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Cyanocharax obi, a new species (Characiformes: Characidae) and the first record of the genus from tributaries of the río Paraná basin, Argentina

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Abstract

Cyanocharax obi is described from tributaries of the arroyo Paranay–Guazú, río Paraná basin, Misiones province, Argentina. This new species can be distinguished from its congeneres by the following combination of characters: lateral line interrupted or with alternated series of perforated and non perforated scales, 22–24 branched anal-fin rays, body depth at dorsal-fin origin (34.5–40.8% SL), and distal border of anal fin in matures males nearly straight. Validity of the new species is also well supported by a molecular phylogenetic analysis (COI, 12S, 16S). *Cyanocharax obi* represents the first record of the genus in the río Paraná basin.

Key words: freshwater fishes, taxonomy, Misiones province, arroyo Paranay–Guazú

Resumen

Cyanocharax obi es descripta de tributarios del arroyo Paranay–Guazú, cuenca del río Paraná en la provincia de Misiones, Argentina. Esta nueva especie puede ser distinguida de las otras especies del género por la siguiente combinación de caracteres: línea lateral interrumpida o con alternancia de series de escamas perforadas y no perforadas, 22–24 radios anales ramificados, altura del cuerpo en el origen de la aleta dorsal (34.5–40.8 % LE), y borde distal de la aleta anal algo recto en machos maduros. La nueva especie se encuentra también bien fundamentada por un análisis filogenético molecular (COI, 12S, 16S). *Cyanocharax obi* representa el primer registro del género para la cuenca del río Paraná.

Introduction

The genus *Cyanocharax* Malabarba & Weitzman, 2003 is known from the río Uruguay basin in Argentina and Brazil and from the Atlantic coastal drainages in Southern Brazil. *Cyanocharax* includes seven nominal species (Malabarba & Weitzman, 2003); *Cyanocharax alburnus*, *C. dicropotamicus*, *C. itaimbe*, *C. tipiaia*, and one undescribed species (C.S. Pavanelli & A.G. Bifi, unplublished) inhabit Atlantic coastal drainages, whereas the remaining species, *C. alegretensis*, *C. lepiclastus*, and *C. uruguayensis* are distributed in the río Uruguay basin.

Cyanocharax belongs to the characid Clade A, characterized by the presence of ii,8 dorsal-fin rays, and four teeth in the inner row of premaxilla (Malabarba & Weitzman, 2003). In the same paper, *Cyanocharax* was diagnosed by the presence of a small maxillary dentigerous surface consisting of 2–8 teeth occupying less than the anterior half of the bone. Incomplete dentition is a character also present in several other members of clade A. Therefore, it can not be assumed as a synapomorphy for *Cyanocharax* until the relationships among the members of the clade A are better understood (Malabarba & Weitzman, 2003). Recently, Javonillo *et al*. (2010) based on molecular sequence data show the Clade A with *Cyanocharax alburnus* clustering inside the genus *Diapoma*. Thus the monophyly of the genus *Cyanocharax* is still uncertain at this time.

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The province of Misiones is one of the regions with the highest diversity in Argentina (Bertonatti & Corcuera, 2000). The Uruguay, Paraná, and Iguazú Rivers form the main boundaries of this province and with their different sets of species offer an ideal setting for the study of faunal evolution in the larger context of the La Plata basin (Piálek *et al*., 2012). Recent collecting trips allowed us to find several new species endemic to this region (Casciotta *et al*., 2010; Piálek *et al*., 2010; Říčan *et al*., 2011).

The aim of this paper is to describe a new species of *Cyanocharax* from the río Paraná basin which represent the first record of that genus for this basin.

Material and methods

Morphological methods. Measurements and counts were taken as described by Malabarba & Weitzman (2003). Measurements were taken as straight line distances with digital caliper to 0.1 mm. Specimens were cleared and counterstained (C&S) following Taylor & Van Dyke (1985). Vertebral counts were taken from C&S specimens and include the four vertebrae of the Weberian apparatus and the last half centrum.

In the description the number of specimens is indicated in parentheses, values of the holotype are indicated by an asterisk. Body length is expressed as standard length (SL). Institutional abbreviations are as listed in Ferraris (2007).

Molecular methods. We used three mitochondrial genes (COI, 12S, 16S) to recover the phylogenetic position of the newly described species, all widely used in phylogenetic studies of the characids. Our molecular phylogenetic analysis includes sequences of 13 terminals (excluding outgroup) representing six species of *Cyanocharax* and two of *Diapoma*. Eighteen sequences of 6 specimens are newly sequenced and the remaining were obtained from GenBank (http://www.ncbi.nlm.nih.gov/genbank). Several successive outgroups based on the study of Javonillo *et al*. (2010) were used to root our phylogeny: *Bryconamericus*, *Hypobrycon*, *Knodus*, and *Odontostoechus* (Table 1).

Genomic DNA was extracted from ethanol-preserved fin tissue using a JETQUICK Tissue DNA Spin Kit (Genomed) following the standard protocol. The analyzed loci were amplified using PCR with the following primers: LCO1490-5' (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2198-3' (TAA ACT TCA GGG TGA CCA AAA AAT CA) for COI, 12s53F-5' (CAC AAA GGC TTG GTC CTG ACT TT) and 12s991R-3' (GGT ACA CTT ACC ATG TTA CGA CT) for 12S, 16SAR-5' (CGC CTG TTT ATC AAA AAC AT) and 16SBR-3' (CCG GTC TGA ACT CAG ATC ACG T) for 16S. Each PCR reaction volume of 25 µl contained 12.5 µl of Combi PPP Master Mix (Top-Bio, http://www.top-bio.cz), 1.0 µl of each primer (10 pmol/µl), and 1 µl of DNA extract. Reaction mixtures were subjected to the following cycling protocol: 3 min. 94 °C, 35 x (15 s 94 °C, 30 s 40/60/49 °C [COI/12S/16S], 60 s 72 °C), 10 min. 72 °C. PCR reactions were performed in a Bioer XP Thermal Cycler and PCR products were purified using the JETQUICK PCR Purification Spin Kit (Genomed). Sequencing reactions were performed following the standard protocol with the use of the same primers, and the products were analyzed in an ABI 3730XL automated sequencer (Applied Biosystems; both steps done by Macrogen Inc., Korea).

