven hooks in each ray, with one hook per segment of each ray ($n = 2$). Males with an incipient horizontal slit along the pelvic-fin musculature separating it from the main hypaxial muscle mass of the body (Figure 4C). Overall size of pelvic girdle in mature males larger than that of females, pelvic bone restricted posteriorly to level of rib of sixth vertebra in females, but reaching further anteriorly (e.g., halfway between ribs of fifth and sixth vertebra, or rib of fifth vertebra) in mature males. Basipterygium of males better ossified, more robust, and closer to contralateral part than in females.

Distribution: *Priocharax piagassu* **sp. nov.** is only known from its type locality, Lago Xaviana, an affluent of Rio Purus (Figure 5) in the surroundings of the Piagaçu-Purus Sustainable Development Reserve, Beruri municipality, Amazonas State.

Ecological notes: *Priocharax piagassu* **sp. nov.** was collected in blackwater system, in a small stream from the upper section of a lake located in an peculiar area that the presence of floodplain areas on one bank and “terra-firme” on the other creates an environmental gradient, and was collected at the margins with moderate slope and substantial amounts of leaf litter, where riparian vegetation was still present (Figure 6B,C). Access to this location is very difficult, so all specimens sampled in this study were collected in the same fieldtrip. At the type locality, *Priocharax piagassu* **sp. nov.** was collected with the anchovy *Anchoviella jamesi* (Jordan & Seale 1926), and a range of characiforms, such as *Aphyocharax* sp., *Oxybrycon parvulus* Géry 1964, *Hemigrammus analis* Durbin 1909, *Hemigrammus durbinae* Ota, Lima & Pavanelli 2015, *Hemigrammus levis* Durbin 1908, *Cyanogaster* sp., *Iguanodectes purusii* (Steindachner 1908), *Nannostomus eques* Steindachner 1876, *Nannostomus unifasciatus* Steindachner 1876, and *Pyrrhulina australis* Eigenmann & Kennedy 1903. Other species recorded include the siluriforms *Loricariichthys nudirostris* (Valenciennes 1840) and *Rineloricaria castroi* Isbrücker & Nijssen 1984, as well as the cyprinodontiforms *Fluviphylax simplex* Costa 1996 and *Anablepsoides ornatus* (Garman 1895). Also found were the sleeper *Microphilypnus ternetzi* Myers 1927; the cichlid *Apistogramma agassizii* (Steindachner 1875) and the leafaffish *Monocirrhus polyacanthus* Heckel, 1840.

Etymology: The name *piagassu* originates from Nheengatu, an Indigenous language, in which *piá* means “heart” and *wa’su* means “big”, symbolizing the “Big heart”—in allusion to the region in the middle of the Amazon basin the type series specimens were collected. A noun in apposition.

3.2. PCA and LDA

Specimens used in the description of *Priocharax piagassu* **sp. nov.** are in poor condition and exhibit several damaged structures, which prevented the inclusion of measurements such as dorsal-fin length, anal-fin length, interorbital distance, orbital diameter, and upper jaw length in the PCA and LDA, as these statistical methods require complete datasets and cannot be directly applied when data are missing [16]. As a result, only 11 measurements from 124 specimens were included in the PCA and LDA (Figure 8).

