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A new species of *Aphyocharax* Günther, 1868 (Characiformes, Characidae) from the Maracaçumé river basin, eastern Amazon

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Abstract

A new species of *Aphyocharax* is described from the Maracaçumé river basin, eastern Amazon, based on morphological and molecular data. The new species differs from all its congeners, mainly by possessing the upper caudal-fin lobe longer than the lower one in mature males, and other characters related to teeth counts, colour pattern, and body depth at dorsal-fin origin. In addition, the new species is corroborated by a haplotype phylogenetic analyses based on the Cytochrome B (Cytb) mitochondrial gene, where its haplotypes are grouped into an exclusive lineage, supported by maximum posterior probability value, a species delimitation method termed the Wiens and Penkrot analysis (WP).

Key Words

Freshwater, integrative taxonomy, Neotropical ichthyology, sexual dimorphism

Introduction

The Neotropical fish genus *Aphyocharax* Günther, 1868 is distributed along the river basins of the Orinoco, Amazon, and La Plata systems, as well as in the river systems drainaing the Guiana Shield (Géry 1977; Taphorn and Thomerson 1991; Tagliacollo et al. 2012; Brito et al. 2018; Fricke et al. 2019), with highest diversity in the Amazon basin (Fricke et al. 2019). According to Brito et al. (2018), the genus comprises 11 valid species: *Aphyocharax agassizii* (Steindachner, 1882), *A. anisitsi* Eigenmann & Kennedy, 1903, *A. avary* Fowler, 1913, *A. colifax* Taphorn & Thomerson, 1991, *A. dentatus* Eigenmann & Kennedy, 1903, *A. erythrurus* Eigenmann, 1912, *A. gracilis* Fowler, 1940, *A. nattereri* (Steindachner, 1882), *A. pusillus* Günther, 1868, *A. rathbuni* Eigenmann, 1907,

and *A. yekwanae* Willink, Chernoff & Machado-Allison, 2003. However, there are at least four undescribed species (Souza-Lima 2007).

Tagliacollo et al. (2012) included seven valid species of *Aphyocharax* in their phylogenetics analysis, and provided a hypothesis of interspecific relationships based on both molecular and morphological datasets. Their parsimony-based total evidence analysis (TE) indicates that *Aphyocharax* and *Prionobrama* Fowler, 1913 form a clade supported by three morphological synapomorphies: (1) interrupted lateral line with a single perforated scale on the posterior region of caudal peduncle; (2) absence or reduction of the fourth infraorbital bone canal; and (3) presence of a single large cusp on anterior maxillary teeth. In addition, three morphological synapomorphies have been proposed for *Aphyocharax*: (1) narrow trigeminofacialis foramen like a cleft with sphenotic almost excluded from its margin; (2) dorsal projection of maxilla overlaping the second infraorbital; and (3) dorsal margin of third postcleithrum not projecting dorsally to posterior region of scapula (Mirande 2010; Tagliacollo et al. 2012). However, several other morphological features have been commonly used to characterize *Aphyocharax* species, such as the red caudal-fin colouration, moderately elongated body, single series of tricuspid teeth on the premaxilla and mandible, and maxilla with teeth on up to two-thirds of its ventral margin (Taphorn and Thomerson 1991; Willink et al. 2003; Tagliacollo et al. 2012; Brito et al. 2018).

During recent fieldwork at the Maracaçumé river basin, eastern Amazon, specimens of an additional undescribed species of *Aphyocharax* were collected and is herein described, based on both morphological and molecular evidence, in accordance to an integrative taxonomy perspective.

Methods

Taxa sampling, specimens collection, and preservation

Individuals collected for this study were euthanized with a buffered solution of Tricaine methanesulfonate MS-222 at a concentration of 250 mg/L for a period of 10 min or more until opercular movements completely ceased. Specimens selected for morphological analysis were fixed in 10% formalin and left for 10 days, after which they were preserved in 70% ethanol and specimens selected for molecular analysis were fixed, and preserved in absolute ethanol.

Specimens for morphological analysis are listed in type and comparative material lists. Specimens for molecular analysis are listed in Table 1. We also retrieved sequences from other species of *Aphyocharax* and allied genera for a comparative analysis from the National Center for Biotechnology Information (NCBI) databases (Table 1).

