

Phylogenetic Evidence for the *Cyphocharax saladensis* Clade with Description of a New Species of *Cyphocharax* Endemic to the Upper Rio Paraguai Basin (Teleostei: Curimatidae)

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New genetic and morphological data support the recognition of a distinct monophyletic group, the *Cyphocharax saladensis* clade, which includes *C. vanderi*, *C. saladensis*, *C. boiadeiro*, and a new species. This four-species group can be recognized by modifications in the laterosensory system with instances of incomplete or interrupted poring, by the ontogenetic development of the lateral line with larger specimens having a more developed laterosensory system, and by the presence of a patch of dark pigmentation over the caudal peduncle. The new species is described from the upper Rio Correntes, an upland tributary of the upper Rio Paraguai in central Brazil and is diagnosed by the presence of a thin longitudinal black stripe, by the variable shapes of the dark blotches on the caudal peduncle, and by morphometric and meristic data. New mitochondrial data from paratypes provide clear evidence of genetic distinction between the new species and congeners, and additionally place it as the sister species to *C. boiadeiro* from the upper Rio Araguaia. Based on the updated molecular phylogeny and biogeographic information, we propose an evolutionary hypothesis with four events of river captures with subsequent allopatric speciation of the new species and *C. boiadeiro* in the Correntes and Araguaia systems. The new species is suggested to be categorized as Near Threatened, living in a strongly impacted region of the Brazilian Cerrado.

Novos dados morfológicos e genéticos suportam o reconhecimento de um distinto grupo monofilético, o clado *Cyphocharax saladensis*, que inclui *C. vanderi*, *C. saladensis*, *C. boiadeiro* e uma nova espécie. Esse grupo de quatro espécies pode ser reconhecido pelas modificações no sistema látero-sensorial com séries incompletas ou interrompidas de poros, pelo desenvolvimento ontogenético da linha lateral com espécimes maiores tendo um sistema látero-sensorial mais desenvolvido, e pela presença de uma mácula escura no pedúnculo caudal. A nova espécie é descrita do alto Rio Correntes, um tributário do planalto no alto Rio Paraguai no Brasil central e é diagnosticada pela presença de uma faixa longitudinal fina e escura, pelos formatos variáveis das manchas escuras do pedúnculo caudal e por dados morfométricos e merísticos. Novos dados mitocondriais de parátipos mostram claras evidências de distinção genética entre a nova espécie e os congêneres, e adicionalmente aloca-a como irmã de *C. boiadeiro* do alto Rio Araguaia. Baseado na filogenia molecular atualizada e informações biogeográficas, nós propomos uma hipótese evolutiva com quatro eventos de capturas de rios com subsequente especiação alopátrica da nova espécie e *C. boiadeiro* nos sistemas dos rios Correntes e Araguaia. A nova espécie é sugerida como categoria Quase Ameaçada vivendo numa região fortemente impactada do Cerrado brasileiro.

CYPHOCHARAX contains 44 species, making it the most species-rich genus of the Neotropical family Curimatidae. The genus is generally characterized by the absence of the morphological synapomorphies defining the other three curimatid genera: *Curimatella*, *Pseudocurimata*, and *Steindachnerina* (Vari, 1989, 1992). *Cyphocharax* possesses a bifurcate laterosensory canal in the sixth infraorbital, absence of elaborate processes in the buccopharyngeal complex, and absence of a dark spot in the proximal portion of the middle unbranched dorsal-fin rays (Vari, 1989, 1992). Species of the genus occur in all major river basins of South America, Panama, and Costa Rica, with a concentration in the Amazon, Orinoco, Guianas, and river basins of the Brazilian Shield such as the Paraná-Paraguai system, São Francisco, and Atlantic coastal rivers (Vari, 1988, 1992, 2003; Sidlauskas and Vari, 2012; Frable, 2017). Because curimatids are detritivores (i.e., feed on the detritus that accumulates on the river bottom), species of *Cyphocharax* play an important

ecological role in carbon and nutrient cycling across the Neotropics (Araujo-Lima et al., 1986).

The understanding of the phylogenetic relationships and the species richness of *Cyphocharax* has improved over the last three decades (Vari, 1992; Melo and Vari, 2014; Dillman et al., 2016; Dutra et al., 2016; Bortolo et al., 2018; Melo et al., 2018; Bortolo and Lima, 2020). The genus has been hypothesized to be non-monophyletic based on phylogenies using extensive examination of anatomical characters (Vari, 1989; Dillman et al., 2016) and by a molecular matrix of mitochondrial and nuclear loci (Melo et al., 2018). The morphological phylogeny resulted in a polytomy within the family with *Cyphocharax*, *Curimatella*, *Pseudocurimata*, and *Steindachnerina*, and it did not result in a monophyletic *Cyphocharax* (Vari, 1989). Subgroups of the genus were tentatively proposed based on body coloration and the degree of development of the laterosensory system (Vari, 1992), but the molecular phylogeny suggests that these groups are polyphyletic (Melo et al., 2018). For example, the

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presence of a patch of dark pigmentation on the lateral surface of the caudal peduncle was proposed to support a putative group of 19 species of *Cyphocharax* (Vari, 1992: 123), but the molecular phylogeny clearly shows that the character state is plesiomorphic, being present in at least one species of every curimatid genus and independently lost in some clades/species along the phylogeny, as for example in the species-rich *Curimatella alburna* clade (Melo et al., 2018).