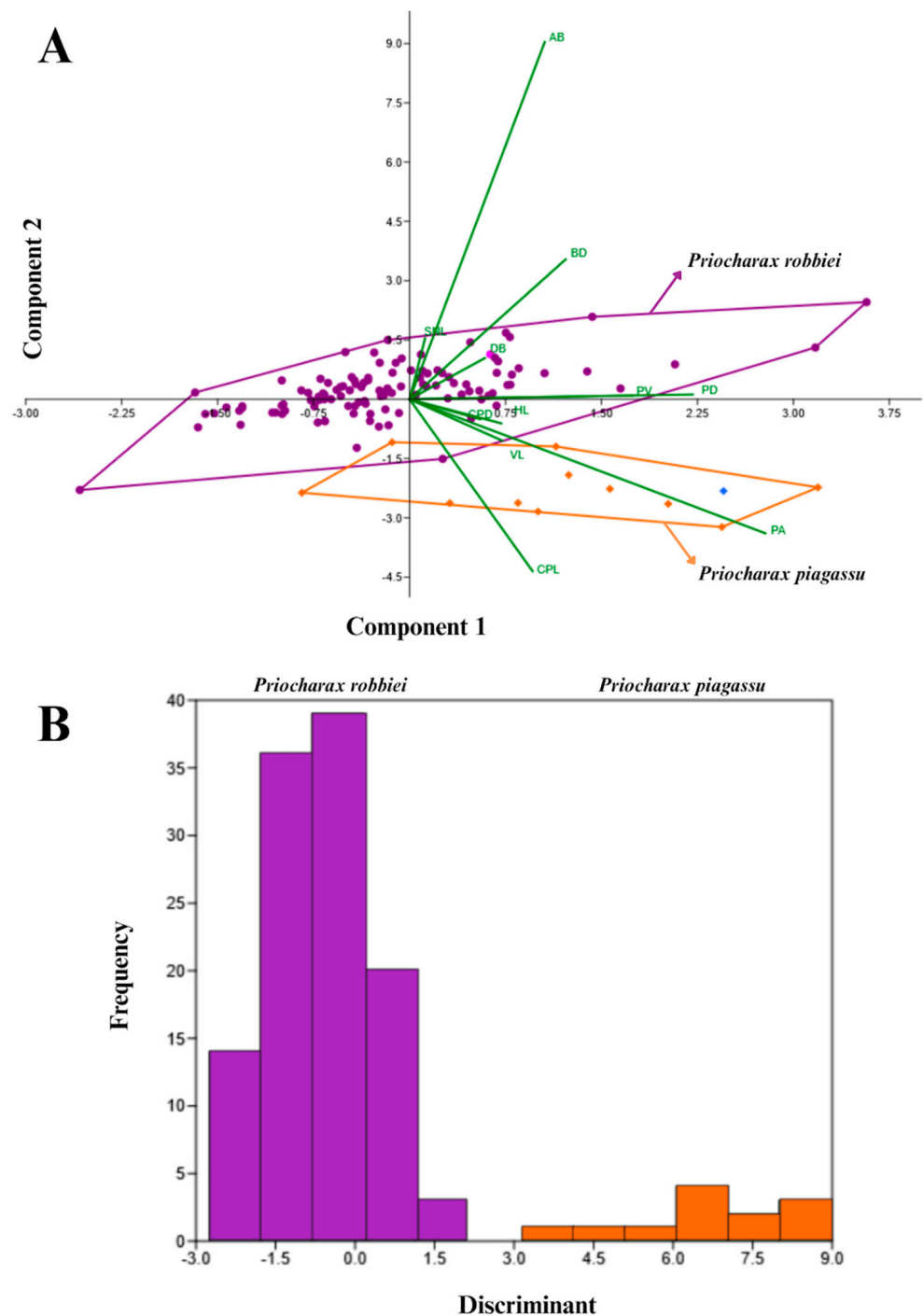


Figure 8. (A) Principal component analysis plot of 11 morphometric measurements, showing the clustering of the 124 specimens in two subsets (i.e., *Priocharax robbiei* sp. nov. and *P. piagassu* sp. nov.) (B) Linear discriminant analysis plot of the same morphometric characters showing the separation of specimens of the two species recognized herein. The pink dot represents the holotype of *P. robbiei* sp. nov. The blue diamond represents the holotype of *P. piagassu* sp. nov. Abbreviations: AB, anal-fin base; BD, body depth; CPD, caudal-peduncle depth; CPL, caudal-peduncle length; DB, dorsal-fin base; HL, head length; PA, pre-anal distance; PD, pre-dorsal distance; PV, pre-pelvic distance; SNL, snout length; VL, pelvic-fin length.

The PCA revealed that the two species are primarily separated along the first principal component (PC1), which accounts for 72.2% of the total variation (Figure S1). This separation is mainly driven by differences in pre-anal (PA), pre-dorsal (PD), and pre-pelvic (PV) distances (Figure S2). As shown in Figure 8A, *Priocharax piagassu* sp. nov. exhibits

higher values for these variables, indicating a more elongated anterior body region compared to *P. robbiei* **sp. nov.** The second principal component (PC2), which accounts for 11.6% of the total variation, was mainly influenced by the length of the anal-fin base (AB) (Figures S1 and S2). As also shown in Figure 8A, *P. robbiei* **sp. nov.** exhibits higher values for this count, which is directly associated with a greater number of anal-fin rays in this species compared to *P. piagassu* **sp. nov.** (ii,20–28 vs. ii,18–23). An LDA was also conducted using the morphometric data to assess classification accuracy, yielding a jackknifed success rate of 99.19%, which strongly supports the morphological distinctiveness between the two species (Figures 8B, S3 and S4).