Contiguous sequences of the gene segments were created by assembling DNA strands (forward and reverse) using BioLign 4.0.6.2 (Hall, 2001) and aligned using MUSCLE ver. 3.8 (Edgar, 2004) with default settings. Separate alignments of individual loci were assembled together into a final phylogenetic matrix by a computer program created in Borland Delphi (Borland Delphi for Microsoft Windows, version 10, 2005. Borland Software Corporation), written by the third author. All sequences were submitted to GenBank under Accession Nos. JN712183 to JN712200 (Table 1).

Phylogenetic analyses. *Morphological data set*. The morphological matrix inludes 25 characters, of which 18 are multistate and 17 are ordered (all except character 5; see Appendices 1 and 2 for details). Morphological data for *Cyanocharax alburnus* were taken from Malabarba's (1983) redescription of *Astyanax hasemani* (actually a junior synonym of *C. alburnus*), from the respective descriptions for the remaining species (Malabarba & Weitzman, 2003), and from examination of our comparative material.

Most of the characters are quantitative characters and these have been coded using the gap weighting method (GW) of Thiele (1993). Thiele's implementation of gap weighting involves finding (for a given character) the mean value of the trait in each species in the analysis, the range of mean species values among taxa (*i.e.* the species with the greatest mean value and the species with the lowest), and then dividing this range into smaller ranges or segments equal to the maximum number of character states allowed by the phylogenetic software program (*e.g.* 32 for PAUP*; Swofford 2003). We have used a less fine grained spacing, thus having in most cases less than 32 states.

Species are then assigned states based on these ranges, and the character is ordered. Evolving from low to high mean trait values (or vice versa) therefore requires passing through many intermediate states and requires many steps, whereas smaller changes in trait values involve fewer state changes and fewer steps. An important advantage of the gap-weighting method is that it incorporates information on the distance between states, weighting the changes according to the difference between mean species values.

We have used the between-state scaling (Wiens, 2001) to weight quantitative characters against qualitative characters. The proportional measurement characters (characters 12–25) were thus down-weighted five times compared to binary characters, because their frequency bins were spaced at one percent and we have arbitrarily taken a 5% change to correspond to one character state among binary characters. Meristic characters (characters 6–11) have been divided into a similar number of character states as proportional meristics and for the sake of parsimony they are down-weighted by the same factor. The consistency index is reported with uninformative characters excluded.

Phylogenetic analyses were performed using PAUP* 4b.10 (Swofford, 2003) with maximum parsimony (MP). Analyses included 500 random sequence additions, 10 trees kept per addition, and a heuristic search on the saved trees to find all the shortest trees. Bootstrap analyses were done using the same approach, with 5 random sequence additions per one bootstrap. Bootstrap analyses were run with 1000 replications.

Since the sister group of *Cyanocharax* is not well established (Javonillo *et al*., 2010), we have used the out-group character states from Malabarba & Weitzman (2003). Character evolution has been studied in Mesquite (Maddison & Maddison, 2004).

Molecular data set. For construction of the molecular phylogenetic tree, Bayesian analysis (BA) of the combined dataset was performed utilizing the software MrBayes 3.1.2 (Huelsenback & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Akaike criterion was used to select a model in MrModelTest 2.2 (Nylander, 2004), a simplified version of ModelTest 3.06 (Posada & Crandall, 1998) for use with MrBayes, and PAUP* ver. 4.0b10 (Swofford, 2003). BA using a Markov chain Monte Carlo simulation was run for 1 million generations, with trees sampled and saved every 100 generations (10000 trees saved per run). Two simultaneous analyses, each with four chains, were performed. The first trees from each run before reaching equilibrium were discarded as burn-in; convergence between the two runs was estimated using diagnostics criteria produced by the ‘sump’ command in MrBayes. The remaining trees were used for reconstruction of a 50% majority-rule consensus tree with the posterior probability (PP) values of the relevant branches displayed by the 'sumt' command.

Results

Phylogenetic analyses

The phylogenetic analysis of the morphological matrix of 25 characters (Appendices 1 and 2) resulted into one MP tree (L=170; CI=0.75; RI=0.70) (Fig. 1). The validity of the new species is well supported by the morphological phylogeny, sharing several diagnostic characters with other *Cyanocharax* species, and being nested well within the genus as the sister species of *C. tipiaia*.

The phylogenetic analysis of the molecular matrix of three genes (COI, 12S, 16S) representing 20 taxa and 2035 characters (COI, 658; 12S, 803; 16S, 574) of which 184 were parsimony-informative (COI, 118; 12S, 36; 16S, 30) is shown in Fig. 2. Translation of the coding COI sequences into amino acids displayed no stop codons or frame shifts. The inferred nucleotide-substitutions models were GTR+I+G (COI, 16S) and HKY+I (12S). Testing the influence of the burn-in value on the consensus BA tree revealed absolute stability both of the tree topology and of the PP values within the whole investigated range (burn-in 500 to 7000).

The results are similar to those from the morphological analysis (Fig. 1), with the topology and node support of the recovered BA tree (Fig. 2) supporting the new species as a unique evolutionary lineage within a polytomy including *Cyanocharax* as well as *Diapoma*.

The trio of species *C. alegretensis*, *C. lepiclastus*, and *C. uruguayensis* are in both morphological and molecular phylogenies recovered as a monophyletic and strongly supported clade. The species *C. dicropotamicus* and *C. tipiaia* were not available for molecular study which makes comparisons between the molecular and morphological phylogenies difficult.

