

# A new miniature species of *Priocharax* (Teleostei: Characiformes: Characidae) from the Rio Madeira drainage, Brazil, with comments on the adipose fin in characiforms

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## Abstract

A new miniature species of the freshwater fish genus *Priocharax* is described from the Rio Jamari, Rio Madeira drainage, Amazon basin. *Priocharax varii* sp. n. is the fourth species currently recognized in the genus and shares with the other three the presence of a conspicuous larval pectoral fin in adults, a fully toothed maxilla, a triangular pseudotympanum situated anterior to the fifth vertebra and a tiny size combined with a translucent body. The new species is diagnosed from congeners in having an adipose fin, a unique feature within *Priocharax*. Molecular identification of the new species and the other valid *Priocharax* species supports the morphological findings. A brief discussion on the presence/absence of the adipose fin in Characiformes is provided.

## Key words

Amazon River basin, barcoding, biodiversity, osteology, taxonomy.

## Introduction

*Priocharax* is a genus originally described by WEITZMAN & VARI (1987) to include two miniature species (sensu WEITZMAN & VARI, 1988): *Priocharax ariel* Weitzman & Vari and *Priocharax pygmaeus* Weitzman & Vari. These species were collected from the upper Rio Orinoco in Venezuela and in the region of Leticia, Rio Amazonas Colombia, respectively. The authors proposed the presence in adults of *Priocharax* of a peculiar, larval-like, rayless pectoral fin as the main character to diagnose the genus. They also listed a set of other diagnostic features to distinguish *Priocharax* from most New World characids except those included in the Characinae and Cynopotaminae (= Characinae): dentary with approximately

30–50 conical teeth in somewhat irregular row, upper jaw (premaxilla and maxilla) with approximately 45–90 conical teeth in somewhat irregular row, and adult body size of 11–17 mm SL, a feature otherwise unknown in the Characinae and Cynopotaminae (= Characinae) at that time. Another feature that according to WEITZMAN & VARI (1987) distinguishes *Priocharax* from the remaining characines is the number of i,5 pelvic-fin rays, with all the remaining members of the subfamily having i,7 pelvic-fin rays.

Based on the presence of numerous conical teeth in the upper and lower jaws and on the elongate maxilla, WEITZMAN & VARI (1987) hypothesized that the relation-

ships of *Priocharax* would be among the Characinae, pending further studies. Twenty-five years later, MATTOX & TOLEDO-PIZA (2012) carried out a phylogenetic study of this subfamily and found out that the Characinae as previously proposed were not monophyletic. Rather, they restricted the Characinae to a small group of seven genera (*Acanthocharax* Eigenmann, *Acestrocephalus* Eigenmann, *Charax* Scopoli, *Cynopotamus* Valenciennes, *Galeocharax* Fowler, *Phenacogaster* Eigenmann and *Roeboides* Günther) and transferred the other genera of more diminutive fishes to the Heterocharacinae (*Gnathocharax* Fowler, *Heterocharax* Eigenmann, *Hoplocharax* Géry, *Lonchogenys* Myers and *Priocharax*), together with *Gilbertolus* Eigenmann and *Roestes* Günther. Similar results were obtained by other morphological (i.e. MIRANDE, 2010) and molecular approaches (i.e., OLIVEIRA *et al.*, 2011). A recent dataset (MIRANDE, 2018) combining both sources of information also partially corroborated the results, albeit not including exactly the same taxa, but none of those studies except that of MATTOX & TOLEDO-PIZA (2012) included *Priocharax* in their analyses. In the same year, BETANCUR-R *et al.* (2018) included *Priocharax ariel* in a molecular analysis of characiforms based on exons and recovered the genus among genera of the Characinae, but did not discuss this result any further.

Since WEITZMAN & VARI'S (1987) publication, a third species of *Priocharax* was discovered in the Rio Negro: *P. nanus* Toledo-Piza *et al.* (2014). *Priocharax nanus* was diagnosed from *P. ariel* and *P. pygmaeus* by the unique presence of i,6 pelvic-fin rays, presence of the claustrum and two post-cleithra. Although still a miniature, *P. nanus* was interpreted as the least ontogenetically truncated species in the genus. Additional specimens of *Priocharax* were reported in the literature from new localities (e.g., QUEIROZ *et al.*, 2013; VIEIRA *et al.*, 2016) or collected during recent expeditions. Upon closer study, samples from the Rio Jamari, Rio Madeira drainage, Amazon basin, Brazil were found to represent a new species, which is described in this paper.

## Material & 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 percentage of caudal-peduncle length. Measurements were taken point to point with a precision of 0.1 mm from digital photographs of specimens taken under the stereomicroscope. Counts of vertebrae, teeth, gill-rakers and procurrent caudal-fin rays were obtained

from five 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 separated 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 branch of the gill arch. Photographs were made with a Zeiss Discovery V20 stereomicroscope with a Zeiss Axiocam digital camera attached. Osteological terminology follows WEITZMAN (1962) except for inner arm of the *os suspensorium* instead of *os suspensorium*, and outer arm of the *os suspensorium* instead of rib of fourth vertebra, following CONWAY & BRITZ (2007), and other 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. Information on meristic and morphometric data of *Priocharax ariel* and *P. pygmaeus* were taken from WEITZMAN & VARI (1987). Specimens examined for this study are deposited in the Coleção de Peixes da Universidade Federal de Rondônia (UFRO-ICT), Instituto Nacional de Pesquisas da Amazônia (INPA), Laboratório de Biologia e Genética de Peixes, Departamento de Biologia Estrutural e Funcional, Universidade Estadual Paulista, Botucatu (LBP), Museu de Zoologia da Universidade de São Paulo (MZUSP), and 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.

### Molecular analysis

Voucher specimens for the molecular study are deposited in the collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Biologia Estrutural e Funcional, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil. This study is in accordance with the National Council for the Control of Animal Experimentation (CONCEA) approved by UNE-SP Ethics Committee on Use of Animals (CEUA), protocol number 1058. Twenty specimens of *Priocharax* were included in the analysis: one specimen of *P. nanus* and six specimens of *P. ariel* from the Rio Negro, nine specimens of *P. pygmaeus* from the Rio Amazonas and four specimens of the new species from the Rio Madeira, described herein. A single specimen of *Acestrocephalus sardina* (Characiformes, Characidae, Characinae) was used to root the trees. Voucher data are summarized in Table 1.

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). PCR amplifications were performed in a total volume of 12.5 µl that included 1.25 µl of 10X buffer, 0.25 µl of MgCl<sub>2</sub> (50 mM), 0.2 µl dNTPs (2 mM), 0.5 µl of

each primer (5 mM), 0.1 µl of PHT Taq DNA polymerase (*Phoneutria*), 1.0 µl of genomic DNA (200 ng) and 8.7 µl ddH<sub>2</sub>O. The thermocycling profile consisted of an initial denaturation (5 min at 94°C), followed by 30 cycles of chain denaturation (40s at 94°C), primer hybridization (30s at 50–54°C) and nucleotide extension (1 min at 68°C), and a final extension (8 min at 68°C). All PCR products were checked on 1% agarose gels and then purified with ExoSap-IT (USB Corporation) following the manufacturer's instructions. The subsequently purified PCR products were submitted to sequencing reactions using BigDye Terminator v 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and purified again by ethanol precipitation. Products were loaded onto an ABI 3130 DNA Analyzer automatic sequencer (Applied Biosystems).

Raw sequences were assembled to consensus using GENEIOUS v7.1.9 (KEARSE *et al.*, 2012) and subsequently aligned with MUSCLE (EDGAR, 2004) under default parameters. To evaluate the occurrence of substitution saturation, we estimated whether the Iss (index of substitution saturation) is significantly lower than Iss.cAsym (asymmetrical topology) and Iss.Sym (symmetrical topology) using the method of XIA *et al.* (2003) in DAMBE v5.3.38 (XIA, 2013). Nucleotide variation, substitution patterns and best-fit model of nucleotide evolution were estimated in MEGA v6.0 (TAMURA *et al.*, 2013).

The overall mean distance (among all specimens), the intraspecific distances (among specimens of each species group) and interspecific distances (among species groups) were estimated using the Kimura-2-parameter model (K2P; KIMURA, 1980) in MEGA v6.0 (TAMURA *et al.*, 2013). The Maximum-likelihood analysis (ML) was generated with 1000 bootstrap replicates in MEGA v6.0 (TAMURA *et al.*, 2013). Species delimitation analyses included two approaches: (a) the Automatic Barcode Gap Discovery analysis (ABGD; PUILANDRE *et al.*, 2012) available in the ABGD webserver (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>), excluding the root *Acestrocephalus sardina* and under default parameters; and (b) Poisson Tree Processes (PTP; ZHANG *et al.*, 2013) using the ML tree and other parameters at default in the PTP webserver (<http://species.h-its.org/ptp/>). Groups were ordered based on ABGD and PTP results.

## Results

### *Priocharax varii*, sp. nov.

ZOOBANK: urn:lsid:zoobank.org:act:811F685B-BBB7-45B2-8294-C1BC837565DB

Figs. 1–4, Table 2

**Holotype.** MZUSP 125786, 12.2 mm SL, Brazil, Rondônia, Candeias do Jamari, Rio Preto approximately 12km South from road BR-364, before bridge crossing the river, Rio Jamari drainage, 08°52'53.5" S, 63°37'50.8" W, G. Mattox & S. Souza, 02 Sep 2018.