3.3. Molecular Data Analysis

The molecular data included 54 sequences with 524 bp and 205 variable sites. The nucleotide frequencies were 23.9% adenine, 16.8% guanine, 33.2% thymine, and 26% cytosine. DAMBE indicated no saturation for either transitions or transversions in both asymmetrical (Iss.cAsym) and symmetrical (Iss.cSym) topologies. The maximum likelihood (ML) tree showed high bootstrap values supporting each of the analyzed species (Figure 9). Both ASAP (best partition asap-score: 2.00) and bPTP analyses recognized 11 species, supporting *Priocharax robbiei* **sp. nov.** and *Priocharax piagassu* **sp. nov.** as new species (Figures 9 and S5). The overall mean of genetic distances (K2P) among *Priocharax* species was 21.6%. The values of interspecific distances ranged from 4.8% between *P. robbiei* **sp. nov.** and *P. britzi* to 31.7% between *P. pygmaeus* and *P. ariel* (Table 3).

Table 3. Pairwise K2P genetic distances among species of *Priocharax*. Intraspecific genetic variations are shown in the diagonal. Numbers below diagonal are values of interspecific distances. Values shown in percentages.

	1	2	3	4	5	6	7	8	9	10	11
1. <i>P. ariel</i>	-										
2. <i>P. varii</i>	22.9	0.1									
3. <i>P. britzi</i>	26.1	26.1	0.1								
4. <i>P. nanus</i>	21.8	21.1	22.1	-							
5. <i>P. phasma</i>	28.5	21	18.9	22	0.2						
6. <i>P. conwayi</i>	24.1	18.3	26.9	22.4	26.3	0.2					
7. <i>P. marupiara</i>	18.5	21.3	19.4	18.7	26.2	20.5	0				
8. <i>P. pygmaeus</i>	31.7	23.2	24.5	22	25.6	30.7	22.1	0			
9. <i>P. toledopizae</i>	29.5	23	22.9	20.7	23.5	25.5	22	8.8	0.1		
10. <i>P. robbiei</i>	24.9	24.6	4.8	21.8	18.8	23.1	20	25	24.3	0	
11. <i>P. piagassu</i>	19.5	19.9	20.8	22.2	25	21.5	8.5	24.7	24.4	18.5	0.1