Morphological analysis

Measurements and counts were made according to Fink and Weitzman (1974) and Brito et al. (2018), except for the count of scale rows below lateral line, which were counted to the insertion of pelvic-fin. Vertical scale rows between the dorsal-fin origin and lateral line do not include the scale of the median predorsal series situated just anterior to the first dorsal-fin ray. Counts of supraneurals, vertebrae, procurrent caudal-fin rays, unbranched dorsal and anal-fin rays, branchiostegal rays, gill-rakers, and teeth were taken only from cleared and stained paratypes (C&S), prepared according to Taylor and Van Dyke (1985). The four modified vertebrae that constitute the Weberian apparatus were not included in the vertebrae counts and the fused PU1 + U1 was considered as a single element. Osteological nomenclature follows Weitzman (1962). Institutional abbreviations are: **ANSP** Academy of Natural Sciences, Philadelphia, Pennsylvania, USA; **BMNH** Natural History Museum, London, UK; **CAS** California Academy of Sciences, San Francisco, California, USA; **CICCAA** Coleção Ictiológica do Centro de Ciências Agrárias Ambientais, Universidade Federal do Maranhão, Chapadinha, Brazil; **FMNH** Division of Fishes, Department of Zoology, Field Museum of Natural History, Chicago, Illinois, USA; **LBP** Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, São Paulo, Brazil; **MNRJ** Museu Nacional, Departamento de Vertebrados, Setor de Ictiología, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; **UFRJ** Coleção Ictiológica do Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; **UFRO** Universidade Federal de Rondônia, Porto Velho, Brazil.

DNA extraction, amplification, and sequencing

DNA extraction was carried out with the Wizard Genomic DNA Purification kit (Promega) following manufacturer's protocol. DNA quality was evaluated by 0.8% agarose gel electrophoresis stained with GelRed (Biotium). DNA was stored in −20 °C until further procedures. Samples (Table 1) were amplified using standard PCR (Polymerase Chain Reaction) for partial Cytochrome B gene (CytB), using primers developed by Ward et al. (2005) (CytB2F 5′ - GTG ACT TGA AAA ACC ACC GTT G-3′ and CytB2R 5′ - AAT AGG AAG TAT CAT TCG GGT TTG ATG-3′).

Amplification reactions were performed in a total volume of 15 µl comprising 1× buffer, 1.5 mM $MgCl_2$, 400 µM dNTP, 0.2 uM of each primer, 1 U of Taq Polymerase (Invitrogen), 100 ηg of DNA template, and ultrapure water. The amplification program consisted of a denaturation of 94 °C for 3 min, followed by 35 cycles

Table 1. List of species, specimens and their respective GenBank sequence accession numbers. Sequences made available by this study in bold.

Species	Catalog number	Genbank accession
Aphyocharacidium bolivianum	LBP9055-42219	HQ289710
Aphyocharax anisitsi	LBP 25524	JQ820081
Aphyocharax anisitsi	LBP3764-22190	HQ289581
Aphyocharax avary	CICCAA2344-1	MK409660
Aphyocharax avary	CICCAA2344-3	MK409661
***Aphyocharax brevicaudatus* sp. nov. (female)**	CICCAA02306	MK409668
***Aphyocharax brevicaudatus* sp. nov. (male)**	CICCAA02308	MK409669
***Aphyocharax brevicaudatus* sp. nov. (male)**	CICCAA02310	MK409670
Aphyocharax dentatus	LBP 26163	JQ820082
Aphyocharax dentatus	LBP 3604	JQ820083
Aphyocharax cf. *erythrurus*	LBP 15819	JQ820076
Aphyocharax cf. *erythrurus*	LBP 15820	JQ820077
Aphyocharax nattereri	LBP 22345	JQ820070
Aphyocharax nattereri	LBP 22132	JQ820071
Aphyocharax pusillus	LBP 23546	JQ820078
Aphyocharax pusillus	LBP4046-22920	HQ289590
Aphyocharax rathbuni	LBP 36496	JQ820079
Aphyocharax rathbuni	LBP 40434	JQ820080
Aphyocharax sp.	LBP1587-11774	HQ289533
Aphyocharax sp.	LBP 16349	JQ820084
Prionobrama paraguayensis	LBP 19465	JQ820073
Prionobrama paraguayensis	LBP 19468	JQ820072
Prionobrama filigera	LBP 23664	JQ820075
Prionobrama filigera	LBP 23663	JQ820074
Leptagoniates steindachneri	LBP 4137-23661	HQ289600
Paragoniates alburnus	LBP9208-43156	HQ289712
Phenagoniates macrolepis	LBP6105-35623	HQ289678
Xenagoniates bondi	LBP3074-19694	HQ289563

of 94 °C for 30 s, 46–48 °C for 45 s, and 72 °C for 80 s, and an extension phase of 5 min at 72 °C. Amplicons were visualised in 1% agarose gel electrophoresis stained with GelRed (Biotium) and purified with Illustra GFX PCR DNA and Gel Purification Kit (GE Healthcare). Samples were sequenced using both forward and reverse primers and BigDye Terminator 3.1 Cycle Sequencing kit in ABI 3730 DNA Analyser (Thermo Fisher Scientific).