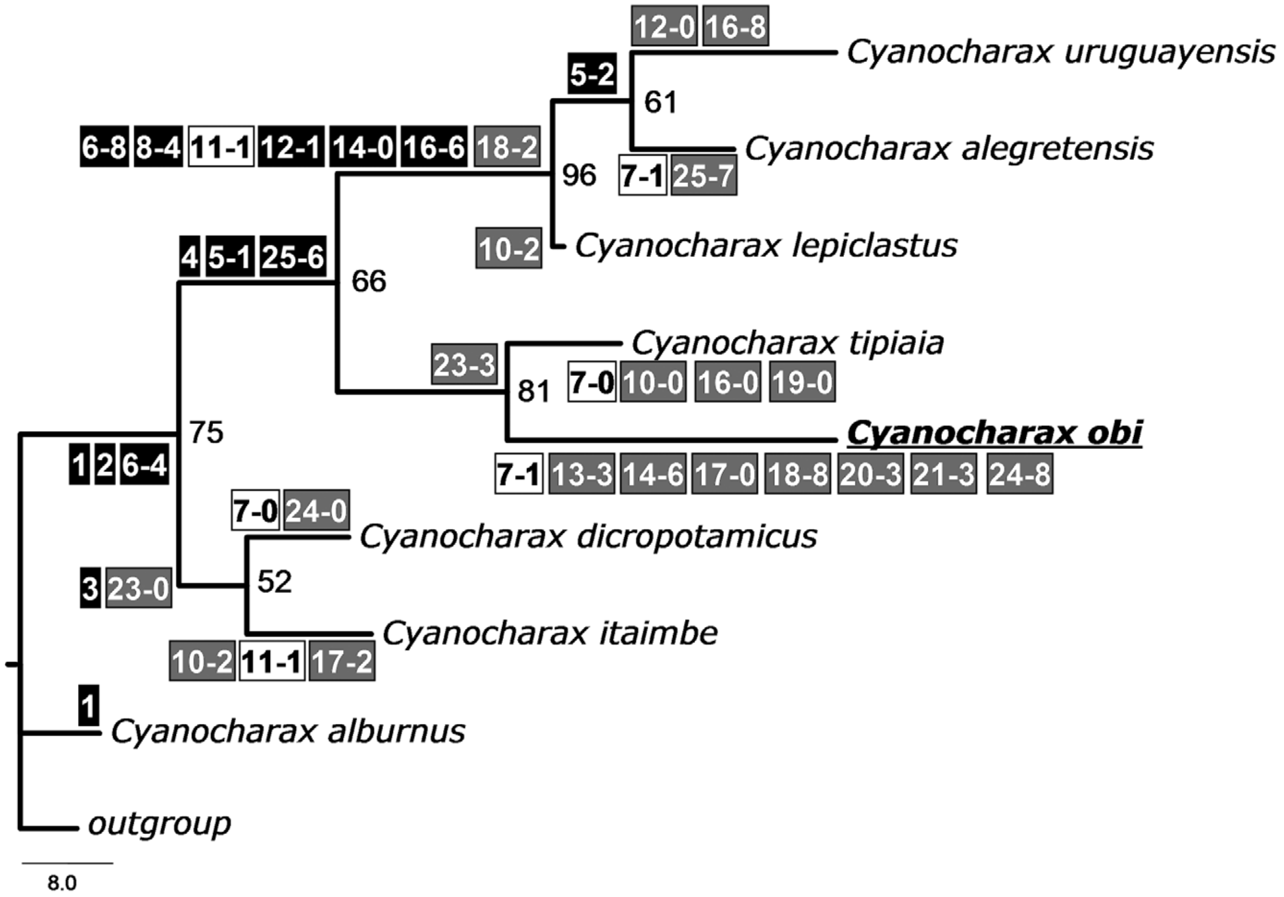

FIGURE 1. Phylogenetic tree obtained from the MP analysis of the 25 morphological characters. Numbers for each recovered node represent bootstrap supports. Character evolution has been studied in Mesquite (Maddison and Maddison 2004) using unambiguous optimization: white numbers in black boxes: unique synapomorphies; white numbers in grey boxes: unique autapomorphies or synapomorphies within complex transformations; black numbers in white boxes: convergent autapomorphies. For multistate characters the character state is shown after the hyphen.

Cyanocharax obi sp. nov.
(Figs. 3–8, Tab. 2)

Holotype. MACN-ict 9560, 53.3 mm; Argentina: Misiones: río Paraná basin, tributary of the arroyo Paranay–Guazú, 26°52’28.9” S, 54°42’22.8” W, col: Casciotta *et al*., December 2010 (Fig. 3).

Paratypes. All from Argentina: Misiones: río Paraná basin:

MACN-ict 9557, 9 ex., 41.0–52.7 mm; same data as holotype. AI 284, 2 ex., 52.2–57.4 mm, tributary of the arroyo Paranay-Guazú in trail from road 14 to Monte Carlo city, 26°49'03.5" S, 54°26'58.9" W, col: Říčan *et al*., December 2007. MACN-ict 9558, 3 ex., 38.1–46.6 mm, tributary of the arroyo Paranay–Guazú, road 11 between Aristóbulo del Valle and El Alcazar, 26°48'27.1" S, 54°45'37.7" W, col: Říčan *et al*., December 2007. MACN-ict 9559, 3 ex., 48.4–56.7 mm, tributary of the arroyo Paranay–Guazú, road 11 between Aristóbulo del Valle and El Alcazar, 26°48'27.1" S, 54°45'37.7" W, col: Casciotta *et al*., December 2010. AI 282, 1 ex. (C&S), 58.7 mm, tributary of the arroyo Paranay–Guazú, road 11 between Aristóbulo del Valle and El Alcazar, 26°48'27.1" S, 54°45'37.7" W, col: Říčan *et a*l., December 2007.

Diagnosis. *Cyanocharax obi* can be distinguished by the following combination of characters: lateral line interrupted or with alternated series of perforated and non perforated scales, 22–24 (mode=24) branched anal-fin rays, body depth at dorsal-fin origin (34.5–40.8, mean=37.9 % SL), and distal border of anal fin in matures males nearly straight.

Cyanocharax obi differs from *C. alburnus*, *C. itaimbe*, and *C. dicropotamicus* in having the lateral line interrupted or with alternated series of perforated and non perforated scales vs. usually complete. Also, *Cyanocharax obi* differs from *C. alburnus* by the number of sheath scales on anal-fin base (7–14 scales vs. 6–8), body depth at dorsal-fin origin (2.5–2.9 vs. 3.1–4.7 in SL), anal-fin base length (2.9–3.4 vs. 3.5–3.8 in SL), and interorbital width (2.7–2.9 vs. 2.9–3.7 in HL); from *C. itaimbe* by the number of scales on longitudinal series (34–38 vs. 37–40), and horizontal eye diameter (35.9–42.1 vs. 43.0–50.0% SL); from *C. dicropotamicus* by the body depth at dorsal-fin origin (34.5–40.8 vs. 26.6–33.1% SL), snout length (24.0–27.3 vs. 19.5–23.5% HL), and upper jaw length (42.0–46.9 vs. 33.3–41.9% HL); from *C. tipiaia* by the number of maxillary teeth (3–6 vs. 2–3), pelvic-fin length (14.5–18.1 vs. 13.1–14.5% SL), snout to pelvic-fin origin (44.4–50.6 vs. 42.3–45.3% SL), and body depth at dorsal-fin origin (34.5–40.8 vs. 28.5–31.3% of SL); from *C. lepiclastus* by a sheath of scales on anal-fin base consisting of row with 7–14 scales vs. 13–20, number of branched anal-fin rays (22–24 vs. 24–29), snout to anal-fin origin (59.4–65.3 vs. 52.1–58.8% SL), and snout to pelvic-fin origin (44.4–50.6 vs. 38.6–44.6% SL); from *C. alegretensis* by the number of branched anal-fin rays (22–24 vs. 23–30), snout to anal-fin origin (59.4–65.3 vs. 53.4–58.2% SL), and snout to pelvic-fin origin (44.4–50.6 vs. 38.9–43.5% SL); and from *C. uruguayensis* by the number of branched anal-fin rays (22–24 vs. 28–35) and distal margin of anal-fin in mature males nearly straight vs. convex.