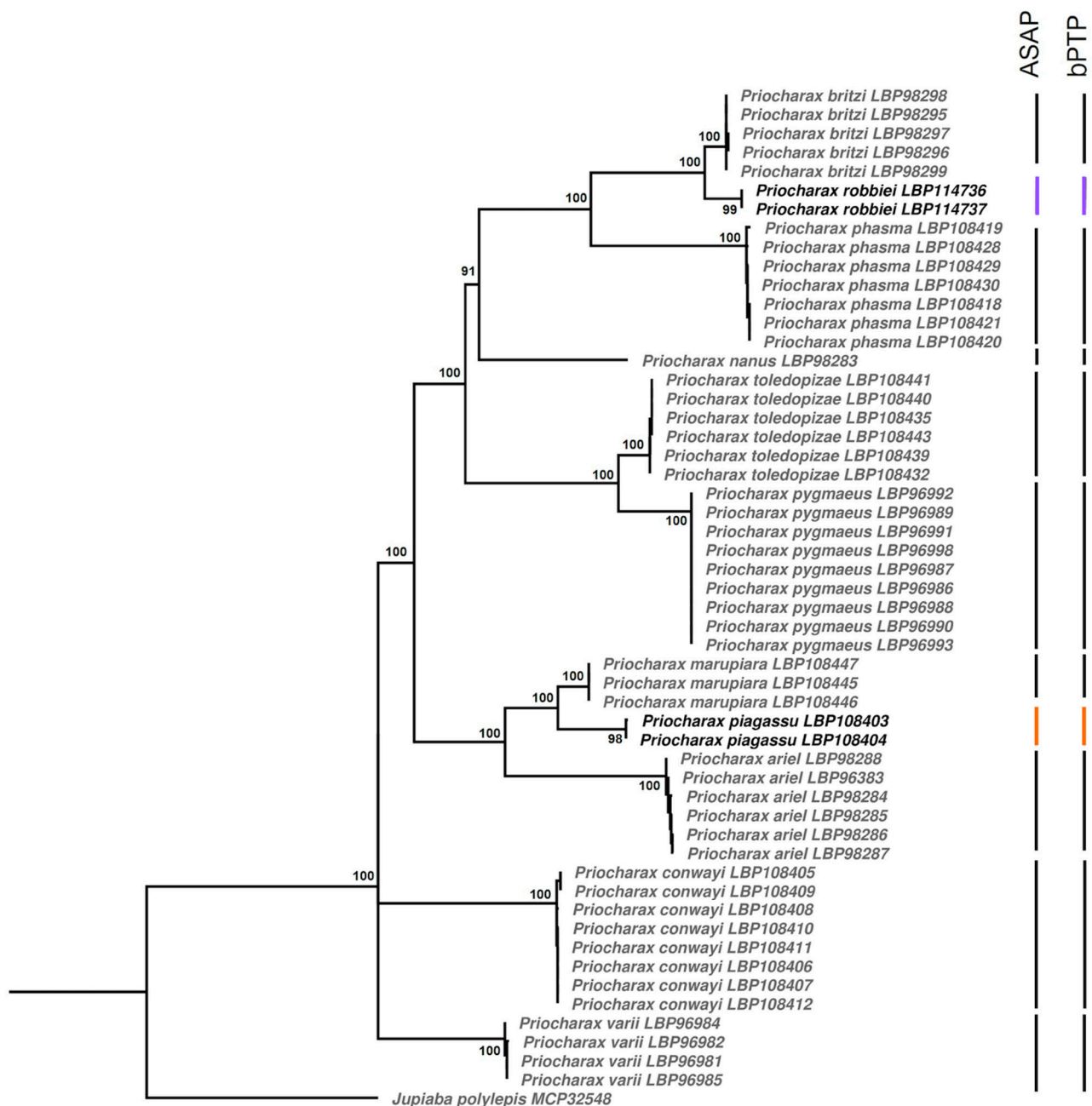


Figure 9. Maximum likelihood tree of the miniature tetra *Priocharax* based on partial sequences of the cytochrome oxidase c subunit 1 gene (524 bp). Vertical bars represent the number of species delimited by ASAP and bPTP. Purple and orange bars represent the new species. Black bars represent the other species of *Priocharax* analyzed. Numbers near nodes represent bootstrap support. Codes after species names represent voucher numbers.

4. Discussion

With the description of these two new species, the genus *Priocharax* now comprises 12 valid species. In addition to osteological and molecular differences, *Priocharax robbiei* **sp. nov.** and *Priocharax piagassu* **sp. nov.** also differ in overall body shape (Figure 8). Notably, as observed with other pairs of species of *Priocharax* from close locations recently described, such as *P. toledopizae* and *P. marupiara* [6] from the Juruá system, Acre State; and *P. conwayi* and *P. phasma* [7] from the vicinities of Santarém municipality, Pará State, *P. robbiei* **sp. nov.** and *P. piagassu* **sp. nov.** also have different body shapes, with the former deeper and the later more streamlined (Figures 1B,C and 8).

The two species described herein have the classic trait of other characiforms in which males present bony hooks on anal fins (Figure 3A–D) as well as a larger pelvic girdle. Just like the other *Priocharax* species recently described (e.g., *P. toledopizae*, *P. conwayi*, *P. phasma* and *P. rex* [6–8]), males of *P. robbiei* **sp. nov.** and *P. piagassu* **sp. nov.** have a horizontal slit separating the hypaxial musculature from the pelvic-fin musculature (Figure 4A,C). It is worth noting that the small size of the hooks in *P. piagassu* **sp. nov.** (Figure 3B,D) may be related to the small size of the individual (13.9 mm SL), and not necessarily a species-specific trait. However, this cannot be confirmed, as only two small adult male specimens of the species were examined.