Data partition, evolution models, and alignment

The dataset included the partial Cytochrome B (CytB) mitochondrial gene (754bp). Sequences were aligned using ClustalW (Chenna et al. 2003), and were translated into amino acids residues to test for the absence of premature stop codons or indels using the program MEGA 7 (Kumar et al. 2016). Substitution Saturation tests were performed in DAMBE5 (Xia 2013) according to the algorithm proposed by Xia et al. (2003). The best-fit evolutionary model (GTR+G) was selected using Akaike Information Criterion (AIC) by jModelTest 2.1.7 (Darriba et al. 2012).

Phylogenetic analysis

A Bayesian inference-based phylogenetic (BI) tree was estimated in MrBayes (Huelsenbeck and Ronquist 2001) plugin in Geneious 9.0.5 to reconstruct the evolutionary relationships among terminals using General Time Reversible (GTR+G) as evolutionary model; and following parameters: two Markov chain Monte Carlo (MCMC) runs of four chains each for 3 million generations and sampling frequency of 1,000. We used sequences of *Aphyocharacidium bolivianum* Géry, 1973, *Leptagoniates steindachneri* Boulenger, 1887, *Paragoniates alburnus* Steindachner, 1876, *Phenagoniates macrolepis* (Meek & Hildebrand, 1913), *Prionobrama filigera* (Cope, 1870), *Prionobrama paraguayensis* (Eigenmann, 1914), and *Xenagoniates bondi* Myers, 1942 as outgroups.

Species concept, species delimitation, and diagnoses

The unified species concept is herein adopted by expressing the conceptual definition shared by all traditional species concepts, “species are (segments of) separately evolving metapopulation lineages”, disentangling opera-

tional criterion elements to delimit taxa from species concepts (de Queiroz 2005, 2007). According to this concept, species are treated as hypothetical units and could be tested by the application of distinct criteria (species delimitation methods) (de Queiroz 2005, 2007). It allows for any criterion to separately provide evidence about species limits and identities, independently from other criteria (de Queiroz 2005, 2007). However, evidence corroborated from multiple operational criteria is considered to produce stronger support for hypotheses of lineage separation (de Queiroz 2007; Goldstein and Desalle 2010), a practice called "integrative taxonomy" (Dayrat 2005; Goldstein and Desalle 2010; Padial et al. 2010).

Two distinct and independent operational criteria for species delimitation, based on morphological and molecular data, were implemented here: the population aggregation analysis (Davis and Nixon 1992) (hereafter PAA); and a tree-based method as proposed by Wiens and Penkrot (2002) (hereafter WP, following Sites and Marshall 2003).

Population aggregation analysis (PAA)

The PAA (Davis and Nixon 1992) is a character-based method, in which species are delimited by unique combination of morphological character states occurring in one or more populations (Costa et al. 2014). The morphological data was based on both examined material and literature (e.g. Günther 1869; Cope 1870; Eigenmann and Kennedy 1903; Eigenmann and Ogle 1907; Fowler 1913; Eigenmann 1915; Fowler 1940; Géry 1977; Taphorn and Thomerson 1991; Britski et al. 1999; Souza-Lima 2003a, 2003b; Willink et al. 2003; Gonçalves et al. 2005; Tagliacollo et al. 2012; Brito et al. 2018).

Wiens and Penkrot analysis (WP)

The WP analysis was based on CytB haplotypes, supported on the direct inspection of the haplotype tree generated by the phylogenetic analysis having as terminals at least two individuals (haplotypes) of each focal species. In this method, the term 'exclusive' is used instead of monophyletic, as the term monophyly is considered inapplicable below the species level (Wiens and Penkrot 2002). Clustered haplotypes with concordant geographic distribution forming mutual and well supported clades (exclusive lineages) are considered strong evidence for species discrimination (absence of gene flow with other lineages). When haplotypes from the same locality fail to cluster together, there is potential evidence of gene flow with other populations (Wiens and Penkrot 2002). Statistical support for clades is assessed by the posterior probability, considered as significant values about 0.95 or higher (Alfaro and Holder 2006). When only one haplotype (specimen) from one putative population was available, the species delimitation was based on the exclusivity of the sister clade of this single haplotype, supported by significant values, allowing us to perform the test in populations with only one haplotype (Wiens and Penkrot 2002). In addition, the method allows recognition of non-exclusive lineages as species if their sister clade is exclusive and supported by significant values (Wiens and Penkrot 2002).