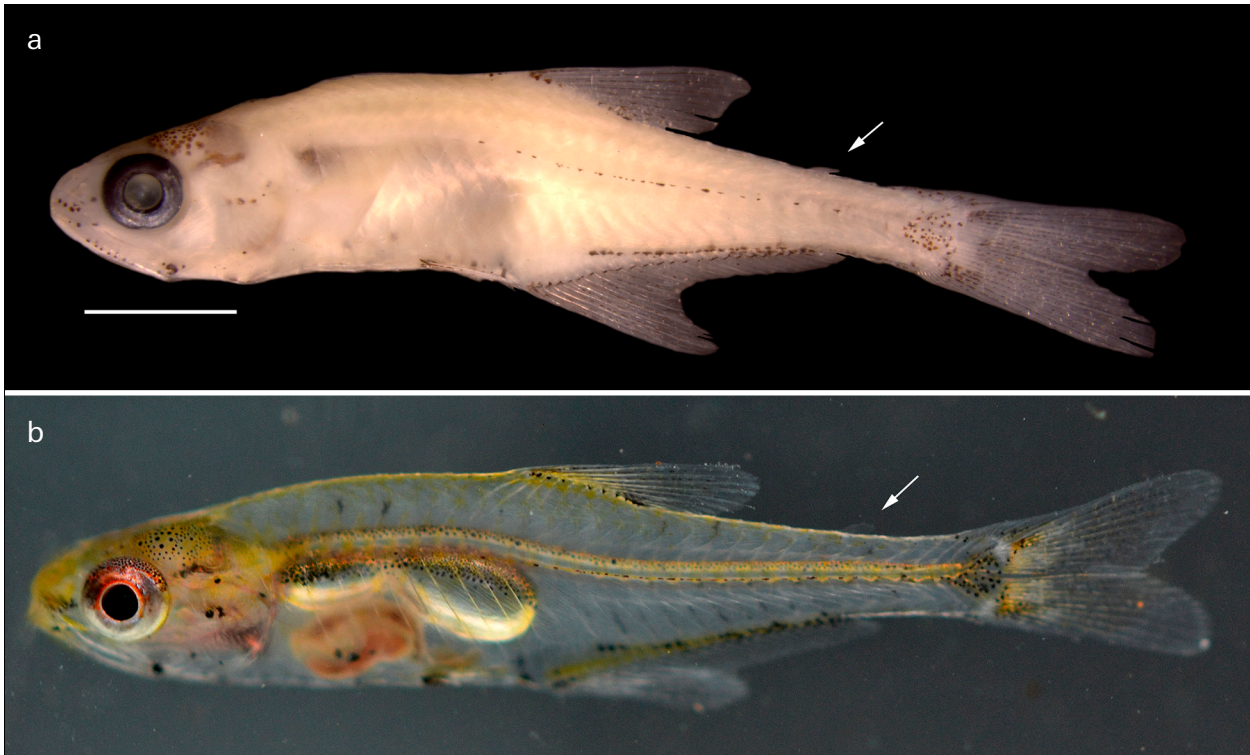
**Paratypes.** LBP 28495, 5, 12.5–12.7 mm SL, collected with holotype. MZUSP 125787, 44, 11.8–14.0 mm SL (5 c&s, 11.8–13.7 mm SL), collected with holotype. UFRO-ICT 027656, 5, 12.2–13.3 mm SL, collected with holotype.

**Diagnosis.** *Priocharax varii* is distinguished from all congeners by the presence of an adipose fin (*vs* adipose fin absent). *Priocharax varii* can be further diagnosed from *P. ariel* and *P. pygmaeus* by the presence of i,6 pelvic-fin rays (i,5 in one out of 50 specimens examined) (*vs* i,5) and of two postcleithra (*vs* postcleithra absent). *Priocharax varii* can be further diagnosed from *P. ariel* by having a shorter caudal peduncle (14–18 % SL *vs* 18–24 % SL).

**Description.** For overall appearance, see Figure 1. Morphometric data of holotype and paratypes are presented in Table 2. Body laterally compressed, 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; straight and posteroventrally sloping 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 blunt in lateral view. Infraorbitals 1 to 6 and supraorbital absent, antorbital present. Mouth terminal with lower jaw slightly shorter than upper jaw. Tip of maxilla elongate, posterior border reaching vertical through posterior border of pupil. Premaxilla with 23(1), 25(2), 26(1) or 29(1) teeth in single series. Maxilla with 34(1), 39(2) or 42(2) teeth. Dentary with 37(2), 40(1), 42(1) or 46(1) teeth. Dentary teeth in single series, with few anterior teeth slightly displaced anteriorly. Conspicuous elongate foramen at anterior portion of dentary. All jaw teeth small, conical and lingually curved to a moderate extent (Figure 2).

Dorsal-fin rays ii,9\*(50). Endoskeletal part and some dermal bones of pectoral fin with structure similar to larval specimens of most characiforms (Figure 3). 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 posttem-



**Fig 1.** *Priocharax varii*, a) holotype, MZUSP 125786, 12.2 mm SL; Brazil, Rondônia, Candeias do Jamari, Rio Preto approximately 12km South from road BR-364, before bridge crossing the river, Rio Jamari drainage; scale bar = 2mm. b) live specimen photographed right after capture. Arrow points to adipose fin.

poral, supracleithrum, cleithrum and two postcleithra. Cleithrum with posteriorly directed, curved process immediately below ventral tip of supracleithrum. Pelvic-fin rays i,5 (1) or i,6\*(49). Posterior tip of pelvic fin falling short of origin of anal fin but extending slightly beyond vent. Anal-fin rays ii, 18(1), 19(1), 20(1), 21(4), 22(11), 23\*(14), 24(14) or 25(2). Anal-fin distal margin concave with anterior lobe formed by elongated fin rays and posterior portion with shorter rays. Caudal-fin rays i,8,7,i (1), i,8,8,i (1) or i,9,8,i\*(42), dorsal procurrent rays 9(5), ventral procurrent rays 7(4) or 8(1). Caudal fin forked. Adipose fin small and situated midway between terminus of dorsal fin and anteriormost dorsal procurrent caudal-fin ray (n=50).

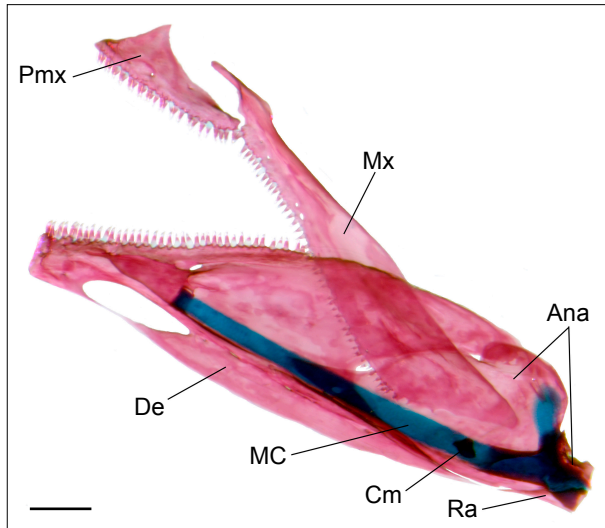
Squamation present in almost all specimens, but scales highly deciduous and easily lost during handling. Scales cycloid, very thin, with no obvious circuli or radii. Scales in midlateral row 26(2), 27\*(14), 28(14), 29(9), 30(6), 31(1) or 32(2); no canal bearing lateral-line scales on body. Horizontal scale rows between dorsal-fin origin and pelvic-fin origin 7(3) or 8\*(7). Horizontal scale rows around caudal peduncle 8\*(5) or 9(5). 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 33(5); abdominal vertebrae 14(1) or 15(4); caudal vertebrae 18(4) or 19(1). Total number of gill-rakers on first branchial arch 8(1), 10(1), 11(2) or 12(1), upper limb gill-rakers 1(1) or 3(4), lower limb gill-rakers 7(2), 8(2) or 9(1). Weberian apparatus well devel-

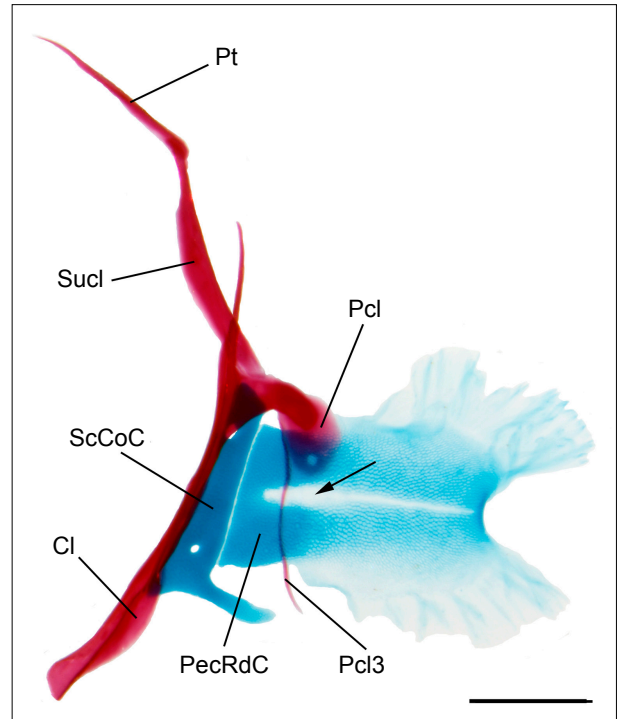
oped, all components ossified except for claustrum (Figure 4), absent in all but one specimen with tiny, vestigial claustrum. Large gap between neural arches 3 and 4, with gap partially filled by dorsally projecting pointed process from vertebral centrum 3. Inner arm of *os suspensorium* large, projecting forward to vertical through middle of second centrum.