As previously mentioned, specimens used in the description of *Priocharax piagassu* **sp. nov.** are in poor condition and exhibit several damaged structures. However, due to the rarity of the species and the unsuccessful attempts to collect additional material (since the locality is very remote and demands complex logistics), we decided to proceed with the species description based on these individuals. Although the designated holotype is not ideal due to some damage, it retains the morphological features necessary for a reliable diagnosis of the species.

Oliveira et al. [24] initially identified some specimens collected in the Catuá-Ipixuna Extractive Reserve as *Priocharax pygmaeus*. However, detailed analyses of the material revealed that these specimens (INPA 27343), although in poor conditions, belong to the new species, described here as *P. robbiei* **sp. nov.**, and not to *P. pygmaeus*. Hence, the latter species does not occur in the Brazilian territory.

So far, the two species described here follow the same pattern observed in other congeners: a distribution restricted to one or a few localities, which makes them vulnerable to anthropogenic impacts such as deforestation, loss of riparian vegetation, habitat fragmentation, land-use change, overfishing, construction of dams, and contamination of water bodies by urban and agricultural pollutants [26]. In this conservation context, it is worth noting that *Priocharax robbiei* **sp. nov.** and *P. piagassu* **sp. nov.** were collected within and near the boundaries of two protected areas in Brazil: the Catuá-Ipixuna Extractive Reserve and the Piagaçu-Purus Sustainable Development Reserve. Similarly, *Priocharax britzi* was collected near the protected area Balata-Tufari National Forest [5], and *P. conwayi* was sampled in the Tapajós-Arapiuns Extractive Reserve. This highlights the importance of protected areas for conservation and knowledge of biodiversity, even more in a context in which species are becoming extinct before being described by science—the so-called dark extinction [27].

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/taxonomy5030036/s1>, Figure S1: Summary of the detailed results of the Principal Component Analysis (PCA); Figure S2: Principal Component Analysis (PCA) loadings; Figure S3: Summary of the detailed results of the Linear Discriminant Analysis (LDA), Axis 1; Figure S4: Results of the reclassification of the Linear Discriminant Analysis (LDA); Figure S5: Maximum Likelihood solution of the Poisson Tree Process model (bPTP) analysis.

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Data Availability Statement: Specimens have been deposited in publicly accessible collections, and the DNA sequences generated in this study are available in GenBank.

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Appendix A

Table A1. Lots, vouchers, locality information, and GenBank accession numbers of the analyzed specimens of *Priocharax*. Specimens from all species included in the molecular analysis are paratypes of their respective species, except for *P. ariel*, *P. nanus*, and *P. pygmaeus*.