Results

***Aphyocharax brevicaudatus* sp. nov.**

http://zoobank.org/C5D86CB2-B51B-4B45-AFF7-6E483533B680

Figs 1, 2

Holotype. CICCAA 02293, (male) 35.9 mm SL, Brazil, Maranhão state, Maracaçumé municipality, Maracaçumé River, 2°3'14"S, 45°57'16"W; 29 Jun 2018, E.C. Guimarães and P.S. Brito.

Paratypes. All from Brazil, Maranhão state: CICCAA 02294, 1 (female), 32.4 mm SL, CICCAA 02295, 35 (males), 20.9–31.7 mm SL,CICCAA 02296, 94 (females), 21–32.1 mm SL, CICCAA 02297, 30 (females) C&S, 22.2–30.8 mm SL, CICCAA 02312, 2 (males) C&S, 28.3–32.1 mm SL, UFRJ 11746, 10 (female), 24.2–30.2 mm SL; all collected with holotype.

Diagnosis (PAA). *Aphyocharax brevicaudatus* sp. nov. differs from all its congeners by possessing the upper lobe of the caudal fin longer than the lower lobe in mature males (vs upper and lower lobes similar in length, see Figs 1, 2; Tagliacollo et al. 2012: fig.4). Additionally, the new species is distinguished from *Aphyocharax avary* and *A. pusillus* by having hyaline middle caudal-fin rays (vs black or dark brown middle caudal-fin rays, Brito et al. 2018: fig. 3); from *Aphyocharax colifax*, *A. yekwanae*, and *A. rathbuni* by having caudal-fin light red colouration never surpassing the vertical line of the adipose-fin (vs red colouration extending to the lateral midline of body, Willink et al. 2003: fig. 1); from *A. gracilis* by having a larger body depth at dorsal-fin origin (body depth), 24.5–29.2% SL (vs 20.1–20.6% SL); and from *A. pusillus* by having teeth along 2/3 of the maxillary extension (vs along proximal half of the bone, Brito et al. 2018: fig. 4).

Description. Morphometric data is presented in Table 2. Body shape is generally fusiform, slightly elongate, greatest body depth slightly anterior to dorsal-fin base; dorsal body profile straight or slightly convex from snout to vertical through anterior nostrils; straight or slightly convex from posterior nostrils to tip of supraoccipital bone; straight or slightly convex from this point to dorsal-fin origin; slightly convex along dorsal-fin base; postdorsal profile straight from base of last dorsal-fin ray to adipose-fin origin; slightly concave from adipose-fin to end of caudal peduncle; ventral profile convex from snout to pelvic-fin insertion; straight or slightly convex from this point to anal-fin origin; straight along anal-fin

Table 2. Morphometric data ($N = 141$) of the holotype and paratypes of *Aphyocharax brevicaudatus* sp. nov. from the Maracaçumé river basin. SD: Standard deviation.

	Holotype (Male)	Paratypes (Male) N = 35	Mean	SD	Paratypes (Female) N = 105	Mean	SD
Standard length (mm)	35.9	20.9–35.9	26.6	–	21.0–32.4	28.0	–
Percentages of standard length							
Depth at dorsal-fin origin (body depth)	25.4	24.5–28.7	25.9	1.0	25.6–29.1	26.3	0.8
Snout to dorsal-fin origin	53.1	51.9–55.6	52.6	1.1	51.8–54.5	52.1	0.7
Snout to pectoral-fin origin	23.2	23.0–27.7	23.9	0.9	22.6–25.2	23.5	0.6
Snout to pelvic-fin origin	46.3	45.1–49.4	45.2	0.6	44.2–47.1	44.6	0.9
Snout to anal-fin origin	67.4	63.9–68.6	64.4	0.9	64.0–68.5	64.2	0.7
Caudal peduncle depth	10.8	10.1–12.5	11.3	0.5	10.9–12.2	11.3	0.3
Caudal peduncle length	13.2	12.2–17.2	14.0	1.2	12.2–14.9	13.1	0.7
Pectoral-fin length	20.4	17.9–22.5	19.7	0.3	18.6–21.1	19.3	0.6
Pelvic-fin length	15.9	14.6–20.6	15.6	0.5	14.0–17.1	15.3	0.7
Dorsal-fin base length	11.6	9.5–13.4	11.3	0.5	10.8–13.0	11.8	0.5
Dorsal-fin height	23.1	21.2–24.8	22.4	0.5	20.8–24.0	22.3	0.7
Anal-fin base length	18.9	16.7–21.1	18.1	0.4	16.8–20.7	18.3	1.0
Eye to dorsal-fin origin	42.6	40.6–54.6	42.1	0.6	41.4–52.4	41.8	1.9
Dorsal-fin origin to caudal-fin base	47.6	46.5–49.5	46.5	0.7	46.4–49.4	46.5	0.7
Head length	24.0	22.3–26.6	24.0	1.7	22.3–24.9	23.1	0.6
Percentages of head length							
Horizontal eye diameter	30.2	28.7 –36.0	31.4	1.5	29.5–34.8	31.6	1.4
Snout length	24.2	19.7 –28.8	23.5	0.6	22.8–29.3	25.4	1.2
Least interorbital width	36.8	32.7 –38.9	34.1	0.1	32.9 –37.0	11.1	1.1
Upper jaw length	34.2	31.9 –37.3	33.4	0.2	32.7–39.9	33.9	1.4