**Colour in alcohol.** Overall ground coloration pale yellow (Fig. 1a). Patch of melanophores on dorsal portion of head and scattered melanophores on opercle. Head with dark stripe radiating anteriorly from eye to tip of snout. Line of melanophores along dentary with concentration of melanophores on articulation between lower jaw and quadrate. Iridophores present in eye of most specimens. Longitudinal line of melanophores along midlateral body, from vertical through dorsal-fin origin to middle portion of caudal peduncle. Triangular to circular patch of melanophores at base of caudal fin forming inconspicuous spot. Bases of anal-fin rays with series of spots forming irregular line along fin. Second dark line, dorsal and more superficial, extending along ventral margin of hypaxial myomeres posteriorly from vertical through fifth branched anal-fin ray. Both lines more evident and well separated anteriorly but approaching each other posteriorly. Two patches of melanophores ventrally on body anterior to pelvic fin: anteriormost in form of an inconspicuous small spot on isthmus and posteriormost patch elongated just anterior to basipterygium. Few melanophores present in region around vent. Melanophores at

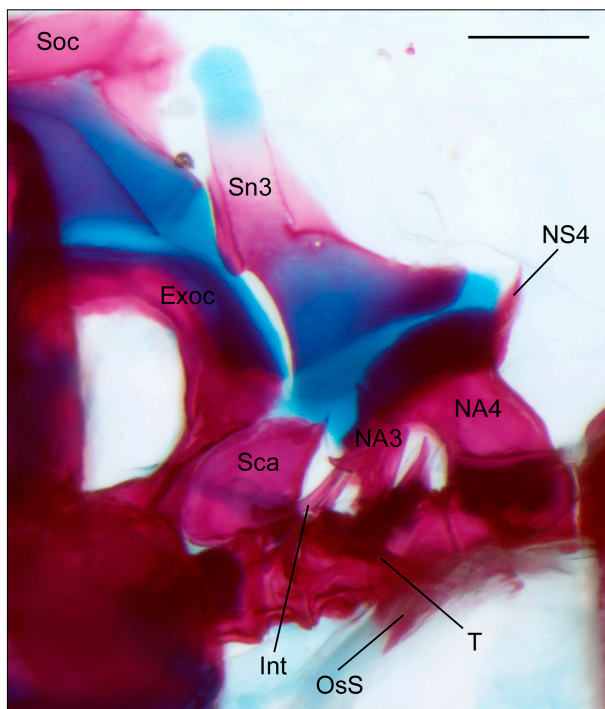




**Fig 2.** *Priocharax varii*, paratype, MZUSP 125787, 13.6 mm SL, c&s; jaws in lateral view. Ana = anguloarticular; Cm = coronomeckelian; De = dentary; MC = Meckel's cartilage; Mx = maxilla; Pmx = premaxilla; Ra = retroarticular. Scale bar = 0.2 mm.



**Fig 3.** *Priocharax varii*, paratype, MZUSP 125787, 13.6 mm SL, c&s; shoulder girdle in lateral view. Cl = cleithrum; PecRdC = pectoral-fin radial cartilage; Pcl = unidentified postcleithrum; Pcl3 = postcleithrum 3; Pt = posttemporal; ScCoC = scapulocoracoid cartilage; Sucl = supraclithrum. Arrow points to middle fissure on pectoral-fin radial cartilage. Tip of larval finfold is bent. Scale bar = 0.5 mm.



**Fig 4.** *Priocharax varii*, paratype, MZUSP 125787, 13.6 mm SL, c&s; Weberian apparatus in lateral view. Exoc = Exoccipital; Int = intercalarium; NA3–4 = neural archs 3–4; NS4 = neural spine 4; OsS = os suspensorium; Sca = scaphium; Sn3 = supra-neural 3; Soc = supraoccipital; T = tripus. Scale bar = 0.2 mm.

origin of dorsal, pelvic, and anal fins. All fins except pectoral and adipose with scattered melanophores along margins of fin rays. Caudal fin with two faint dark blotches on bases of four dorsal- and four ventralmost rays.

**Colour in life.** Body mostly translucent (Fig. 1b). Pattern of melanophores generally as described above for alco-

hol specimens. Melanophores also scattered on dorsal surface of swim bladder and along neural cord. Melanophores in line along base of dorsal-fin rays. Melanophores arranged along neural and haemal spines deep inside body forming few oblique lines. Dorsal portion of head and snout predominantly golden yellow. Xanthophores along dorsal profile of body, especially along dorsal midline and dorsal-fin base. Few scattered xanthophores on dorsal flanks of body. Xanthophores also present and abundant along neural chord and ventral margin of vertebral column, dorsal surface of swim bladder, anal-fin base and forming irregular patches on dorsal and ventral bases of caudal-fin lobes. Scattered melanophores on dorsal and caudal fins, all other fins hyaline including adipose fin. Eye silvery, dorsal half predominantly red and dorsal margin covered with melanophores.

**Sexual dimorphism.** Hooks, typical of many mature male characids, absent in dorsal-, pectoral-, pelvic-, and anal-fins of all examined specimens (n=50), but gonads not checked for maturity and sex.

**Genetics.** The alignment of the 21 terminals contained 652 bp with 208 variable sites. The nucleotide frequencies were 23.1% adenine, 17.8% guanine, 31.8% thymine, and 27.2% cytosine. DAMBE indicated no saturation in either transitions or transversions in both asymmetrical

**Table 1.** Vouchers, locality information, and GenBank accession numbers of the analyzed specimens of *Priocharax*.

Lot	Voucher	Species	Basin	Locality	Coordinates	GenBank n.
LBP 28495	LBP 96981	<i>Priocharax varii</i>	Rio Madeira	Rio Preto, affluent of Rio Jamari, Candeias do Jamari, RO, Brazil	08°52'53.5" S 63°37'50.8" W	MT754786
LBP 28495	LBP 96982	<i>Priocharax varii</i>	Rio Madeira	Rio Preto, affluent of Rio Jamari, Candeias do Jamari, RO, Brazil	08°52'53.5" S 63°37'50.8" W	MT754785
LBP 28495	LBP 96984	<i>Priocharax varii</i>	Rio Madeira	Rio Preto, affluent of Rio Jamari, Candeias do Jamari, RO, Brazil	08°52'53.5" S 63°37'50.8" W	MT754783
LBP 28495	LBP 96985	<i>Priocharax varii</i>	Rio Madeira	Rio Preto, affluent of Rio Jamari, Candeias do Jamari, RO, Brazil	08°52'53.5" S 63°37'50.8" W	MT7547864
LBP 28442	LBP 98284	<i>Priocharax ariel</i>	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
LBP 28442	LBP 98285	<i>Priocharax ariel</i>	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
LBP 27704	LBP 98286	<i>Priocharax ariel</i>	Rio Negro	Igarapé Tapage, Rio Urubaxi, approximately 1 hour from mouth of river, S. I. Rio Negro, AM, Brazil	00°30'05.3" S 64°49'11.7" W	MT754778
LBP 27704	LBP 98287	<i>Priocharax ariel</i>	Rio Negro	Igarapé Tapage, Rio Urubaxi, approximately 1 hour from mouth of river, S. I. Rio Negro, AM, Brazil	00°30'05.3" S 64°49'11.7" W	MT754782
LBP 27704	LBP 98288	<i>Priocharax ariel</i>	Rio Negro	Igarapé Tapage, Rio Urubaxi, approximately 1 hour from mouth of river, S. I. Rio Negro, AM, Brazil	00°30'05.3" S 64°49'11.7" W	MT754779
LBP 25858	LBP 96383	<i>Priocharax ariel</i>	Rio Negro	Igarapé Uacatuna, São Gabriel da Cachoeira, AM, Brazil	00°03'38.0" S 67°05'45.0" W	MT754777
LBP 28490	LBP 98283	<i>Priocharax nanus</i>	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
LBP 22464	LBP 96986	<i>Priocharax pygmaeus</i>	Rio Amazonas	Quebrada La Ponderosa, Letícia, Colombia	04°08'24.4" S 69°56'53.4" W	MT754771
LBP 22464	LBP 96987	<i>Priocharax pygmaeus</i>	Rio Amazonas	Quebrada La Ponderosa, Letícia, Colombia	04°08'24.4" S 69°56'53.4" W	MT754774
LBP 22464	LBP 96988	<i>Priocharax pygmaeus</i>	Rio Amazonas	Quebrada La Ponderosa, Letícia, Colombia	04°08'24.4" S 69°56'53.4" W	MT754769
LBP 22464	LBP 96998	<i>Priocharax pygmaeus</i>	Rio Amazonas	Quebrada La Ponderosa, Letícia, Colombia	04°08'24.4" S 69°56'53.4" W	MT754768
LBP 22739	LBP 96989	<i>Priocharax pygmaeus</i>	Rio Amazonas	Quebrada Pichuna, Letícia, Colombia	04°07'33.8" S 70°00'28.9" W	MT754772
LBP 22739	LBP 96990	<i>Priocharax pygmaeus</i>	Rio Amazonas	Quebrada Pichuna, Letícia, Colombia	04°07'33.8" S 70°00'28.9" W	MT754773
LBP 22739	LBP 96991	<i>Priocharax pygmaeus</i>	Rio Amazonas	Quebrada Pichuna, Letícia, Colombia	04°07'33.8" S 70°00'28.9" W	MT754776
LBP 22739	LBP 96992	<i>Priocharax pygmaeus</i>	Rio Amazonas	Quebrada Pichuna, Letícia, Colombia	04°07'33.8" S 70°00'28.9" W	MT754770
LBP 22739	LBP 96993	<i>Priocharax pygmaeus</i>	Rio Amazonas	Quebrada Pichuna, Letícia, Colombia	04°07'33.8" S 70°00'28.9" W	MT754775
LBP 17833	LBP 70161	<i>Acestrocephalus sardina</i>	Rio Negro	Santa Isabel do Rio Negro, AM, Brazil	00°30'5.3" S 64°49'12.2" W	MT754767

(Iss.cAsym) and symmetrical (Iss.cSym) topologies. The overall mean of K2P genetic distances without outgroup was 18.1%. The values of interspecific distances among the four species ranged from 20.7% between *Priocharax varii* and *P. nanus* to 30.5% between *P. ariel* and *P. pygmaeus* (Table 3). Intraspecific genetic variation ranged from 0% within *Priocharax pygmaeus* to 0.2% within *P. ariel* and *P. varii* (Table 3). The convergence of initial and recursive partitions of the ABGD analysis suggested four species, *Priocharax ariel*, *P. nanus*, *P. pygmaeus*,

and *P. varii* (Fig. 5; Supplementary Fig. 1). The ML solution of the PTP analysis returned identical results (Fig. 5; Supplementary Fig. 2).