Species	Lot	Voucher	Basin	Locality	Coordinates	GenBank n.
<i>Priocharax robbiei</i>	LBP 36836	114736	Rio Solimões	“Igarapezinho” upstream from the São Sebastião da Água Branca community, a tributary of the Água Branca stream, affluent of Ipixuna stream, São Francisco, Coari, AM, Brazil	3°54'20.7" S 63°52'20.5" W	PV698181
<i>Priocharax robbiei</i>	LBP 36836	114737	Rio Solimões	“Igarapezinho” upstream from the São Sebastião da Água Branca community, a tributary of the Água Branca stream, affluent of Ipixuna stream, São Francisco, Coari, AM, Brazil	3°54'20.7" S 63°52'20.5" W	PV698181
<i>Priocharax piagassu</i>	LBP 31205	108403	Rio Purus	Lago Xaviana, Rio Purus, Beruri, AM, Brazil	4°20'18.70" S 61°49'43.20" W	PV698179
<i>Priocharax piagassu</i>	LBP 31205	108404	Rio Purus	Lago Xaviana, Rio Purus, Beruri, AM, Brazil	4°20'18.70" S 61°49'43.20" W	PV698180
<i>Priocharax conwayi</i>	LBP 31740	108405	Rio Tapajós	Igarapé do Henrique, Rio Maró, affluent of Rio Arapiuns, Santarém, PA, Brazil	02°41'9.30" S 55°40'32.97" W	PP902468
<i>Priocharax conwayi</i>	LBP 31740	108406	Rio Tapajós	Igarapé do Henrique, Rio Maró, affluent of Rio Arapiuns, Santarém, PA, Brazil	02°41'9.30" S 55°40'32.97" W	PP902469
<i>Priocharax conwayi</i>	LBP 31740	108407	Rio Tapajós	Igarapé do Henrique, Rio Maró, affluent of Rio Arapiuns, Santarém, PA, Brazil	02°41'9.30" S 55°40'32.97" W	PP902470
<i>Priocharax conwayi</i>	LBP 31741	108408	Rio Tapajós	Igarapé do Henrique, Rio Maró, affluent of Rio Arapiuns, Santarém, PA, Brazil	02°41'9.30" S 55°40'32.97" W	PP902471
<i>Priocharax conwayi</i>	LBP 31741	108409	Rio Tapajós	Rio Mentaí, in the vicinity of the community at mouth of Rio Mentaí, affluent of Rio Arapiuns, Santarém, PA, Brazil	02°37'52.51" S 55°34'40.98" W	PP902472
<i>Priocharax conwayi</i>	LBP 31741	108410	Rio Tapajós	Rio Mentaí, in the vicinity of the community at mouth of Rio Mentaí, affluent of Rio Arapiuns, Santarém, PA, Brazil	02°37'52.51" S 55°34'40.98" W	PP902473
<i>Priocharax conwayi</i>	LBP 31741	108411	Rio Tapajós	Rio Mentaí, in the vicinity of the community at mouth of Rio Mentaí, affluent of Rio Arapiuns, Santarém, PA, Brazil	02°37'52.51" S 55°34'40.98" W	PP902474
<i>Priocharax conwayi</i>	LBP 31741	108412	Rio Tapajós	Rio Mentaí, in the vicinity of the community at mouth of Rio Mentaí, affluent of Rio Arapiuns, Santarém, PA, Brazil	02°37'52.51" S 55°34'40.98" W	PP902475
<i>Priocharax phasma</i>	LBP 31742	108418	Rio Amazonas	Lago Santana, Ilha de Marimarituba, Rio Amazonas, Santarém, PA, Brazil	02°11'12.31" S 55°02'12.00" W	PP902476