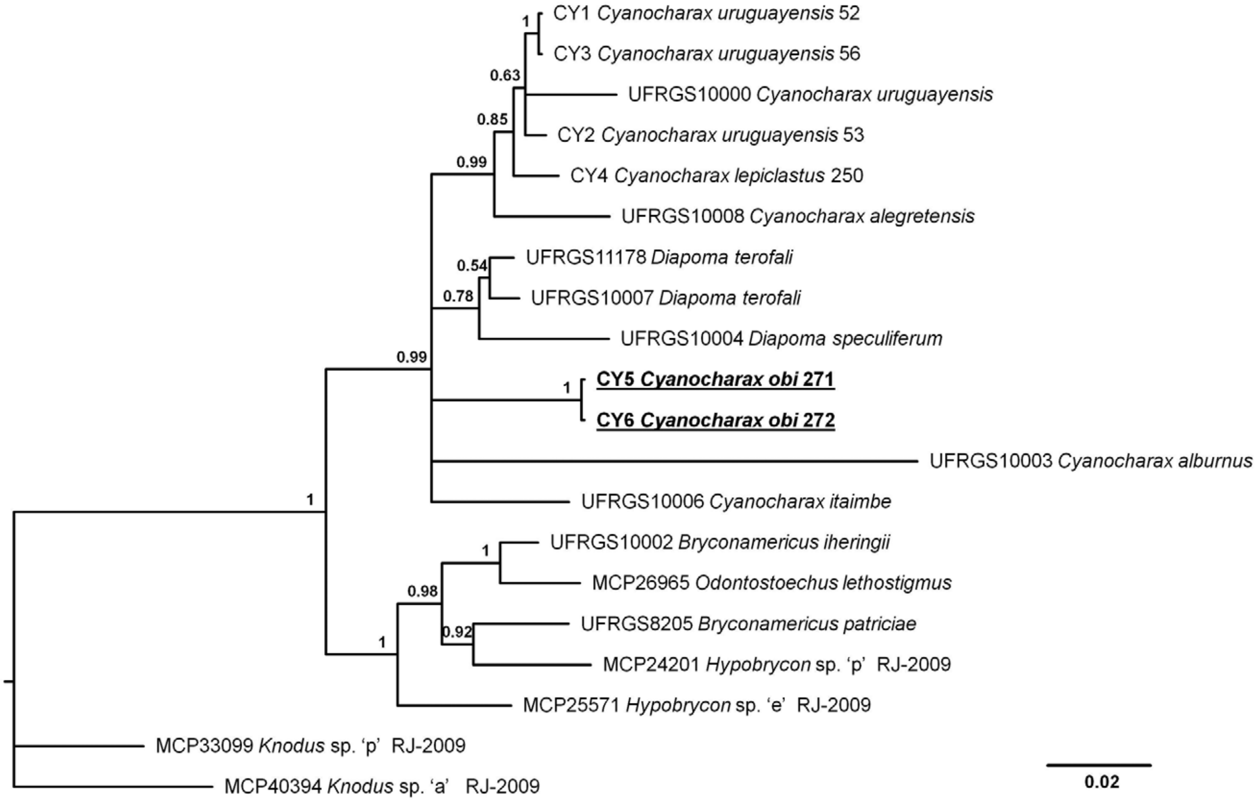

FIGURE 2. Phylogenetic tree obtained from the BA analysis (burnin, 1000) of the combined molecular dataset (COI, 12S, 16S). Numbers for each recovered node represent Bayesian posterior probability.

Description. Morphometric data of the holotype and paratypes are given in Table 2. Body moderately elongate and compressed. Predorsal body profile convex, usually with a concavity at nape. Dorsal body profile nearly straight from dorsal fin to caudal peduncle. Ventral body profile convex from snout tip to anal-fin origin. Nearly straight in females and slightly convex in mature males along anal-fin base. Ventral and dorsal borders of caudal peduncle slightly concave.

Head small, posterior tip of maxilla surpassing the anterior margin of orbit.

Two series of premaxillary teeth. Teeth of outer series tricuspid, smaller than those of inner series (Fig. 4). Four teeth with 3 to 5 cusps in inner series (19* ex.). Three to six maxillary teeth with 1 to 4 cusps, four tricuspid teeth in the holotype (Fig. 4). Dentary with three large anterior teeth followed by one medium-size teeth and 8 smaller ones, ranging from tricuspid to unicuspid, from anteriormost to posteriormost teeth (Fig. 5). Anterior larger dentary teeth tri- or pentacuspid. Second dentary tooth shorter and inserted at lower position in jaws (1 ex., C&S), tip of its longest cusp reaches only as high as tip of second largest cusp of first and third dentary teeth.

TABLE 2. Morphometrics data of the holotype and 18 paratypes of *Cyanocharax obi.* Standard length is expressed in mm. SD: standard deviation.

	Holotype	Range	Mean	SD
Standard length	53.3	38.1–58.7		
Percents of SL				
Snout to anal-fin origin	63.2	59.4–65.3	62.8	1.60
Snout to dorsal-fin origin	57.6	54.1–59.1	56.8	1.56
Snout to pelvic-fin origin	49.4	44.4–50.6	47.9	1.52
Dorsal-fin base length	11.7	10.2–12.6	11.7	0.65
Anal-fin base length	30.8	29.2–34.1	31.6	1.36
Caudal peduncle length	12.7	10.0–12.9	11.9	0.82
Caudal peduncle depth	11.1	9.7–11.7	11.0	0.53
Depth at dorsal-fin origin	40.2	34.5–40.8	37.9	2.23
Dorsal-fin height	23.4	20.5–23.9	22.5	1.12
Pelvic-fin length	17.2	14.5–18.1	16.8	0.97
Pectoral-fin length	23.3	21.3–24.5	23.3	0.90
Bony head length	22.9	21.3–24.3	22.7	0.70
Percents of head length				
Snout length	25.8	24.0–27.3	25.7	0.91
Upper jaw length	43.4	42.0–46.9	45.1	1.42
Horizontal eye diameter	38.5	35.9–42.1	39.2	1.77
Least interorbital width	36.7	34.3–37.5	35.9	1.04

FIGURE 3. *Cyanocharax obi,* male, holotype, MACN-ict 9560, 53.3 mm; Argentina: Misiones: río Paraná basin, tributary of the arroyo Paranay-Guazú, 26°52’ 28.9”S, 54°42’22.8” W.