**Distribution.** *Priocharax varii* is known to date from the type locality in the Rio Preto, a tributary of the Rio Jamari, itself an affluent of the Rio Madeira (Fig. 6). Specimens from several other localities in the Madeira system in Brazil, including some of its large tributaries (e.g., Jaciparaná, Jamari, Marmelos) as well as some are-

**Table 2.** Morphometric data of *Priocharax varii* sp. nov. (n=50, except for anal-fin length and upper jaw length with n=49, range includes holotype); SD = Standard Deviation.

	Holotype	Range	Mean	SD
Standard length (SL) (mm)	12.2	11.8–14.0	—	—
Percentages of SL				
Depth at dorsal-fin origin	24	18–31	23.5	1.7
Snout to dorsal-fin origin	54	49–58	54.3	1.5
Snout to pelvic-fin origin	41	39–44	40.5	0.9
Snout to anal-fin origin	53	52–57	53.8	1.0
Dorsal-fin length	25	23–29	25.6	1.1
Dorsal-fin base	13	10–14	12.0	1.1
Pelvic-fin length	11	9–13	11.5	0.8
Anal-fin length	35	32–38	35.5	1.3
Anal-fin base	32	29–36	32.4	1.2
Caudal-peduncle depth	8	7–9	8.0	0.4
Caudal-peduncle length	16	14–18	15.6	0.9
Head length (HL)	26	24–27	25.3	0.6
Percentages of HL				
Orbital diameter	36	32–57	34.8	3.5
Interorbital distance	38	34–47	39.1	2.9
Snout length	21	20–27	23.0	1.3
Upper jaw length	54	48–62	54.7	3.5
Percentage of caudal-peduncle length				
Caudal-peduncle depth	51	43–63	51.4	4.0

**Table 3.** Pairwise K2P genetic distances among species of *Priocharax*. Intraspecific genetic variations are highlighted in bold. Numbers below diagonal are values of interspecific distances. Values are shown in percentages, followed by standard deviation. Number of sequences: *P. ariel* = 6, *P. nanus* = 1, *P. pygmaeus* = 9, *P. varii* = 4.

	1	2	3	4
1 <i>P. ariel</i>	<b>0.2±0.1</b>			
2 <i>P. pygmaeus</i>	30.5±3.6	<b>0</b>		
3 <i>P. nanus</i>	21.9±2.8	21.1±2.9	—	
4 <i>P. varii</i>	22.7±2.9	23.5±2.8	20.7±2.8	<b>0.2±0.1</b>

as near the main channel (e.g., Lago Cuniã) were examined herein and identified as *P. ariel*. Figure 6 also shows the known distribution of all congeners.

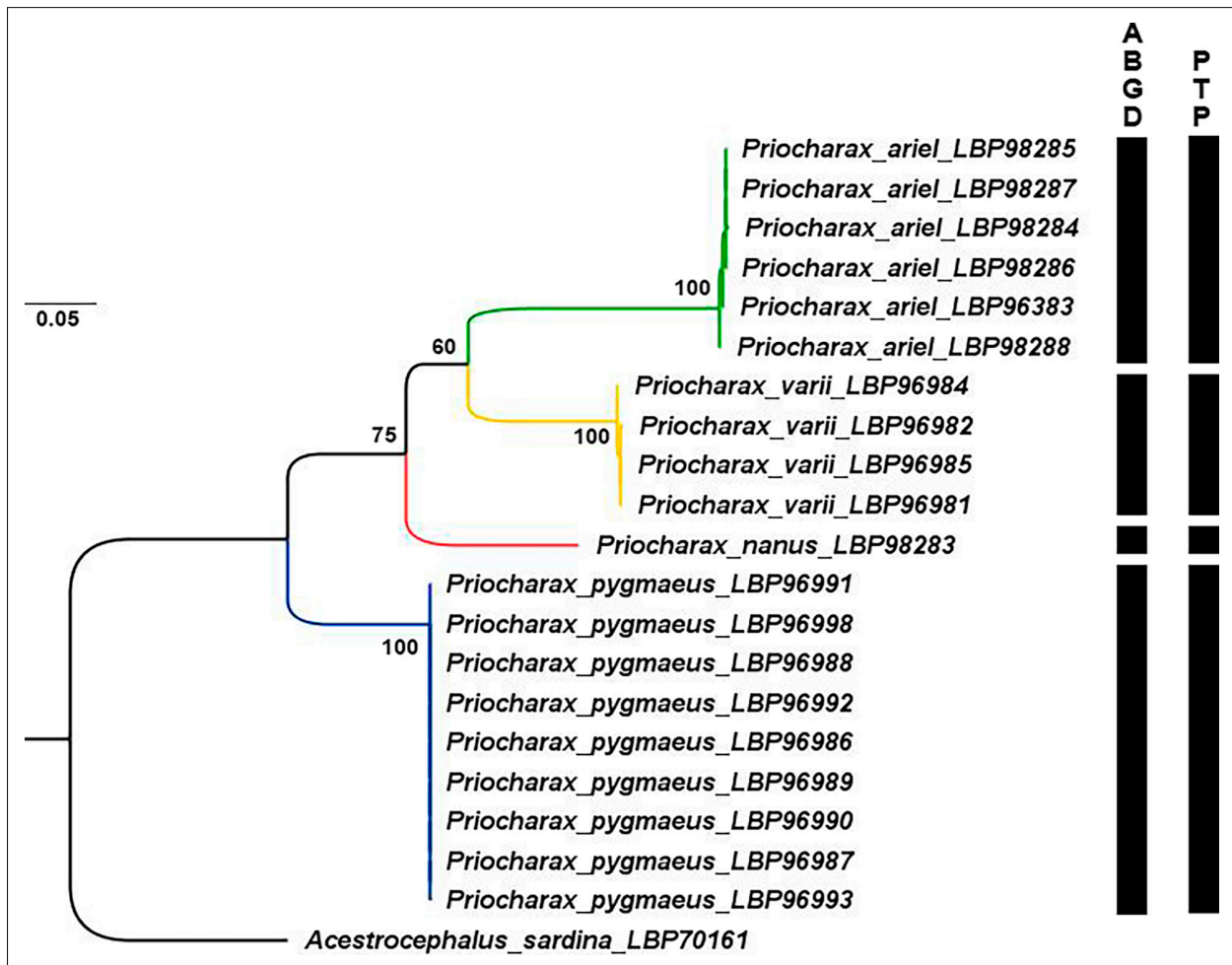
**Ecological notes.** Specimens were collected between 14:00 and 17:00 in a small tributary of the Rio Jamari (Fig. 7). They were all collected close to a bush of submerged macrophytes in a slow flowing part of the Rio Preto, approximately 200m above rapids. *Priocharax varii* was collected with *Apistogramma resticulosa* Kullander 1980, *Axelrodia lindeae* Géry 1973, *Axelrodia stigmatias* (Fowler 1913), *Hemigrammus bellottii* (Steindachner 1882), *Hyphessobrycon* aff. *sweglesi* (Géry 1961), *Leporinus* sp., *Nannostomus trifasciatus* Steindachner 1876, *Odontocharacidium aphanes* (Weitzman & Kanazawa 1977) and *Satanoperca* sp. Three of these species are considered miniature (*sensu* WEITZMAN & VARI, 1988): the characids *A. lindeae*, *A. stigmatias*

and the crenuchid *O. aphanes*. In addition to *Priocharax varii* and those three miniatures which are mostly transparent, also the larger *H. bellottii* has a predominantly translucent body in life, lending support to the idea of an “almost invisible league” (Fig. 8) for the Rio Negro proposed by CARVALHO *et al.* (2006).

**Etymology.** *Priocharax varii* honours the late Richard P. Vari, our dear friend and mentor, and one of the most influential researchers in Neotropical ichthyology of the past 40 years. A noun in genitive. In addition to being one of the authors of the genus *Priocharax*, he continuously encouraged the first author to study this taxon further and in more detail.

## Discussion

WEITZMAN & VARI (1987) referred to the retention of the larval pectoral-fin structure in adults of *Priocharax* as the main diagnostic feature of genus (e.g., Figure 3). Based on the hypothesis of phylogenetic relationships of *Priocharax* with *Gnathocharax*, *Heterocharax*, *Hoplocharax* and *Lonchogenys* (MATTOX & TOLEDO-PIZA, 2012:880) this feature was corroborated as autapomorphic for the genus (TOLEDO-PIZA *et al.* 2014:234). In addition to retaining the larval rayless structure of the pectoral fin, *Priocharax varii* possesses most other features listed by WEITZMAN & VARI (1987) to diagnose *Priocharax*, except for the number of pelvic-fin rays.



**Fig. 5.** Maximum likelihood tree of four known species of *Priocharax*, based on the COI gene (652 pb). Values < 50% are not shown. Bars represent the number of species obtained by the ABGD and bPTP analyses. Numbers near nodes represent bootstrap support.