Figure 1. *Aphyocharax brevicaudatus* sp. nov. **a.** CICCAA 02293, holotype (male), 35.9 mm SL; **b.** CICCAA 02294, paratype (female), 32.4 mm SL, Brazil: Maranhão state: Maracaçumé river basin. (Photographed by Erick Guimarães).

Figure 2. Caudal-fin of *Aphyocharax brevicaudatus*, holotype, CICCAA 02293, (male).

base; long snout, with its length larger than orbital diameter; five infraorbital bones; fourth infraorbital absent and sixth infraorbital reduced; posterior border of maxilla rounded, extending vertically through anterior margin of orbit, not reaching third infraorbital.

All teeth unicuspid or tricuspid and lateral cusps, when present, much smaller; premaxillary teeth in one rows with 6(9), 7(23) tricuspid teeth; maxilla with 11(3), 12(12), 13(14), or 14(3) unicuspid teeth; dentary with 6 (2) or 7 (30) larger tricuspid teeth followed by 6(26) or 7(6) smaller tricuspid teeth.

Scales cycloid and same size over entire body generally. Predorsal scales mostly regular, but sometimes irregular just posterior to supraoccipital and/or slightly anterior to dorsal-fin. Scales covering anterior third of caudal-fin, with up to two, three, or four scales beyond posterior margin of hypural plate. Lateral line interrupted; last scale on caudal-fin base, with 9+1(12),10+1(74), 11+1(50), or 12+1(5). Longitudinal scales series including lateral-line scales 35(3), 36(3), 37(56), 38(49), or 39(30). Longitudinal scales rows between dorsal-fin origin and lateral line 5(1), 6(93) or 7(47). Horizontal scale rows between lateral line and pelvic-fin origin 4 (141), Axillary scale present. Scales in median series between tip of supraoccipital spine and dorsal-fin origin 13+1(24),14+1(65), 15+1(26), or 16+1(26). Circumpeduncular scales 13(18), 14(115), or 15(8).

Dorsal-fin rays i+10(99) or ii+10(42). Dorsal-fin origin situated posterior to vertical through pelvic-fin insertion, near middle of body. First dorsal-fin pterygiophore main body located of 8th and 9th vertebrae. Adipose-fin present. Anal-fin i+14(20), iii+15(18), ii+16(61), iii+16(24), ii+17(10), iii+17(5), ii+18 (3). Anteriormost anal-fin pterygiophore inserting at 14th and 15th vertebrae. Anterior anal-fin margin slightly convex, with anteriormost rays more elongate and slightly more thickened than remaining rays, forming a distinct lobe. Remaining rays smaller with straight distal margin. Pectoral-fin rays i+9(8), i+10(113), or i+11(20). Tip of pectoral-fin not reaching pelvic-fin origin, when adpressed. Pelvic-fin rays i+7(120) or ii+7(21). Tip of pelvic-fin not reaching anal-fin origin, when