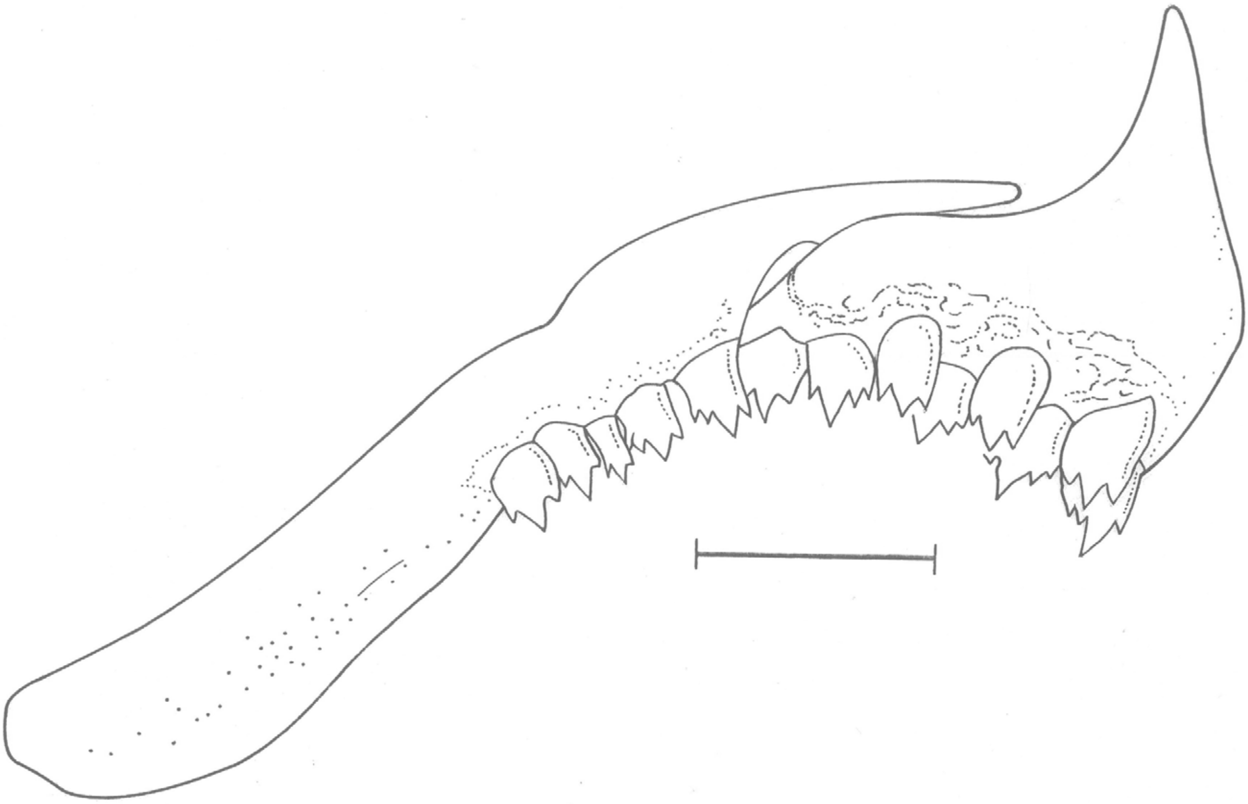

FIGURE 4. *Cyanocharax obi*, AI 282, paratype, 58.7 mm, lateral view of right upper jaw. Scale bar: 1 mm.

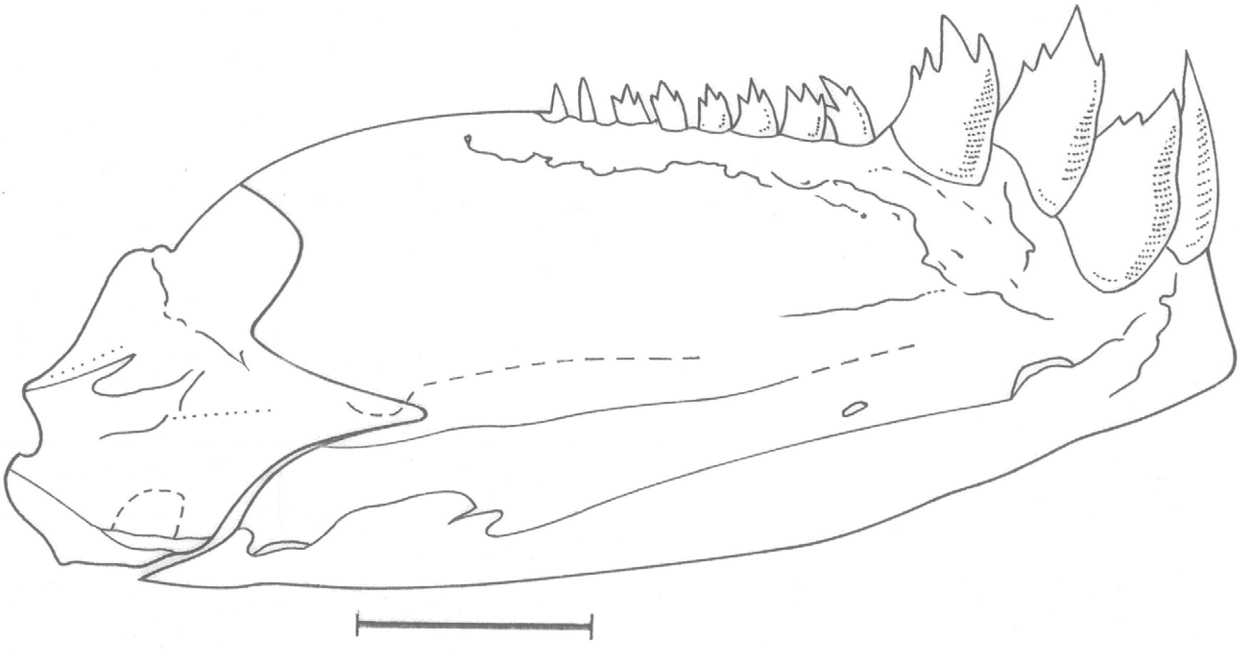

FIGURE 5. *Cyanocharax obi*, AI 282, paratype, 58.7 mm, lateral view of right lower jaw. Scale bar: 1 mm.