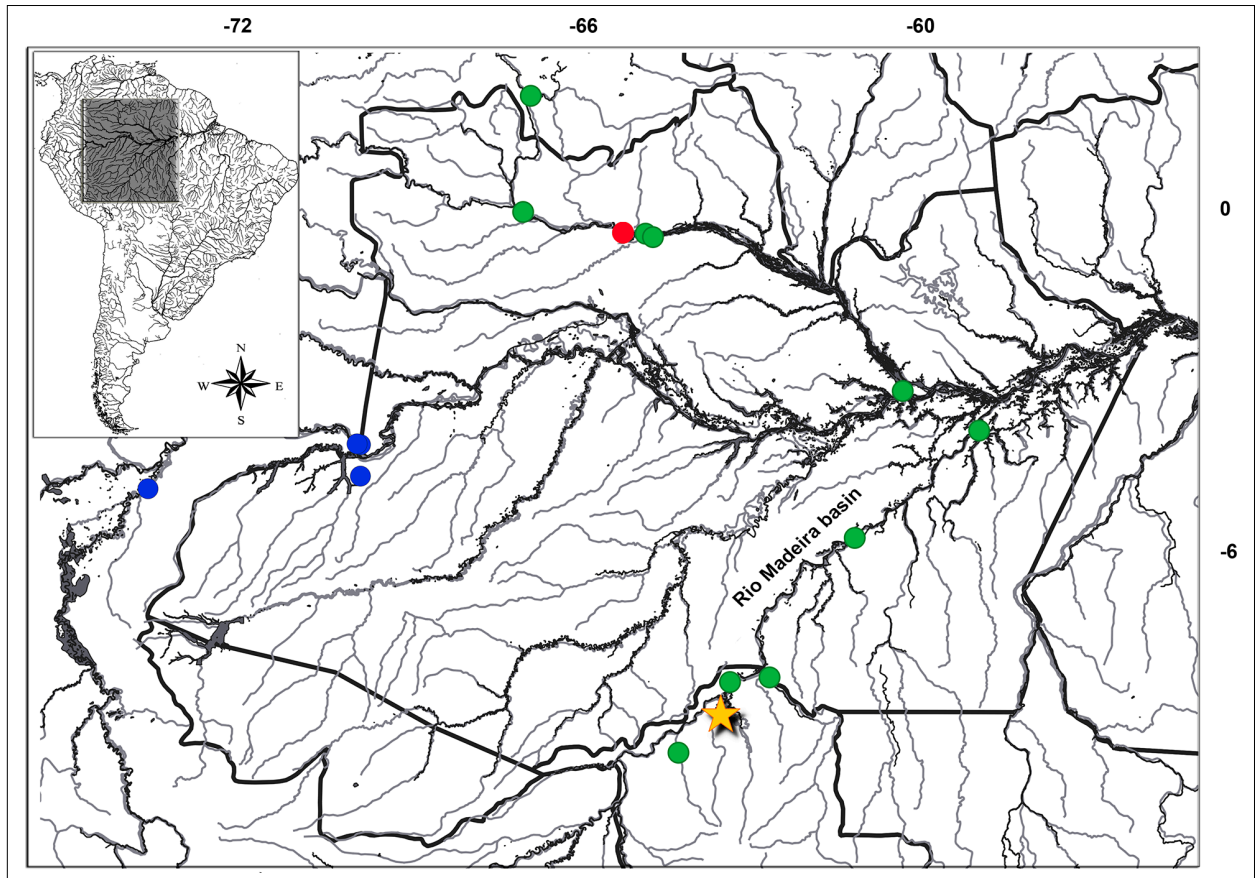
Among the diagnostic characters of *Priocharax* listed by WEITZMAN & VARI (1987), *Priocharax nanus*, described years later (TOLEDO-PIZA *et al.*, 2014), has a claustrum (vs. claustrum absent) and has six branched pelvic-fin rays (vs. five). Five to six branched pelvic-fin rays are still diagnostic for *Priocharax* among the Heterocharacinae, which invariably have seven. We recognize *Priocharax varii* as a member of the genus *Priocharax* based on the following features which are in accordance with the diagnostic features of the genus mentioned by WEITZMAN & VARI (1987) and discussed by TOLEDO-PIZA *et al.* (2014): the retention of larval pectoral fin in adults (Figure 3), upper and lower jaws with somewhat irregular single rows of conical teeth (57–71 and 37–46, respectively) (Figure 2), and low number of branched anal-fin (18–25) and branched pelvic-fin rays (6 – one specimens out of 50 with 5 branched rays).

Like the other species of the genus, *Priocharax varii* also has a number of reductive anatomical characters associated with miniaturization: e.g. loss of the latero-sensory canal system on the head and body, loss of the bones of the infraorbital series and the presence of a gap in the Weberian apparatus between neural arches 3 and 4 (WEITZMAN & VARI, 1987: fig. 3; MATTOX *et al.*,

2013:313, TOLEDO-PIZA *et al.*, 2014: fig. 4). However, other anatomical complexes present different degrees of reduction among *Priocharax* species. For example, *Priocharax varii* and *P. nanus* have two postcleithra in the pectoral girdle, while in *P. ariel* and *P. pygmaeus* all postcleithra are absent. *Priocharax varii* and *P. nanus* have six branched pelvic-fin rays (with the exception of one specimen of *P. varii* with five) and *P. ariel* and *P. pygmaeus* have only five. On the other hand, the claustrum in the Weberian apparatus is absent in most specimens of *Priocharax varii* (n=4 out of 5), and in all specimens of *P. ariel* and *P. pygmaeus* (WEITZMAN & VARI, 1987) while it is present in *P. nanus* (TOLEDO-PIZA *et al.*, 2014).

*Priocharax ariel*, *P. nanus* and *P. pygmaeus* all lack the adipose fin, a feature interpreted herein as a developmental truncation since the adipose fin does not develop from the larval fin fold, but forms *de novo* in the ontogeny of Characiformes (FUIMAN, 1984; BENDER & MORITZ, 2014; MARINHO, 2017). *Priocharax varii* can be distinguished from all three congeners by the presence of a small adipose fin located midway between the dorsal-fin terminus and the anterior most caudal-fin ray (Figure 1). Presence of the adipose fin has been used as one of the





**Fig 6.** Map of South America with a close-up of upper and central Amazon basin, Brazil, illustrating the distribution of the four species of *Priocharax*: *P. varii* (yellow star) described herein from the Rio Madeira basin, *P. ariel* (green dots), *P. nanus* (red dot), and *P. pygmaeus* (blue dots). Colours are the same as in Figure 5. Some dots may represent more than one lot.

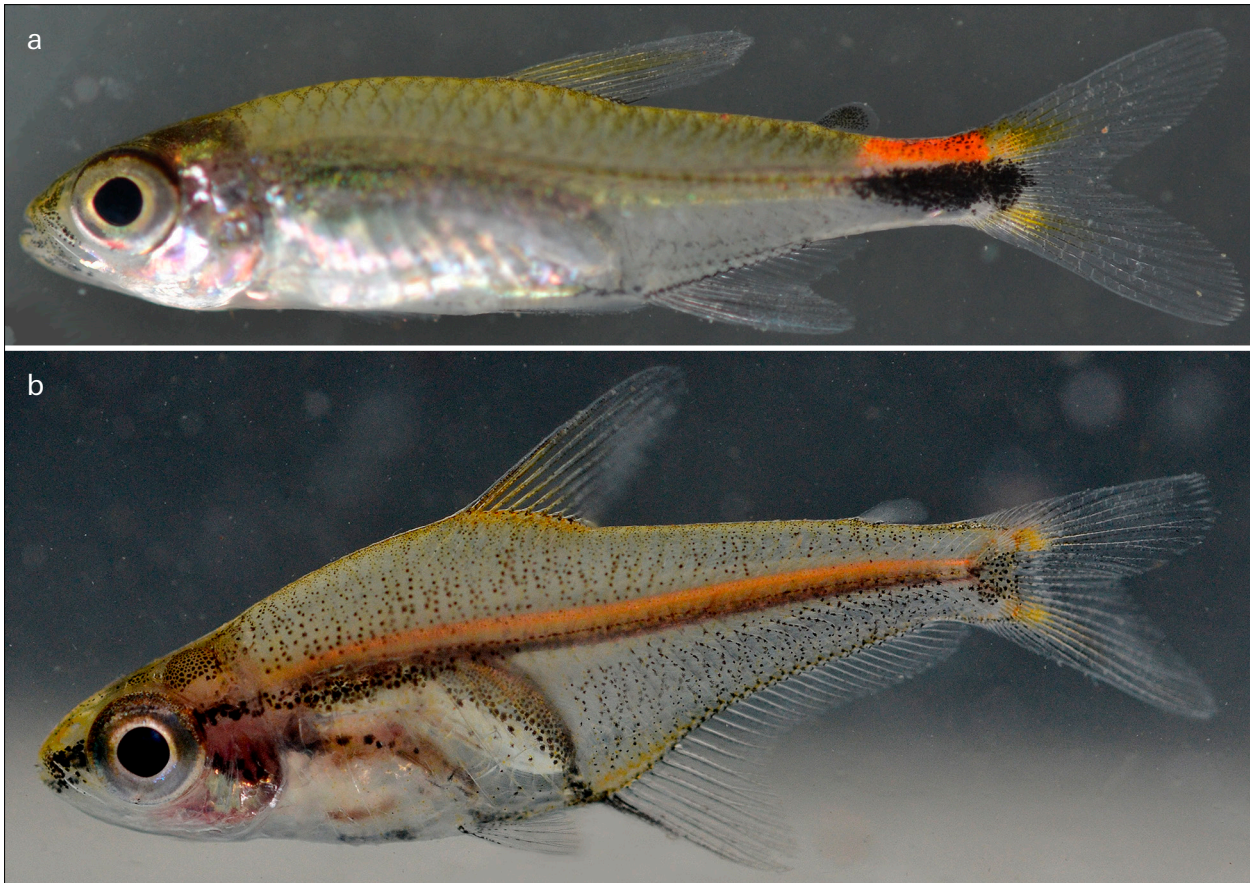


**Fig. 7.** Rio Preto approximately 12km South from road BR-364, Candeias do Jamari, Rondônia, Brazil, the type locality of *Priocharax varii*.

characters to diagnose species in other genera of Characiformes composed of miniature species that otherwise lack such structure: for example, *Elachocharax pulcher*

Myers is the only one of four miniatures in the crenuchid genus *Elachocharax* Myers that has an adipose fin (BUCKUP, 1993) and *Xenurobrycon pteropus* Weitzman &





**Fig. 8.** Examples of fishes found with *Priocharax varii* that are translucent in life: a) *Axelrodia stigmatias*; b) *Axelrodia lindeae*. Live specimens photographed right after capture.

Fink is the only one of six miniatures in the genus that has an adipose fin (MENDONÇA *et al.*, 2016). In addition to *Elachocharax*, *Priocharax* and *Xenobrycon* Myers & Miranda-Ribeiro of which the majority of species lack an adipose fin, the following miniature genera are currently composed solely of species lacking the adipose fin: *Dundocharax* Poll, *Iotabrycon* Roberts and *Scopaeocharax* Weitzman & Fink, and two other genera of Characidae are composed exclusively of miniature species, some of them also lacking the adipose fin: *Tyttobrycon* Géry and *Tyttocharax* Fowler (see Table 4 for details).