<i>Priocharax phasma</i>	LBP 31742	108419	Rio Amazonas	Lago Santana, Ilha de Marimarituba, Rio Amazonas, Santarém, PA, Brazil	02°11'12.31" S 55°02'12.00" W	PP902477
<i>Priocharax phasma</i>	LBP 31742	108420	Rio Amazonas	Lago Santana, Ilha de Marimarituba, Rio Amazonas, Santarém, PA, Brazil	02°11'12.31" S 55°02'12.00" W	PP902478

Table A1. Cont.

Species	Lot	Voucher	Basin	Locality	Coordinates	GenBank n.
<i>Priocharax phasma</i>	LBP 31742	108421	Rio Amazonas	Lago Santana, Ilha de Marimarituba, Rio Amazonas, Santarém, PA, Brazil	02°11'12.31" S 55°02'12.00" W	PP902479
<i>Priocharax phasma</i>	LBP 31743	108428	Rio Amazonas	Lago Pajaú, Ilha Nazareth, Rio Amazonas, Santarém, PA, Brazil	02°11'28.53" S 54°51'27.93" W	PP902480
<i>Priocharax phasma</i>	LBP 31743	108429	Rio Amazonas	Lago Pajaú, Ilha Nazareth, Rio Amazonas, Santarém, PA, Brazil	02°11'28.53" S 54°51'27.93" W	PP902481
<i>Priocharax phasma</i>	LBP 31743	108430	Rio Amazonas	Lago Pajaú, Ilha Nazareth, Rio Amazonas, Santarém, PA, Brazil	02°11'28.53" S 54°51'27.93" W	PP902482
<i>Priocharax toledopizae</i>	LBP 31744	108432	Rio Juruá	Igarapé Preto, tributary of Rio Moa, Cruzeiro do Sul, AC, Brazil	07°35'11" S 72°45'20" W	OP257279
<i>Priocharax toledopizae</i>	LBP 31744	108435	Rio Juruá	Igarapé Preto, tributary of Rio Moa, Cruzeiro do Sul, AC, Brazil	07°35'11" S 72°45'20" W	OP257280
<i>Priocharax toledopizae</i>	LBP 31745	108439	Rio Juruá	Balneário at Igarapé Preto near road BR-307, Cruzeiro do Sul, AC, Brazil	07°35'45" S 72°45'16" W	OP257278
<i>Priocharax toledopizae</i>	LBP 31745	108440	Rio Juruá	Balneário at Igarapé Preto near road BR-307, Cruzeiro do Sul, AC, Brazil	07°35'45" S 72°45'16" W	OP257282
<i>Priocharax toledopizae</i>	LBP 31745	108441	Rio Juruá	Balneário at Igarapé Preto near road BR-307, Cruzeiro do Sul, AC, Brazil	07°35'45" S 72°45'16" W	OP257281
<i>Priocharax toledopizae</i>	LBP 31746	108443	Rio Juruá	Igarapé das Piabas, tributary of Rio Moa, Cruzeiro do Sul, AC, Brazil	07°31'15" S 72°53'48" W	OP257283
<i>Priocharax marupiara</i>	LBP 31747	108445	Rio Juruá	Igarapé Canela Fina, Cruzeiro do Sul, AC, Brazil	07°34'02" S 72°39'40" W	OP257285
<i>Priocharax marupiara</i>	LBP 31747	108446	Rio Juruá	Igarapé Canela Fina, Cruzeiro do Sul, AC, Brazil	07°34'02" S 72°39'40" W	OP257286
<i>Priocharax marupiara</i>	LBP 31747	108447	Rio Juruá	Igarapé Canela Fina, Cruzeiro do Sul, AC, Brazil	07°34'02" S 72°39'40" W	OP257284
<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