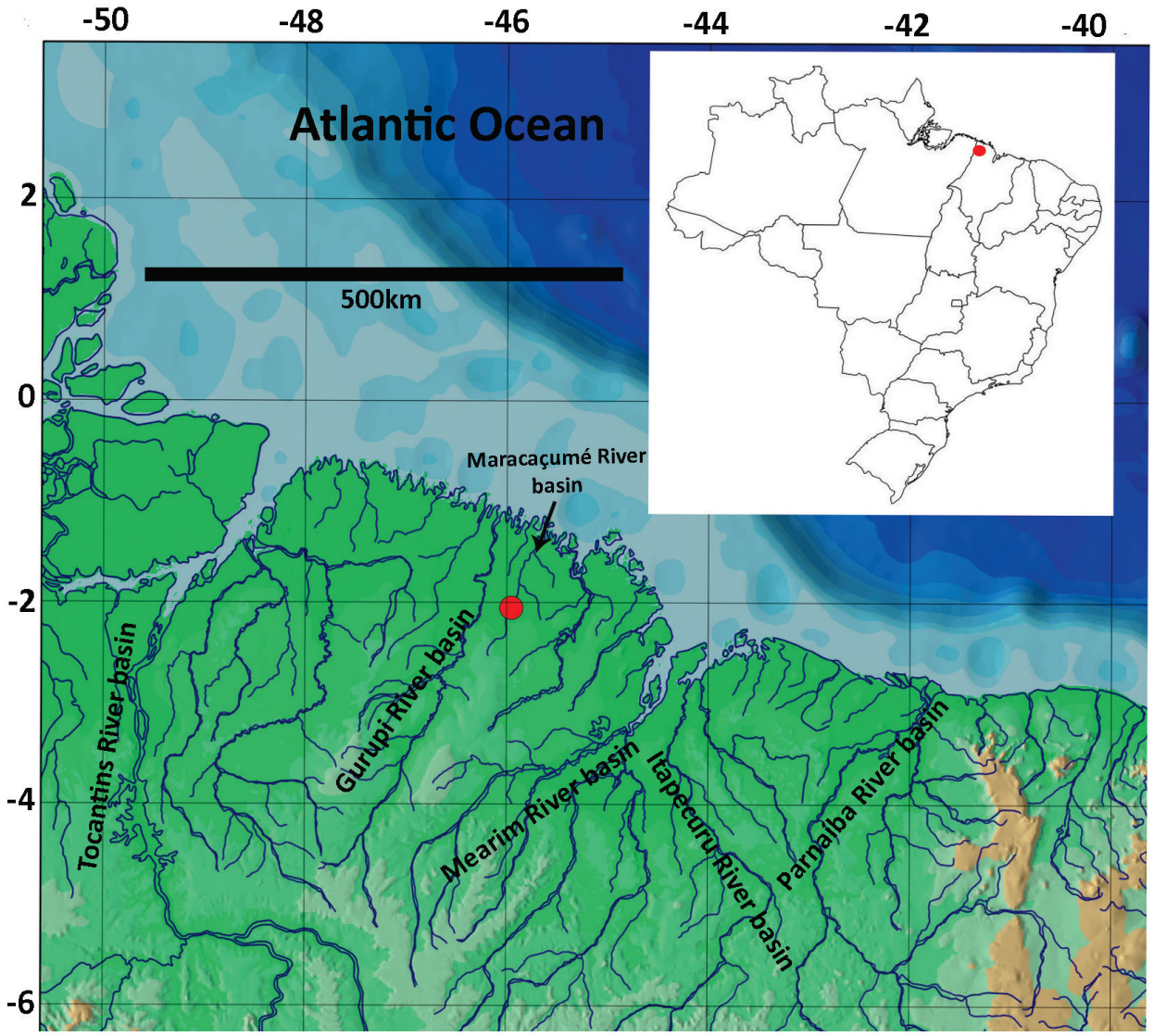

Figure 3. Type locality of *Aphyocharax brevicaudatus* sp. nov.

adpressed. Caudal-fin with a sexually dimorphic pattern, described below (Fig. 1). Principal caudal-fin rays 10+9(130) or 10+10(11); dorsal procurrent rays 8(2), 9(3) or 10(27) and ventral procurrent rays 7(2), 8(3) or 9(27).

Branchiostegal rays 4(32). Supraneurals 6(4) 7(27) or 8(1). Total vertebrae 31 (1), 32(30) or 33(1).

Colour in alcohol. Ground colouration light brown to yellowish brown. Inconspicuous light brown to light gray stripe from humeral spot to caudal-fin base, more conspicuous on posterior half. Humeral region with one conspicuous dark brown to black humeral spot. Smaller dark brown or black chromatophores homogeneously scattered. Smaller dark brown or black chromatophores homogeneously scattered along body, except on chest. Head ground colouration similar to trunk, with dark brown chromatophores present on jaws, tip of snout, opercle, and dorsal portion of head. Dorsal, adipose, anal, caudal, pectoral, and pelvic fins hyaline to light brown.