In some other genera that are not composed solely of miniature species *sensu* WEITZMAN & VARI (1988), the adipose-fin is absent in both miniature and some non-miniature taxa. Such is the case in *Carnegiella* Eigenmann, *Characidium* Reinhardt, *Nannostomus* Günther, and *Neolebias* Steindachner (Table 4). Moreover, the lack of the adipose fin is not exclusive to genera with miniature species. There are small to medium sized characiforms that also lack this fin such as the two species of the crenuchid *Poecilocharax* Eigenmann, the distichodontid *Nannocharax* Günther, the lebiasinids *Copeina* Fowler, *Copella* Myers, *Pyrrhulina* Valenciennes and some species of *Lebiasina* Valenciennes (Table 4). Among characids, the adipose fin is lacking in species of *Coptobrycon* Géry, *Corynopoma* Gill, *Grundulus* Valenciennes, *Gymnocharacinus* Steindachner, *Hasemania*

Ellis, *Hyphessobrycon* Durbin, *Monotocheirodon* Eigenmann, *Nematobrycon* Eigenmann, *Phenagoniates* Eigenmann & Wilson, and *Spintherobolus* Eigenmann (Table 4). Finally, the adipose fin is absent in all 17 species of the Erythrinidae (Table 4), including some of the largest characiforms (MATTOX *et al.*, 2006; OYAKAWA & MATTOX, 2009; 2018). This is evidence that the loss of the adipose fin is not necessarily related to small size.

Intraspecific variation in the presence of the adipose fin and its development was mentioned for some characiforms. WEITZMAN & COBB (1975) cited intraspecific variation in *Nannostomus eques* and *N. trifasciatus*, TRAVASSOS (1949) in *Characidium lauroi*, BUCKUP & HAHN (2000) in *C. vestigipinne* and MELO *et al.* (2016) in *C. oiticicai*. GÉRY (1977) reported variation in the presence/absence of the adipose fin in the African *Neolebias trilineatus* and *N. unifasciatus*. Among the Characidae, intraspecific variation in the size or presence of the adipose fin was reported for *Hasemania nana* (GÉRY, 1977:518; DAGOSTA *et al.*, 2014) *Hyphessobrycon diastatos* (DAGOSTA *et al.*, 2014), *H. negodagua* (LIMA & GERHARD, 2001), *H. eilyos* (LIMA & MOREIRA, 2003), and *H. uaiso* (CARVALHO & LANGEANI, 2013). All examined specimens of *Priocharax varii* (n=50) consistently have a small adipose fin and no intraspecific variation was observed.

The molecular analysis from samples of all four taxa supports the conclusion that each represents a species

**Table 4.** List of species of Characiformes that lack the adipose fin, organized in families according to Miranda (2018). Families and species are listed in alphabetical order. The second column refer to number of species lacking the adipose fin in relation to the total number of species in the genus. References are those that summarize information on the lack of the adipose fin for a species or group of species. Species in bold are miniature according to Weitzman & Vari (1987), with Neotropical fishes updated according to Toledo-Piza *et al.* (2014) or described after that. Species with intraspecific variation in the absence/presence of the adipose fin are not listed here, but are mentioned in the text.

Taxon		Reference
Characidae		
<i>Coptobrycon bilineatus</i> (Ellis, 1911)	1/1	GÉRY, 1977
<i>Corynopoma riisei</i> Gill, 1858	1/1	GÉRY, 1977
<i>Grundulus bogotensis</i> Humboldt, 1821	3/3	ROMÁN-VALENCIA <i>et al.</i> , 2003
<i>Grundulus cochae</i> Román-Valencia <i>et al.</i> , 2003	3/3	ROMÁN-VALENCIA <i>et al.</i> , 2003
<i>Grundulus quitoensis</i> Román-Valencia <i>et al.</i> , 2005	3/3	ROMÁN-VALENCIA <i>et al.</i> , 2005
<i>Gymnocharacinus bergii</i> Steindachner, 1903	1/1	GÉRY, 1977
<i>Hasemania crenuchoides</i> Zarske & Géry, 1999	8/9	SERRA & LANGEANI, 2015
<i>Hasemania hanseni</i> (Fowler, 1949)	8/9	SERRA & LANGEANI, 2015
<i>Hasemania kalunga</i> Bertaco & Carvalho, 2010	8/9	SERRA & LANGEANI, 2015
<i>Hasemania maxillaris</i> Ellis, 1911	8/9	SERRA & LANGEANI, 2015
<i>Hasemania melanura</i> Ellis, 1911	8/9	SERRA & LANGEANI, 2015
<i>Hasemania nambiquara</i> Bertaco & Malabarba, 2007	8/9	SERRA & LANGEANI, 2015
<i>Hasemania piatan</i> Zanata & Serra, 2010	8/9	SERRA & LANGEANI, 2015
<i>Hasemania uberaba</i> Serra & Langeani, 2015	8/9	SERRA & LANGEANI, 2015
<i>Hyphessobrycon taurocephalus</i> Ellis, 1911	1/161	LIMA & GERHARD, 2001
<b><i>Iotabrycon praecox</i> Roberts, 1973</b>	<b>1/1</b>	<b>ROBERTS, 1973</b>
<i>Monotocheiroduon drilos</i> Menezes <i>et al.</i> , 2013	3/3	MENEZES <i>et al.</i> , 2013
<i>Monotocheiroduon kontos</i> Menezes <i>et al.</i> , 2013	3/3	MENEZES <i>et al.</i> , 2013
<i>Monotocheiroduon pearsoni</i> Eigenmann, 1924	3/3	MENEZES <i>et al.</i> , 2013
<i>Nematobrycon lacortei</i> Weitzman & Fink, 1971	2/2	GÉRY, 1977
<i>Nematobrycon palmeri</i> Eigenmann, 1911	2/2	GÉRY, 1977
<i>Phenagoniates macrolepis</i> (Meek & Hildebrand, 1913)	1/1	GÉRY, 1977
<b><i>Priocharax ariel</i> Weitzman &amp; Vari, 1987</b>	<b>3/4</b>	<b>WEITZMAN &amp; VARI, 1987</b>
<b><i>Priocharax nanus</i> Toledo-Piza <i>et al.</i>, 2014</b>	<b>3/4</b>	<b>TOLEDO-PIZA <i>et al.</i>, 2014</b>
<b><i>Priocharax pygmaeus</i> Weitzman &amp; Vari, 1987</b>	<b>3/4</b>	<b>WEITZMAN &amp; VARI, 1987</b>
<b><i>Scopaeocharax atopodus</i> (Böhlke, 1958)</b>	<b>2/2</b>	<b>WEITZMAN &amp; FINK, 1985</b>
<b><i>Scopaeocharax rhinodus</i> (Böhlke, 1958)</b>	<b>2/2</b>	<b>WEITZMAN &amp; FINK, 1985</b>
<i>Spintherobolus ankoseion</i> Weitzman & Malabarba, 1999	4/4	WEITZMAN & MALABARBA, 1999
<b><i>Spintherobolus brocuae</i> Myers, 1925</b>	<b>4/4</b>	<b>WEITZMAN &amp; MALABARBA, 1999</b>
<i>Spintherobolus leptoura</i> Weitzman & Malabarba, 1999	4/4	WEITZMAN & MALABARBA, 1999
<i>Spintherobolus papilliferus</i> Eigenmann, 1911	4/4	WEITZMAN & MALABARBA, 1999
<b><i>Tyttobrycon hamatus</i> Géry, 1973</b>	<b>2/6</b>	<b>MARINHO <i>et al.</i>, 2013</b>
<b><i>Tyttobrycon spinosus</i> Géry, 1973</b>	<b>2/6</b>	<b>MARINHO <i>et al.</i>, 2013</b>
<b><i>Tyttocharax metae</i> Román-Valencia <i>et al.</i>, 2012</b>	<b>2/4</b>	<b>ROMÁN-VALENCIA <i>et al.</i>, 2012</b>
<b><i>Tyttocharax tambopatensis</i> Weitzman &amp; Ortega, 1995</b>	<b>2/4</b>	<b>WEITZMAN &amp; ORTEGA, 1995</b>
<b><i>Xenobrycon coracoralinae</i> Moreira, 2005</b>	<b>5/6</b>	<b>MENDONÇA <i>et al.</i>, 2016</b>
<b><i>Xenobrycon heterodon</i> Weitzman &amp; Fink, 1985</b>	<b>5/6</b>	<b>MENDONÇA <i>et al.</i>, 2016</b>
<b><i>Xenobrycon macropus</i> Myers &amp; Miranda Ribeiro, 1945</b>	<b>5/6</b>	<b>MENDONÇA <i>et al.</i>, 2016</b>
<b><i>Xenobrycon polyancistrus</i> Weitzman, 1987</b>	<b>5/6</b>	<b>MENDONÇA <i>et al.</i>, 2016</b>
<b><i>Xenobrycon varii</i> Mendonça <i>et al.</i>, 2016</b>	<b>5/6</b>	<b>MENDONÇA <i>et al.</i>, 2016</b>
Crenuchidae		
<b><i>Characidium chicoi</i> Graça <i>et al.</i>, 2019</b>	<b>9/73</b>	<b>GRAÇA <i>et al.</i>, 2019</b>
<i>Characidium helmeri</i> Zanata <i>et al.</i> , 2015	9/73	MELO <i>et al.</i> , 2016
<b><i>Characidium mirim</i> Netto-Ferreira <i>et al.</i>, 2013</b>	<b>9/73</b>	<b>MELO <i>et al.</i>, 2016</b>
<b><i>Characidium nana</i> Mendonça &amp; Netto-Ferreira, 2015</b>	<b>9/73</b>	<b>MELO <i>et al.</i>, 2016</b>
<i>Characidium nupelia</i> Graça <i>et al.</i> , 2008	9/73	MELO <i>et al.</i> , 2016
<i>Characidium sterbai</i> Zarske, 1997	9/73	MELO <i>et al.</i> , 2016
<i>Characidium stigmatosum</i> Melo & Backup, 2002	9/73	MELO <i>et al.</i> , 2016
<i>Characidium travassosi</i> Melo <i>et al.</i> , 2016	9/73	MELO <i>et al.</i> , 2016
<i>Characidium xavante</i> Graça <i>et al.</i> , 2008	9/73	MELO <i>et al.</i> , 2016
<b><i>Elachocharax geryi</i> Weitzman &amp; Kanazawa, 1978</b>	<b>3/4</b>	<b>BUCKUP, 1993</b>

Table 4 continued.