The molecular phylogenetic study provided support for the recognition of the *Cyphocharax sensu lato* clade (CSLC) that incorporates the monophyletic *Steindachnerina* and the polyphyletic *Curimatella* interspersed within the paraphyletic *Cyphocharax* (Melo et al., 2018). With the exception of three Amazonian species at the base of the CSLC (*C. abramoides*, *C. multilineatus*, and *C. nigripinnis*), four major subclades exist in the paraphyletic genus *Cyphocharax*: the *C. gilbert*, the *C. magdalenae*, the *C. spilurus*, and the *Curimatella alburna* clades (Melo et al., 2018). Within the *Cyphocharax spilurus* clade, a subclade contains two species that occur in the Rio Paraná-Paraguai system, *C. saladensis* and *C. vanderi*, and one species that occurs in the separate Rio Araguaia, *C. boiadeiro* (Melo et al., 2018). However, there is no available biogeographic or evolutionary hypothesis explaining the disjunct distribution of species in this clade.

During expeditions to the upper Rio Correntes in the upper Rio Paraguai basin, we discovered a species of *Cyphocharax* sharing some morphological patterns with the subclade within the *C. spilurus* clade: modifications in the laterosensory system with instances of interrupted poring and the modifications in the body and caudal-peduncle pigmentation pattern. A deeper morphological analysis of these specimens revealed diagnostic features related to the development of the lateral-line system and the caudal peduncle color pattern, distinguishing it not only from *C. boiadeiro* but from all congeners. From this evidence, we formally describe a species from the uplands of the Rio Paraguai basin within the Cerrado biome, central Brazil, the largest and most species-rich savanna on Earth (Eiten, 1972; Ribeiro and Walter, 1998). These morphological data were also integrated with newly generated mitochondrial sequences to assess its phylogenetic placement, which revealed the need for an additional clade of *Cyphocharax*.

MATERIALS AND METHODS

Morphological and distribution data.—Counts and measurements follow Vari (1992), Melo and Vari (2014), and Melo (2017) and were taken on the left side of specimens. Measurements are presented as proportions of standard length (SL), except for subunits of the head, which are given as proportions of head length (HL). Frequency of each count is provided in parentheses after the respective count; asterisks indicate holotype values. Counts of vertebrae, unbranched-fin rays, and procurrent rays were taken from radiographed (rd) specimens using x-ray equipment Faxitron LX60 DC12 at LIRP, Ribeirão Preto, Brazil. Fused PU1+U1 was considered a single bone, and the vertebrae associated with the Weberian apparatus were counted as four elements. Museum abbreviations follow Sabaj (2020).

Distribution data for the species of the *Cyphocharax saladensis* clade were obtained from museum databases (FishNet2, SpeciesLink, LBP fish collection) with suspicious records (i.e., inaccurate coordinates, records outside the

known distribution) removed accordingly. The conservation status of the new species was suggested using the categories and criteria of the International Union for Conservation of Nature guidelines (IUCN Standards and Petitions Subcommittee, 2019). The Extent of Occurrence was estimated through the software GeoCAT (Geospatial Conservation Assessment Tool; <http://geocat.kew.org>).

Molecular data and phylogenetic analyses.—Twenty-nine species of *Cyphocharax* used in the molecular phylogeny of the Curimatidae (Melo et al., 2018) were included in this reanalysis. These include eight species of the *C. gilbert* clade, two species of the *C. magdalenae* clade, 11 species of the *Curimatella alburna* clade, and eight species of the *C. spilurus* clade. To assess the phylogenetic placement of the new species, we sequenced partial fragments of the *cytochrome oxidase c subunit I (COI)* for two specimens of the new species and incorporated them in the original matrix (Melo et al., 2018). This new matrix included a total of six genes: *16S*, *Cytb*, *COI*, *Myh6*, *Rag1*, and *Rag2* sequenced for 151 specimens plus 65 additional barcode sequences (Melo et al., 2018). Voucher information, locality, and GenBank numbers for specimens of the *C. saladensis* clade are available in Supplementary Table 1 (see Data Accessibility).