Sexual dimorphism. Caudal-fin of mature males with upper lobe longer (about 2/3 longer) than lower one, while both cauldal-fin lobes have similar leght in females (Fig. 1). Gill glands were found in all analyzed mature males of *Aphyocharax brevicaudatus* sp. nov. and were always absent in females. They were always located on anteriormost portion of lower branch of first gill arch, extending posteriorly through variable number of gill filaments.

Etymology. The name *brevicaudatus* is a contraction of the *Latin* words *brevis* meaning “short” and *cauda* meaning “tail”, an allusion to the shorter caudal-fin lower lobe in the mature males of the new species.

Geographic distribution. *Aphyocharax brevicaudatus* sp. nov. is currently known only from a single locality, the Maracaçumé river basin, a small and isolated coastal river basin of the eastern Amazon region (Fig. 3).

Discussion

Several authors supported *Aphyocharax* as a monophyletic genus within Aphyocharacinae (Mirande 2010; Oliveira et al. 2011, Tagliacollo et al. 2012, Betancur-R. et al. 2018, Mirande 2018) and also the sister-group relationship between *Aphyocharax* and *Prinobrama* (e.g. Oliveira et al. 2011; Tagliacollo et al. 2012; Betancur-R et al. 2018).

On the other hand, few studies focused on the intrageneric phylogenetic relationships within *Aphyocharax* (e.g Tagliacollo et al. 2012), and its diversity is probably underestimated, with at least four undescribed species (Souza-Lima 2007) and several populations or species waiting for a taxonomic revision (Lima et al. 2013; Ohara et al. 2017; Brito et al. 2018).

Aphyocharax brevicaudatus sp. nov. is described here based on two distinct criteria and assumptions (PAA and WP). As mentioned in the Diagnosis (PAA), *Aphyocharax brevicaudatus* sp. nov. is unique among its valid congeners possessing the upper lobe of the caudal fin longer than the lower lobe in mature males (Souza-Lima 2003b; this study). This feature is generally rare among species of Characidae (Mirande 2010).

In our Bayesian inference phylogenetic analysis (Fig. 4), haplotypes of *A. brevicaudatus* sp. nov. clustered as an exclusive lineage with high node support (maximum posterior probability value) (WP). The hypothesis of this new species is strengthened from an integrative taxonomy perspective, since it was based on evidence obtained from two independent criteria of species delimitation (see Dayrat 2005; de Queiroz 2007; Goldstein and Desalle 2010; Padial et al. 2010).

The closer relationship between *A. brevicaudatus* sp. nov. and *A. avary* is recovered with maximum posterior probability value. However the relationship between this clade (*A. brevicaudatus* sp. nov. and *A. avary*) and other congeners have low phylogentic resolution, and discussions related to the phylogenetic positioning of this clade would be speculative with the data at hand.

Comparative material

Aphyocharax avary: ANSP 39217, 1 (Holotype), Madeira River, about 200 miles east, Brazil. UFRO 018489, 3, Guajará municipality, Rondônia state, Brazil. UFRO016159, 62, Porto Velho municipality, Rondônia state, Brazil. UFRO 014317, 7, Novo Aripuanã municipality, Amazonas state, Brazil. MNRJ 10968, 11, Borba municipality, lago de Borba (Madeira River Basin), Amazonas state, Brazil. CICCAA 02394, 38, Sororó River, Marabá municipality, Pará state, Brazil. ***Aphyocharax anisitsi***: CICCAA 00867, 14, Pontes Lacerda municipality, Mato Grosso state, Brazil. CICCAA 01267, 6 C&S, Pontes Lacerda municipality, Mato Grosso state, Brazil. CAS 59697, 1, Asuncion municipality (radiograph and photograph of holotype), Par-