Taxon		Reference
Crenuchidae		
<i>Elachocharax junki</i> (Géry, 1971)	3/4	BUCKUP, 1993
<i>Elachocharax mitopterus</i> Weitzman, 1986	3/4	BUCKUP, 1993
<i>Poecilocharax bovaliorum</i> Eigenmann, 1909	2/2	BUCKUP & VAN DER SLEEN, 2018
<i>Poecilocharax weitzmani</i> Géry, 1965	2/2	BUCKUP & VAN DER SLEEN, 2018
Distichodontidae		
<i>Dundocharax bidentatus</i> Poll, 1967	1/1	GÉRY, 1977
<i>Nannocharax machadoi</i>	1/41	GÉRY, 1977
<i>Neolebias ansorgii</i> Boulenger, 1912	5/12	GÉRY, 1977
<i>Neolebias axelrodi</i> Poll & Gosse, 1963	5/12	GÉRY, 1977
<i>Neolebias lozii</i> Winemiller & Kelson-Winemiller, 1993	5/12	WINEMILLER & KELSON-WINEMILLER, 1993
<i>Neolebias philippeii</i> Poll & Gosse, 1963	5/12	GÉRY, 1977
<i>Neolebias powelli</i> Teugels & Roberts, 1990	5/12	TEUGELS & ROBERTS, 1990
Erythrinidae		
<i>Erythrinus erythrinus</i> (Bloch & Schneider, 1801)	2/2	OYAKAWA & MATTOX, 2018
<i>Erythrinus kessleri</i> Steindachner, 1877	2/2	OYAKAWA & MATTOX, 2018
<i>Hoplerythrinus cinereus</i> (Gill, 1858)	3/3	OYAKAWA & MATTOX, 2018
<i>Hoplerythrinus gronovii</i> (Valenciennes, 1847)	3/3	OYAKAWA & MATTOX, 2018
<i>Hoplerythrinus unitaeniatus</i> (Spix & Agassiz, 1829)	3/3	OYAKAWA & MATTOX, 2018
<i>Hoplias aimara</i> (Valenciennes, 1847)	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias argentinensis</i> Rosso <i>et al.</i> , 2018	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias australis</i> Oyakawa & Mattox, 2009	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias brasiliensis</i> (Spix & Agassiz, 1829)	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias curupira</i> Oyakawa & Mattox, 2009	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias intermedius</i> (Günther, 1864)	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias lacerdae</i> Miranda Ribeiro, 1908	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias malabaricus</i> (Bloch, 1794)	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias mbigua</i> Azpelicueta <i>et al.</i> , 2015	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias microlepis</i> (Günther, 1864)	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias misionera</i> Rosso <i>et al.</i> , 2016	12/12	OYAKAWA & MATTOX, 2018
<i>Hoplias teres</i> (Valenciennes, 1847)	12/12	OYAKAWA & MATTOX, 2018
Gasteropelecidae		
<i>Carnegiella marthae</i> Myers, 1927	4/4	WEITZMAN, 1960
<i>Carnegiella myersi</i> Fernández-Yépez, 1950	4/4	WEITZMAN, 1960
<i>Carnegiella schereri</i> Fernández-Yépez, 1950	4/4	WEITZMAN, 1960
<i>Carnegiella strigata</i> (Günther, 1864)	4/4	WEITZMAN, 1960
Lebiasinidae		
<i>Copeina guttata</i> (Steindachner, 1876)	2/2	NETTO-FERREIRA, 2018
<i>Copeina osgoodi</i> Eigenmann, 1912	2/2	NETTO-FERREIRA, 2018
<i>Copella arnoldi</i> (Regan, 1912)	6/6	MARINHO & MENEZES, 2017
<i>Copella callolepis</i> (Regan, 1912)	6/6	MARINHO & MENEZES, 2017
<i>Copella compta</i> (Myers, 1927)	6/6	MARINHO & MENEZES, 2017
<i>Copella eigenmanni</i> (Regan, 1912)	6/6	MARINHO & MENEZES, 2017
<i>Copella nattereri</i> (Steindachner, 1876)	6/6	MARINHO & MENEZES, 2017
<i>Copella vilmae</i> Géry, 1963	6/6	MARINHO & MENEZES, 2017
<i>Lebiasina bimaculata</i> Valenciennes, 1847	7/26	NETTO-FERREIRA, 2010
<i>Lebiasina marilynnae</i> Netto-Ferreira, 2012	7/26	NETTO-FERREIRA, 2012
<i>Lebiasina melanoguttata</i> Netto-Ferreira, 2012	7/26	NETTO-FERREIRA, 2012
<i>Lebiasina minuta</i> Netto-Ferreira, 2012	7/26	NETTO-FERREIRA, 2012
<i>Lebiasina multimaculata</i> Boulenger, 1911	7/26	NETTO-FERREIRA, 2010
<i>Lebiasina taphorni</i> Ardila Rodríguez, 2004	7/26	ARDILA RODRIGUEZ, 2004
<i>Lebiasina uruyensis</i> Fernández-Yépez, 1967	7/26	NETTO-FERREIRA, 2010
<i>Nannostomus anduzei</i> Fernandez & Weitzman, 1987	6/20	FERNANDEZ & WEITZMAN, 1987
<i>Nannostomus beckfordi</i> Günther, 1872	6/20	WEITZMAN & COBB, 1975
<i>Nannostomus bifasciatus</i> Hoedeman, 1954	6/20	WEITZMAN & COBB, 1975
<i>Nannostomus limatus</i> Weitzman, 1978	6/20	WEITZMAN, 1978

Table 4 continued.

Taxon		Reference
<i>Nannostomus marginatus</i> Eigenmann, 1909	6/20	WEITZMAN & COBB, 1975
<b><i>Nannostomus minimus</i> Eigenmann, 1909</b>	<b>6/20</b>	<b>WEITZMAN &amp; COBB, 1975</b>
<i>Pyrhulina australis</i> Eigenmann & Kennedy 1903	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina beni</i> Pearson 1924	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina brevis</i> Steindachner 1876	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina capim</i> Vieira & Netto-Ferreira 2019	19/19	VIEIRA & NETTO-FERREIRA 2019
<i>Pyrhulina eleanorae</i> Fowler 1940	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina elongata</i> Zarske & Géry 2001	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina filamentosa</i> Valenciennes 1847	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina laeta</i> (Cope 1872)	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina lugubris</i> Eigenmann 1922	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina marilynae</i> Netto-Ferreira & Marinho 2013	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina maxima</i> Eigenmann & Eigenmann 1889	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina melanostomus</i> (Cope 1870)	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina obermulleri</i> Myers 1926	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina rachoviana</i> Myers 1926	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina semifasciata</i> Steindachner 1876	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina spilota</i> Weitzman 1960	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina stoli</i> Boeseman 1953	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina vittata</i> Regan 1912	19/19	NETTO-FERREIRA, 2018
<i>Pyrhulina zigzag</i> Zarske & Géry 1997	19/19	NETTO-FERREIRA, 2018

(Figure 5). The genetic distance between the four species ranges from 20.7% between *P. nanus* and *P. varii* to 30.5% between *P. ariel* and *P. pygmaeus*, a high genetic divergence compared with other Neotropical fishes which have an average of 6.8% (e.g., CARVALHO *et al.*, 2011; PEREIRA *et al.*, 2011a; 2013). Considering only characiforms, the range in genetic divergence between species is 1–22% (BENINE *et al.*, 2009; MELO *et al.*, 2011; PEREIRA *et al.*, 2011b; BELLAFRONTI *et al.*, 2013; CASTRO PAZ *et al.*, 2014; SILVA *et al.*, 2013; RAMIREZ & GALETTI JR, 2015; ROSSINI *et al.*, 2016).

Two hypotheses could explain the higher genetic differences in *Priocharax*: either its species have been evolving separately for a long time and have accumulated a large amount of mutations, or they have an accelerated evolutionary rate due to intrinsic features (e.g., CASTRO PAZ *et al.*, 2014). The first hypothesis predicts that *Priocharax* has a long evolutionary and biogeographic history, something that needs to be tested in the future when more data on the taxonomy and phylogeny of the genus are available. The second hypothesis predicts that *Priocharax* has biological features that would increase its evolutionary rate. In fact, smaller organisms tend to have higher nucleotide substitution rates (GILLOOLY *et al.*, 2005) because of their shorter generation time and therefore accumulate more genetic divergence compared to larger organisms in the same time period. ROBERTS (1984) already pointed out that miniature fishes could reproduce year-round continuously increasing the number of generations per time unit and, therefore, their mutation rate. The hypothesis raised by ROBERTS (1984) is particularly interesting and should be tested for *Priocharax* and other miniature taxa. The results for the miniatur-

ized and highly developmentally truncated cypriniforms *Danionella* and *Paedocypris* which both sit at the end of very long branches in molecular phylogenetic trees (see Supplementary figure 7 in BRITZ *et al.*, 2014) is a further example of a higher mutation rate in highly miniaturized fish taxa.