Anal-fin rays iv–v,22–24 (mode=iv,24*, n=19). Anal-fin origin posterior to vertical through dorsal-fin origin at middle of body length. Anal-fin distal border concave in females and juveniles, nearly straight in mature males. Anal-fin rays of males with small retrorse bony hooks present on 2 to 19 branched rays. Hooks mostly present on posterior branches of rays and posterior border of lepidotrichia. One or two pairs of bony hooks per ray segment.

Pectoral-fin rays i,10–12 (mode: i,11*, n=19). Distal ends of longest rays may or may not reach pelvic-fin insertion in both males and females. Pelvic-fin rays i,6. Pelvic-fin origin anterior to dorsal-fin origin. Distal ends of longest rays not reaching anal-fin origin. Pelvic fins with ventromedial, usually unpaired retrorse bony hooks on third to sixth branched rays in sexually mature males. Principal caudal-fin rays 10/9. Scales cycloid. Lateral line never complete, with anterior series of 8 to 12* pored scales (mode=11, n=19), followed by a median non-perforated scales series, and a posterior perforated series of 1 to 3 scales (in 7 ex.). Total number of scales in longitudinal series 34–38 (mode=36*, n=19). Scale rows between dorsal-fin origin and lateral line 5–6* (mode=6, n=19). Scale rows between lateral line and pelvic-fin origin 4. Predorsal scales 13–17 (mode=14*, n=19). Males and females with sheath of scales on anal-fin base consisting of row with 7–14 scales (mode=11*, n=19), covering bases of unbranched rays and first 12 to 14 branched rays. Vertebrae 37 (1 ex., C&S).

Color in alcohol. Body pale brownish. Lateral body stripe broad and dark posteriorly, becoming pale anterior to dorsal-fin origin. Dark humeral spot vertically elongated. Scales of dorsal portion of body with scattered dark chromatophores. Fins translucent, with scattered black chromatophores on distal third of anal fin, middle caudal-fin rays, dorsal and ventral caudal-fin lobes, along branched pelvic-fin rays, along unbranched and branched pectoral-fin rays, and on the distal two thirds of dorsal-fin rays. Tip of all unbranched and 1st to 3rd branched anal-fin rays unpigmented.

Adipose fin darkly pigmented with an unpigmented area in the middle. Head gray dorsally, especially dark near nape. Sides of head and opercle pale brown.

Color in life. Body between silvery and sky-blue. Black humeral spot elongated vertically. A wide silvery lateral band more evident at the caudal peduncle. Scattered black chromatophores on all fins without additional obvious markings. Base of caudal-fin lobes orange (Fig. 6).

Sexual dimorphism. Males of *C. obi* differ from females in the nearly straight or slightly convex distal border of the anal fin (vs. concave in females), first branched pelvic-fin produced in a short filament (4* ex., n=10 males), hooks on anal and pelvic-fin rays.

FIGURE 6. *Cyanocharax obi*, live specimen, female, paratype MACN-ict 9559, 56.7 mm.

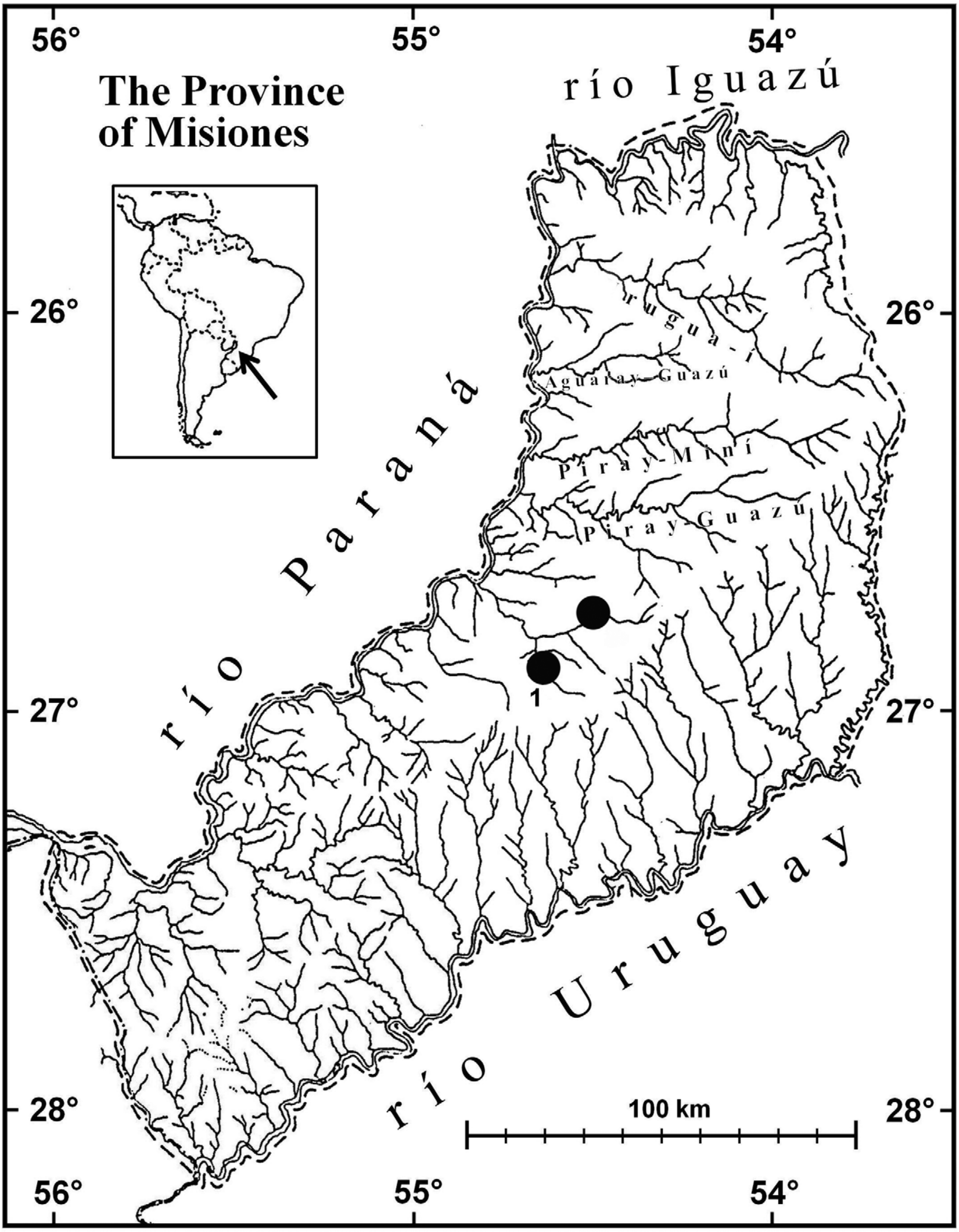

FIGURE 7. Hydrological map of the Misiones Province. The type locality is marked with number 1.