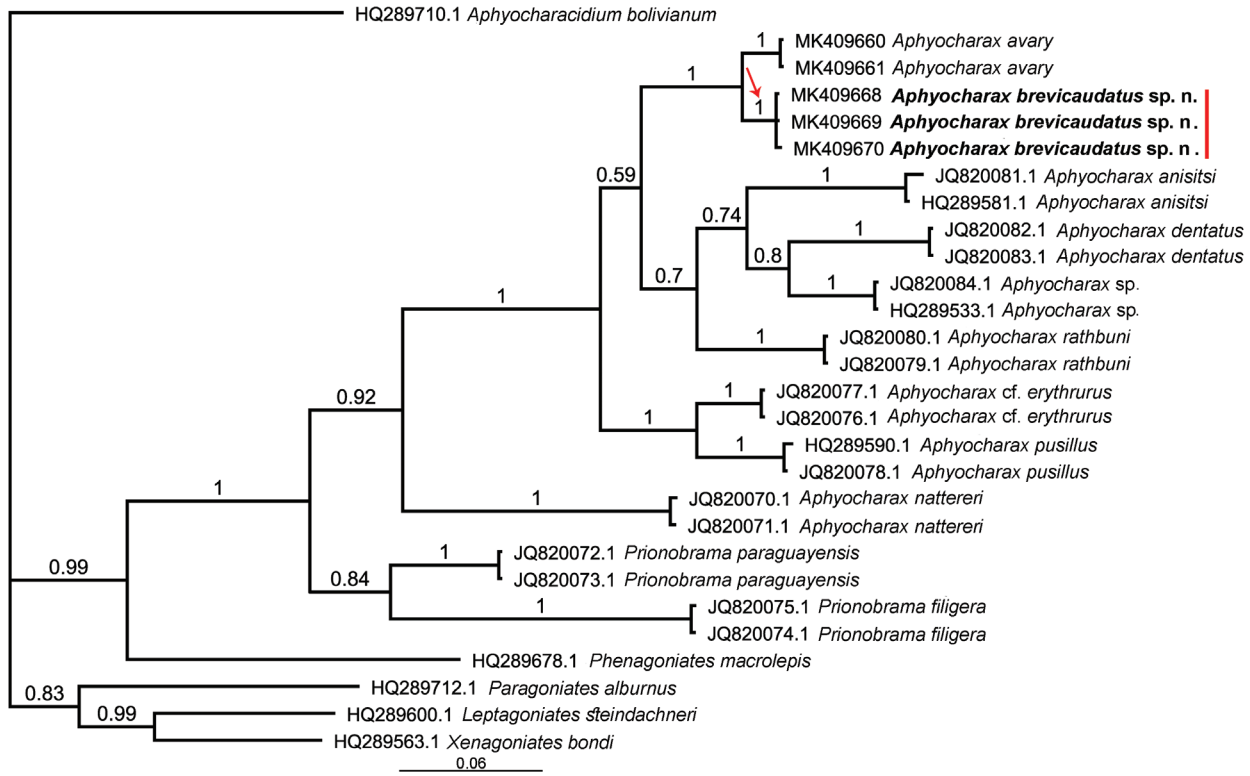

Figure 4. Bayesian inference tree including *Aphyocharax brevicaudatus* sp. nov. (red bar) and other congeners. Red arrow indicates the posterior probability of *A. brevicaudatus* node. Number above branches are posterior probability values.

aguay. ***Aphyocharax dentatus***: ANSP 128718, 21, Lake Mozambique, Colombia. UFRJ 5571, 2, Rio Verde municipality, Mato Grosso do Sul state, Brazil. CAS 59722, 1, Laguna del Río Paraguay (radiograph and photograph of holotype), Asuncion municipality, Paraguay. ***Aphyocharax erythrurus*:** FMNH 53406, 1, Rockstone sandbank (photograph of paratype), Guyana. ***Aphyocharax nattereri***: UFRJ 5783, 2, Poconé municipality, Mato Grosso state, Brazil. ***Aphyocharax pusillus*:** ANSP 178013, 4 (photographs of recently preserved specimens), Rio Napo (Amazon river basin), right bank just upstream from mouth of Mazan River, near town of Mazan, Loreto, Peru. BMNH 1867.6.13.46, 1 (syntype), Amazon river basin, Huallaga and Xeberos, Peru. BMNH 1867.6.13.58-59, 2 (syntypes), Amazon river basin, Huallaga and Xeberos, Peru. BMNH 1869.5.21.10, 1 (lectotype of *Chirodon alburnus*), Amazon River, Peru. BMNH 1869.5.21.11-13, 3 (paralectotypes of *Chirodon alburnus*), Amazon River, Peru. ***Aphyocharax rathbuni***: CAS 76467, 1 (Radiograph and photograph of a Holotype), Paraguay basin, Arroyo Chagalalina, Paraguay. ***Aphyocharax yekwanae*:** FMNH 109278, 1 (radiograph of paratype), Bolivarian Republic, Venezuela. ***Aphyocharax* sp.**: CICCAA 00865, 11, Pontes e Lacerda municipality, Mato Grosso state, Brazil. CICCAA 00865, 4 C&S, Pontes e Lacerda municipality, Mato Grosso state, Brazil.

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