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## Comparative material examined

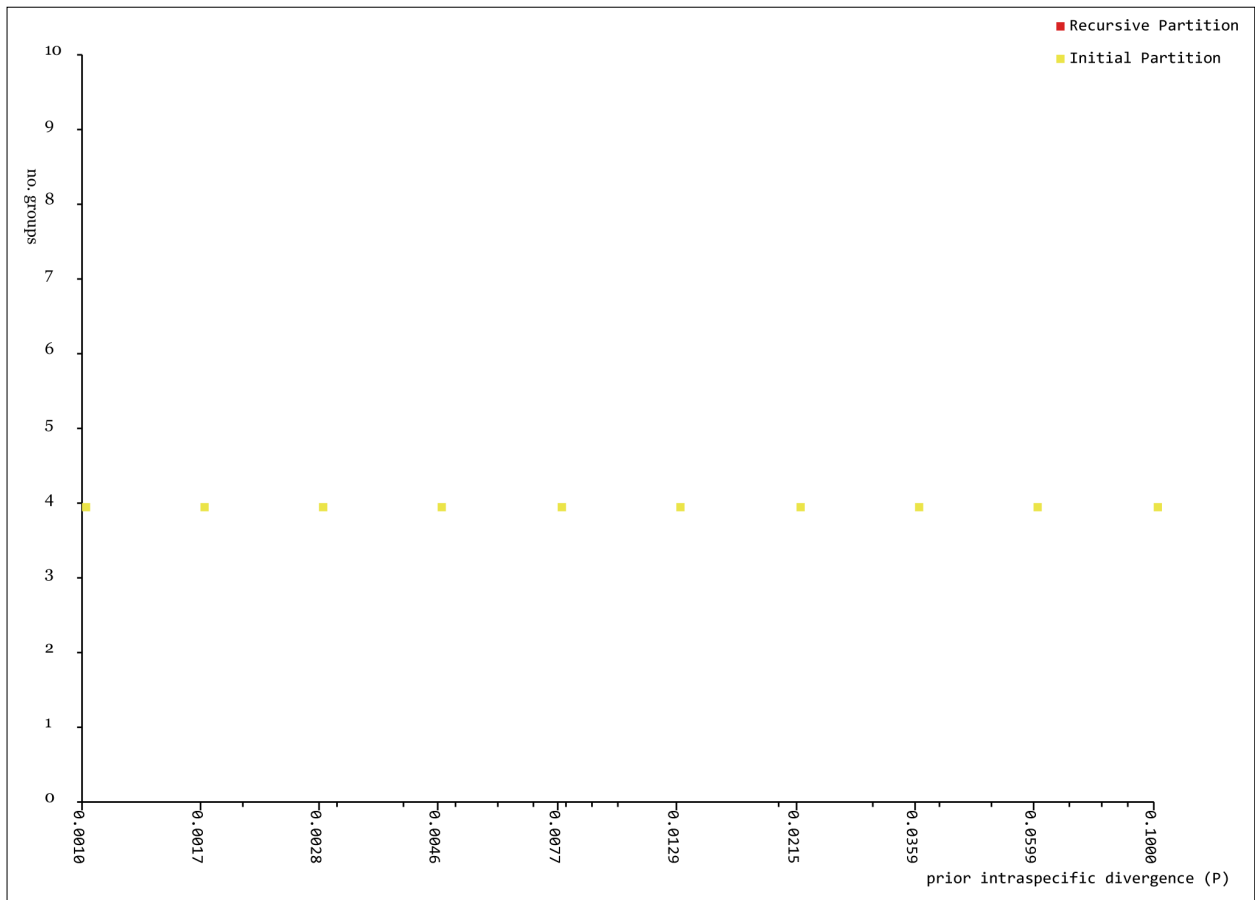
*Priocharax ariel*: Brazil: Amazonas, Santa Isabel do Rio Negro, rio Negro basin: MZUSP 39778, 4, 13.5–14.6 mm SL, rio Urubaxi; MZUSP 55099, 8, 12.4–14.2 mm SL, Igarapé at São João, near Santa Isabel do Rio Negro; MZUSP 55097, 4 of 6, 12.2–12.7 mm SL, lagoon near Paricatuba; MZUSP 62230, 2 of 4, 15.1–15.2 mm SL, lagoon in island near Paricatuba. Rio Madeira basin: UFRO-ICT 5761, 1, 14.5 mm SL, Uirapurá, Nova Olinda do Norte. UFRO-ICT 19660, 1, 10.9 mm SL, Rio Marmelos upstream from Vila do Escondido, Manicoré. UFRO-ICT 20954, 8, 10.1–11.8 mm SL, rocks on the right margin of Rio Marmelos, Manicoré. Rondônia: UFRO-ICT 2151, 11, 13.7–16.1 mm SL (4 c&s, 13.7–16.1 mm SL), Lago Madalena, mouth of Rio Jaciparaná, Porto Velho. UFRO-ICT 5744, 3, 12.1–12.5 mm SL, unnamed tributary of Rio Machado, Porto Velho. UFRO-ICT 5745, 3, 11.6–12.9 mm SL, Lago Cuniã, Porto Velho. UFRO-ICT 8449, 1, 17.1 mm SL, Rio Jaciparaná, tributary of Rio Madeira, Porto Velho. UFRO-ICT 13825, 1, 14.2 mm SL, Igarapé C, ESEC Cuniã, Porto Velho. UFRO-ICT 17412, 2, 11.6–12.4 mm SL, Arrozal, Lago Cuniã, Porto Velho. UFRO-ICT 20075, 1, 17.4 mm SL, Igarapé A, ESEC Cuniã, Porto Velho. Venezuela: Territorio Federal Amazonas: MZUSP 36497, 50, 11.8–15.2 mm SL, MZUSP 55142, 12 paratypes, 12.0–14.7 mm SL (5 c&s, 12.0–14.0 mm SL), Caño Manu, tributary of río Casiquiare approximately 250 m upstream from Solano.

*Priocharax nanus*: MZUSP 114014, 13.8 mm SL, holotype, Brazil, Amazonas, Santa Isabel do Rio Negro, rio Negro basin, lake at right bank of rio Urubaxi, near Igarapé Tapage, 0°33'44.2" S 64°49'40.8" W, 26 Oct 2011, M. Toledo-Piza, G. Mattox, M. Marinho & R. Britz. All paratypes: MZUSP 114015, 9,

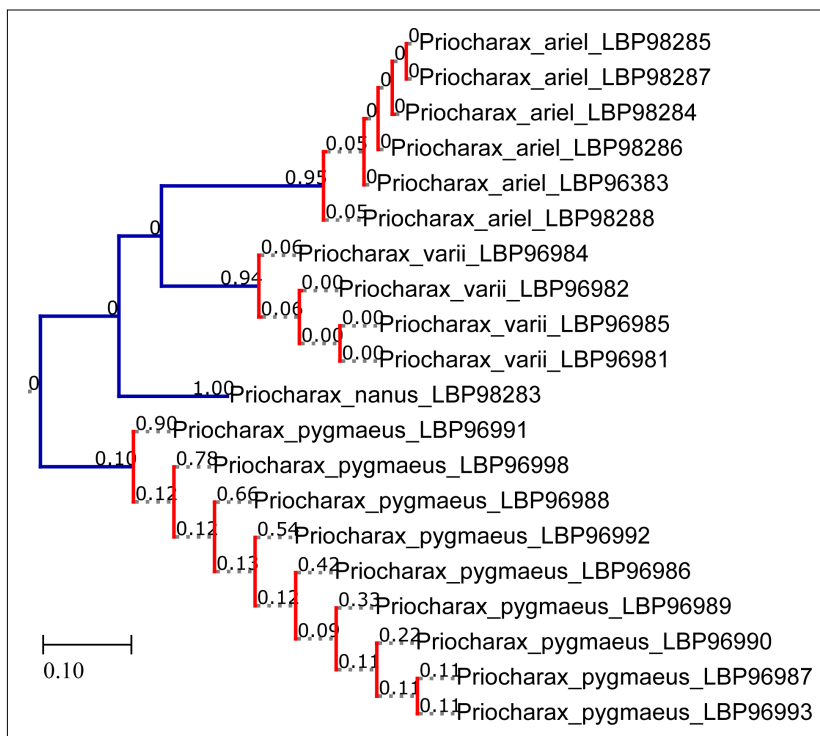
12.1–15.3 mm SL (3 c&s, 14.1–15.3 mm SL), same data as holotype. MZUSP 114016, 5, 12.6–14.6 mm SL (2 c&s, 13.4–13.8 mm SL), Brazil, Amazonas, Santa Isabel do Rio Negro, rio Negro basin, Igarapé Tapage at left bank of rio Urubaxi, 0°30'5.3" S 64°49'11.7" W, 26 Oct 2011, M. Toledo-Piza, G. Mattox, M. Marinho & R. Britz. MZUSP 114017, 3, 13.5–14.6 mm SL (1 c&s, 14.6 mm SL), Brazil, Amazonas, Santa Isabel do Rio Negro, rio Negro basin, first tributary of rio Negro above rio Daraá, 0°27'24.2" S 64°46'54.1" W, 27 Oct 2011, M. Toledo-Piza, G. Mattox, M. Marinho & R. Britz. INPA 39891, 4, 12.5–13.9 mm SL; MZUSP 114018, 11, 11.1–15.4 mm SL (5 c&s, 12.0–14.0 mm SL); USNM 427007, 4, 12.1–13.3 mm SL; Brazil, Amazonas, Santa Isabel do Rio Negro, rio Negro basin, rio Negro and tributaries near Santa Isabel do Rio Negro, 23–30 Oct 2011, M. Toledo-Piza, G. Mattox, M. Marinho & R. Britz.

*Priocharax pygmaeus*: Colombia: Departamento Amazonas: MZUSP 36498, 5 paratypes, 10.2–10.7 mm SL, Quebrada Parajito, tributary of Quebrada Bacada, tributary of Quebrada Matamata, tributary of rio Amazonas, northwest of Leticia, about 04°41' S 69°57' W. Peru: Loreto, Requena, rio Ucayali basin: MZUSP 85644, 1, 16.5 mm SL, small “quebrada”, tributary of Quebrada Fierro Caño, ca. 4 km North of IIAP (2.7 km east of Jenaro Herrera).

Supplementary material



**Fig. 1.** Automatic partition of the dataset reporting the number of groups inside the initial and recursive partitions. Red symbols are overlapped by yellow symbols.



**Fig. 2.** Poisson Tree Processes (PTP) delimitation tests of species using the maximum likelihood tree.