FIGURE 8. Tributary of the arroyo Paranay-Guazú on a side trail from national road 14 to the town of Monte Carlo, 26°49'03.5" S, 54°26'58.9" W.

Distribution. *Cyanocharax obi* is only known from tributaries of the arroyo Paranay–Guazú, río Paraná basin, Misiones Province, Argentina (Fig. 7).

Ecological notes. The localities where *Cyanocharax obi* was collected had a turbid and rapidly flowing water after heavy rain. The depth of the arroyo Paranay–Guazú is variable, 0.40 to 2 m. The bottom consists of mud, sand, and stones. Some areas have scarce submerged vegetation (Fig. 8).

Etymology. The specific epithet *obi* is a Guaraní word that means blue in allusion to the ground color of the body.

Discussion

According to Malabarba & Weitzman (2003), the genus *Cyanocharax* could not be diagnosed based on unique derived features. However, they noted that the relationships among the species are supported by derived characters. Taking into account the cladogram depicted by Malabarba & Weitzman (2003, Fig. 2) and our morphological phylogeny (Fig. 1), *Cyanocharax obi* is the sister species of *C. tipiaia*, with which it shares several derived features: presence of an intense blue colour in sexually mature specimens (character 2), lateral line interrupted or alternating series of perforated and non-perforated scales (character 4), and 7 to 14 scales covering the basal portion of the anal-fin rays (character 6 [state1]), but no synapomorphies among the 25 scored characters are unique to this species pair (Fig. 1). Additionally, since monophyly of the genus *Cyanocharax* is not conclusive due to postulated relationship of *Cyanocharax* with *Diapoma* in the molecular phylogeny (Fig. 2) the definitive relationship of *C. obi* is still pendant.

The high diversity of ichthyofauna and high endemism in the Argentinean province of Misiones has already been noted (Casciotta *et al*., 2010; Piálek *et al*., 2010; Říčan *et al*., 2011). Several new taxa have recently been described from the río Paraná, Iguazú and Uruguay tributaries in that province (*Crenicicha hu* Piálek *et al*., 2010, *Crenicichla ypo* Casciotta *et al*., 2010, Paraná basin; *Astyanax ita*, Almirón *et al*., 2002, *Bryconamericus ikaa* Casciotta *et al*., 2004, *Australoheros kaaygua* Casciotta *et al*., 2006, Iguazú basin; and *Astyanax saguazu* Casciotta *et al*., 2003, *Australoheros ykeregua* Říčan *et al*. 2011, Uruguay basin). Several additional new species are known to us and still undescribed (Piálek *et al*., 2012).

Despite recent increased collecting effort in the province of Misiones *Cyanocharax obi* was only found in the arroyo Paranay–Guazú, a tributary of the the río Paraná in Misiones province and represents the first occurence of this genus in the río Paraná basin. The arroyo Paranay–Guazú is located in the center of Misiones province and as it was mentioned, the central and northern tributaries of the río Paraná in that province have a great number of endemic fishes (Říčan *et al*., 2011; Piálek *et al*., 2012).

The genus *Cyanocharax* was until now restricted to the río Uruguay basin and Atlantic coastal drainages of Southern Brazil. At present the best hypothesis for a sister species of *C. obi* is with *C. tipiaia* (Fig. 1) from the Atlantic coastal Rio Jacuí (Laguna dos Patos drainage). If the monophyly of *Cyanocharax* is upheld then a former connection between the río Uruguay basin and the Atlantic coastal drainages with the río Paraná basin before the evolution of *C. obi* is assumed. Today, there is a connection between the río Paraná and río Uruguay through the Río de la Plata, far south of the type locality of *C. obi*. *Cyanocharax* species have never been registered in the Río de la Plata and this connection is probably out of the question for the dispersal of fishes like *C. obi*, which probably require higher temperatures than those present at the Río de la Plata latitude. Therefore, fish dispersal followed by speciation could have occurred through head water river captures and drainage translocations in northern tributaries of the Paraná and Uruguay Rivers, and the Atlantic coast drainages (Říčan *et al*., 2011). The postulated drainage translocation (op. cit.) could also have involved the arroyo Paranay–Guazú, type locality of *Cyanocharax obi*, where the headwaters of this drainage are less than 5 km away from those of the Uruguay tributaries. Extinctions could however have been a significant factor that has contributed to the present distribution patterns (Albert & Carvalho, 2011).

Comparative material

Argentina: Misiones province: río Uruguay basin: *Cyanocharax lepiclastus*: MACN-ict 9682, 47 ex., 29.3–42.0 mm, arroyo Fortaleza, 26º45.5' S, 54º10' W. AI 103, 5 (paratypes), 35.1–43.9 mm, arroyo Fortaleza, 26º45.5' S, 54º10' W. AI 286, 13 ex. (C&S), 33.0–44.5 mm, arroyo Fortaleza, 26º45.5' S, 54º10' W. *Cyanocharax uruguayensis*: MACN-ict 9681, 7 ex., 32.0–33.6 mm, arroyo Itacaruaré, 27º52'33.8" S, 55º16'35.1" W. Brazil: *Cyanocharax alburnus*: MCP 25957, 10 ex., 29.8–39.3 mm, arroio Seival, 30º44'26" S, 53º42'35" W. MCP 12028, 1 ex. (C&S) 60.4 mm, Río Canoas, 27º35'11" S, 51º24'09" W. *Cyanocharax alegretensis*: MCP 27517, 1 ex., 37.4 mm, arroio Taquari, 29º23'47" S, 55º08'52" W. MCP 11232, 1 paratype (C&S), 42.0 mm, afluente del río Ibirapuitã, 30º11'00" S, 55º39'00" W. *Cyanocharax dicropotamicus*: MCP19516, 10 ex., 30.9–43.2 mm, Río Taquari, 29º28'00" S, 51º57'00" W. MCP 19510, 1 paratype (C&S), 36.7 mm, Río Taquari, 29º28'00" S, 51º57'00" W. *Cyanocharax itaimbe*: MCP 14290, 10 ex., 39.4–44.6 mm, Río Três Forquilhas, 29º25'00" S, 50º10'00" W. MCP 14263, 1 ex. (C&S), 44.3 mm, Río Três Forquilhas, 29º25'00" S, 50º10'00" W. *Cyanocharax tipiaia*: MCP 22766, 10 paratypes, 19.0–35.0 mm, arroio Tapiáia, 29º06'49" S, 53º39'04" W. MCP 22712, 1 paratype (C&S), 35.5 mm, arroio Felicio, 29º19'06" S, 53º37'59" W.