Table A1. Cont.

Species	Lot	Voucher	Basin	Locality	Coordinates	GenBank n.
<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

References

- Weitzman, S.H.; Vari, R.P. Miniaturization in South American freshwater fishes: An overview and discussion. *Proc. Biol. Soc. Wash.* **1988**, *101*, 444–465.
- Weitzman, S.H.; Vari, R.P. Two new species and a new genus of miniature characid fishes (Teleostei: Characiformes) from northern South America. *Proc. Biol. Soc. Wash.* **1987**, *100*, 640–652. [[CrossRef](#)]
- Toledo-Piza, M.; Mattox, G.M.T.; Britz, R. *Priocharax nanus*, a new miniature characid from the rio Negro, Amazon basin (Ostariophysi: Characiformes), with an updated list of miniature Neotropical freshwater fishes. *Neotrop. Ichthyol.* **2014**, *12*, 229–246. [[CrossRef](#)]
- Mattox, G.M.T.; Souza, C.S.; Toledo-Piza, M.; Britz, R.; Oliveira, C. A new miniature species of *Priocharax* (Teleostei: Characiformes: Characidae) from the Rio Madeira drainage, Brazil, with comments on the adipose fin in characiforms. *Vertebr. Zool.* **2020**, *70*, 417–433. [[CrossRef](#)]
- Mattox, G.M.T.; Souza, C.S.; Toledo-Piza, M.; Oliveira, C. A new miniature species of *Priocharax* (Characiformes: Characidae) from the upper rio Ipixuna, Purus drainage, Brazil. *Neotrop. Ichthyol.* **2021**, *19*, e210048. [[CrossRef](#)]
- Mattox, G.M.T.; Britz, R.; Souza, C.S.; Casas, A.L.S.; Lima, F.C.T.; Oliveira, C. Two new species of the miniature tetras *Priocharax* from the Rio Juruá drainage, Acre, Brazil (Teleostei: Characiformes: Characidae). *Can. J. Zool.* **2023**, *101*, 248–266. [[CrossRef](#)]
- Mattox, G.M.T.; Lima, F.C.T.; Britz, R.; Souza, C.S.; Oliveira, C. Two new miniature species of the fish genus *Priocharax* from the Rio Tapajós and Amazonas drainages, Pará, Brazil (Teleostei: Characiformes: Characidae). *Vertebr. Zool.* **2024**, *74*, 533–550. [[CrossRef](#)]
- Mattox, G.M.T.; Acosta-Santos, A.; Bogotá-Gregory, J.; Agudelo, E.; Lima, F.C.T. A new miniature *Priocharax* from the rio Putumayo drainage, Amazonas State, Colombia (Teleostei: Characiformes: Characidae) with an unusual skin flap between pelvic fins. *Zootaxa* **2025**, *5575*, 545–554. [[CrossRef](#)]
- Fink, W.L.; Weitzman, S.H. The so-called cheirodontin fishes of Central America with descriptions of two new species (Pisces: Characidae). *Smithson. Contrib. Zool.* **1974**, *172*, 1–46. [[CrossRef](#)]
- Menezes, N.A.; Weitzman, S.H. Two new species of *Mimagoniates* (Teleostei: Characidae: Glandulocaudinae), their phylogeny and biogeography and a key to the glandulocaudin fishes of Brazil and Paraguay. *Proc. Biol. Soc. Wash.* **1990**, *103*, 380–426.
- Taylor, W.R.; Van Dyke, G.C. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* **1985**, *9*, 107–109.
- Weitzman, S.H. The osteology of *Brycon meeki*, a generalized characid fish, with an osteological definition of the family. *Stanford Ichthyol. Bull.* **1962**, *8*, 1–77.
- Mattox, G.M.T.; Britz, R.; Toledo-Piza, M. Skeletal development and ossification sequence of the characiform *Salminus brasiliensis* (Teleostei: Ostariophysi: Characidae). *Ichthyol. Explor. Freshwaters* **2014**, *25*, 103–158.
- Mattox, G.M.T.; Britz, R.; Toledo-Piza, M. Osteology of *Priocharax* and remarkable developmental truncation in a miniature Amazonian fish (Teleostei: Characiformes: Characidae). *J. Morphol.* **2016**, *277*, 65–85. [[CrossRef](#)]
- Mattox, G.M.T.; Kubicek, K.M.; Britz, R. Notes on the skeletal anatomy of *Priocharax ariel* Weitzman & Vari, 1987 with implications for its taxonomy (Teleostei: Characiformes). *Zootaxa* **2022**, *5138*, 597–599. [[CrossRef](#)]
- Manly, B.F.J. *Métodos Estatísticos Multivariados—Uma Introdução*, 3rd ed.; Bookman: Porto Alegre, Brazil, 2008; 229p.
- Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 4.
- Javonillo, R.; Malabarba, L.R.; Weitzman, S.H.; Burns, J.R. Relationships among major lineages of characid fishes (Teleostei: Ostariophysi: Characiformes), based on molecular sequence data. *Mol. Phylogenet. Evol.* **2010**, *54*, 498–511. [[CrossRef](#)] [[PubMed](#)]
- Ward, R.D.; Zemlak, T.S.; Innes, B.H.; Last, P.R.; Hebert, P.D. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. B* **2005**, *360*, 1847–1857. [[CrossRef](#)] [[PubMed](#)]
- Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [[CrossRef](#)]
- Xia, X.; Xie, Z.; Salemi, M.; Chen, L.; Wang, Y. An index of substitution saturation and its application. *Mol. Phylogenet. Evol.* **2003**, *26*, 1–7. [[CrossRef](#)]
- Xia, X. DAMBE6: New tools for microbial genomics, phylogenetics and molecular evolution. *J. Hered.* **2017**, *108*, 431–437. [[CrossRef](#)] [[PubMed](#)]
- Zhang, J.; Kapli, P.; Pavlidis, P.; Stamatakis, A. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **2013**, *29*, 2869–2876. [[CrossRef](#)] [[PubMed](#)]
- Oliveira, R.R.; Rocha, M.S.; Anjos, M.B.; Zuanon, J.; Py-Daniel, L.H.R. Fish fauna of small streams of the Catua-Ipixuna Extractive Reserve, State of Amazonas, Brazil. *Check List* **2009**, *5*, 154–172. [[CrossRef](#)]

25. Venticinque, E.; Forsberg, B.; Barthem, R.; Petry, P.; Hess, L.; Mercado, A.; Canas, C.; Montoya, M.; Durigan, C.; Goulding, M. An explicit GIS-based river basin framework for aquatic ecosystem conservation in the Amazon. *Earth Syst. Sci. Data* **2016**, *8*, 651–661. [[CrossRef](#)]
26. Pelicice, F.M.; Bialecki, A.; Camelier, P.; Carvalho, F.R.; García-Berthou, E.; Pompeu, P.S.; Mello, F.T.; Pavanelli, C.S. Human impacts and the loss of Neotropical freshwater fish diversity. *Neotrop. Ichthyol.* **2021**, *19*, e210134. [[CrossRef](#)]
27. Boehm, M.M.A.; Cronk, Q.C.B. Dark extinction: The problem of unknown historical extinctions. *Philos. Trans. R. Soc. B* **2021**, *376*, 20200376. [[CrossRef](#)]

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