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APPENDIX 1. Character list.

1. Maxilla with an incomplete dentition of 2-8 teeth (Malabarba and Weitzman, 2003, character 1): **(0)** absent; **(1)** present.
2. Intense blue color in mature specimens (Malabarba and Weitzman, 2003, character 2): **(0)** absent; **(1)** present.
3. Black pigmented adipose fin (Malabarba and Weitzman, 2003, character 3): **(0)** absent; **(1)** present.
4. Interrupted lateral line (Malabarba and Weitzman, 2003, character 4): **(0)** absent; **(1)** present.
5. Shape of anal fin (Malabarba and Weitzman, 2003, character 5): **(0)** anal fin with a concave distal border; **(1)** nearly straight distal anal-fin margin; **(2)** deeply convex margin.

Counts

6. Number of scales in scale sheath of anal fin (Malabarba and Weitzman, 2003, character 6, more detailed coding here): **(0)** 6–7 ; **(1)** 7–8 ; **(2)** 8–9; **(3)** 9–10; **(4)** 10–11; **(5)** 11–12; **(6)** 12–13; **(7)** 13–14; **(8)** 14–15; **(9)** 15–16; **(A)** 16–17; **(B)** 17–18; **(C)** 18–19; **(D)** 19–20; **(E)** 20–21; **(F)** 21–22; **(G)** 22–23; **(H)** 23–24; **(J)** 24–25.
7. Anal fin rays unbranched: **(0)** 2–3 ; **(1)** 3–4 ; **(2)** 4–5.
8. Anal fin rays branched (Malabarba and Weitzman, 2003, character 7): **(0)** 22–23 ; **(1)** 23–24 ; **(2)** 24–25; **(3)** 25–26; **(4)** 26–27; **(5)** 27–28; **(6)** 28–29; **(7)** 29–30; **(8)** 30–31.
9. Total number of scales in lateral-line row: **(0)** 35–36 ; **(1)** 36–37 ; **(2)** 37–38; **(3)** 38–39.
10. Scale rows between dorsal fin origin and lateral line: **(0)** 4–5 ; **(1)** 5–6 ; **(2)** 6–7.
11. Scale rows between lateral line and pelvic fin origin: **(0)** 3–4 ; **(1)** 4–5 .

Percents of standard length (%SL)

12. Snout to anal-fin origin: **(0)** 54–55; **(1)** 55–56; **(2)** 56–57; **(3)** 57–58; **(4)** 58–59; **(5)** 59–60; **(6)** 60–61; **(7)** 61–62; **(8)** 62–63.
13. Snout to dorsal-fin origin: **(0)** 53–54; **(1)** 54–55; **(2)** 55–56; **(3)** 56–57.
14. Snout to pelvic-fin origin: **(0)** 41–42; **(1)** 42–43; **(2)** 43–44; **(3)** 44–45; **(4)** 45–46; **(5)** 46–47; **(6)** 47–48.
15. Dorsal-fin base length: **(0)** 10–11; **(1)** 11–12.
16. Anal-fin base length: **(0)** 29–30; **(1)** 30–31; **(2)** 31–32; **(3)** 32–33; **(4)** 33–34; **(5)** 34–35; **(6)** 35–36; **(7)** 36–37; **(8)** 37–38.
17. Caudal peduncle length: **(0)** 11–12; **(1)** 12–13; **(2)** 13–14.
18. Depth at dorsal-fin origin: **(0)** 29–30; **(1)** 30–31; **(2)** 31–32; **(3)** 32–33; **(4)** 33–34; **(5)** 34–35; **(6)** 35–36; **(7)** 36–37; **(8)** 37–38.
19. Dorsal-fin height: **(0)** 21–22; **(1)** 22–23; **(2)** 23–24; **(3)** 24–25; **(4)** 25–26.
20. Pelvic-fin length: **(0)** 13–14; **(1)** 14–15; **(2)** 15–16; **(3)** 16–17.
21. Pectoral-fin length: **(0)** 20–21 ; **(1)** 21–22 ; **(2)** 22–23; **(3)** 23–24.
22. Bony head length: **(0)** 22–23 ; **(1)** 23–24.

Percents of head length (%HL)

23. Snout length: **(0)** 21–22 ; **(1)** 22–23 ; **(2)** 23–24; **(3)** 24–25; **(4)** 25–26.
24. Upper jaw length: **(0)** 37–38 ; **(1)** 38–39 ; **(2)** 39–40; **(3)** 40–41; **(4)** 41–42; **(5)** 42–43; **(6)** 43–44; **(7)** 44–45; **(8)** 45–46.
25. Least interorbital width: **(0)** 29–30; **(1)** 30–31; **(2)** 31–32; **(3)** 32–33; **(4)** 33–34 ; **(5)** 34–35 ; **(6)** 35–36; **(7)** 36–37.

APPENDIX 2. Character matrix.

	0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2
	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5
outgroup	0 0 0 0 0 ?
Cyanocharax obi	1 1 0 1 1 4 1 1 0 1 0 8 3 6 1 2 0 8 1 3 3 0 4 8 6
C. uruguayensis	1 1 0 1 2 J 2 8 2 1 1 0 1 0 0 8 1 4 4 0 1 1 1 1 6
C. alegretensis	1 1 0 1 2 8 1 4 1 1 1 1 0 0 0 6 0 3 4 1 2 0 1 2 7
C. lepiclastus	1 1 0 1 1 A 2 4 2 2 1 1 0 0 0 6 1 2 3 0 1 0 1 1 6
C. tipiaia	1 1 0 1 1 2 0 0 1 0 0 5 1 3 ? 0 1 0 0 0 1 0 3 6 6
C. dicropotamicus	1 1 1 0 0 4 0 2 2 1 0 4 1 2 0 2 1 0 2 1 1 1 0 0 5
C. itaimbe	1 1 1 0 0 3 2 1 3 2 1 3 0 1 1 4 2 0 3 1 0 0 0 2 4
C. alburnus	1 0 0 0 0 0 2 0 2 1 0 ? ? ? ? ? ? ? 4 2 2 1 1 5 0