For the two newly sequenced individuals, DNA was extracted from muscle tissues preserved in 95% ethanol with a DNeasy Tissue kit (Qiagen Inc.) according to the manufacturer's instructions. Partial sequences of *COI* were obtained by polymerase chain reaction (PCR) using the primers L6252-Asn and H7271-COXI (Melo et al., 2011). Total volume included 12.5 μ l with 9.075 μ l of double-distilled water, 1.25 μ l 5X buffer, 0.375 μ l $MgCl_2$ (50 mM), 0.25 μ l dNTP mix, 0.25 μ l of each primer at 10 μ M, 0.05 μ l PHT DNA polymerase enzyme (Phoneutria, Belo Horizonte, Brazil), and 1.0 μ l genomic DNA (10–50 ng). The PCR program consisted of an initial denaturation (4 min at 95°C) followed by 30 cycles of chain denaturation (30 s at 95°C), primer hybridization (30–60 s at 52°C), and nucleotide extension (45 s at 72°C). Amplicons were visualized through 1% agarose gel and used to perform the sequencing reaction using dye terminators (BigDye™ Terminator v 3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems), and purified through ethanol precipitation. Samples were loaded onto an automatic sequencer ABI 3130-Genetic Analyzer (Applied Biosystems) at the Instituto de Biotecnologia at the Universidade Estadual Paulista, Botucatu, Brazil.

Consensus sequences were assembled and edited in Geneious v7.1.9 (Kearse et al., 2012) and aligned with MUSCLE algorithm (Edgar, 2004) under default parameters. We assembled specimens in two matrices. The first represented the multilocus matrix (Melo et al., 2018), including six genes and was used to access the phylogenetic placement of the new species within the Curimatidae and within the CSLC (following Talavera et al., 2022). The second represents a subsample of the first matrix, including only the *COI* gene for members of the *Cyphocharax saladensis* clade and this matrix was used to calculate genetic distances. The index of substitution saturation in asymmetrical (Iss.cAsym) and symmetrical (Iss.cSym) topologies were estimated using DAMBE v5.3.38 (Xia, 2013), and the best-fitting model of nucleotide evolution for each matrix and genetic distances were obtained in MEGA v7.0 (Tamura et al., 2013). A maximum likelihood (ML) tree using GTRGAMMA was



Fig. 1. *Cyphocharax caboclo*, MNRJ 52506, holotype, 59.1 mm SL, Brazil, Mato Grosso, Itiquira, Rio Correntes, upper Rio Paraguai basin.

obtained in RAxML v8.2.10 (Stamatakis, 2014). RAxML executed five inferences on the original dataset using five distinct randomized maximum-parsimony trees and 1,000 nonparametric bootstraps pseudo-replicates using the autoMRE function that estimated 200 pseudo-replicates (Patengale et al., 2009).

RESULTS

Morphological and genetic differentiation.—The recognition of this entity as a true species follows the unified species concept proposed by de Queiroz (2007) as a separately evolving metapopulation lineage. It contains a combination of elements from three major species concepts: the interbreeding between individuals of a population (biological species concept: Wright, 1940; Mayr, 1942), the unique evolutionary role, tendencies, and historical fate (evolutionary species concept: Simpson, 1951; Wiley, 1978; Mayden, 1997), and the monophyly and phenotypic diagnosability (phylogenetic species concept: Rosen, 1979; Nelson and Platnick, 1981; Cracraft, 1983).

This study incorporates a phenotypic investigation of the internal and external morphology in combination of new and available genetic data for the family. The morphological comparisons between the newly discovered species and other species of the *Cyphocharax saladensis* clade (*C. boiadeiro*, *C. saladensis*, and *C. vanderi*) revealed important differences in body coloration, degree of development of the laterosensory system, and morphometry. The new species (Rio Paraguai) is distinguished from *C. boiadeiro* (Rio Araguaia), the most similar species, by the possession of an interrupted lateral line instead of incomplete and ontogenetically lengthened lateral line of *C. boiadeiro*, as well as by the morphology of the dark pigmentation blotches of the caudal peduncle (see Diagnosis and Color in alcohol). Genetic distances between the new species and congeners concord with morphology and are 3.5% for *C. boiadeiro*, 4.1% for *C. saladensis*, and 4.0% for *C. vanderi* (see Molecular results and phylogenetic relationships). Taken together, these data provide combined evidence that the upper Rio Paraguai population represents a novel species, which we describe formally below.

***Cyphocharax caboclo* Melo, Tencatt, and Oliveira, new species**

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Figure 1, Table 1

Holotype.—MNRJ 52506, 59.1 mm SL, rd, Brazil, Mato Grosso, Itiquira, Ribeirão Comprido, tributary of Rio Correntes, upper Rio Paraguai basin, 17°32'05"S, 54°25'36"W, L. F. C. Tencatt, M. N. Souza, V. Carvalho G., and F. R. Moura, 5 April 2019.

Paratypes.—All from Brazil, upper Rio Paraguai basin, Rio Correntes basin. Mato Grosso: LBP 28496, 23, 44.0–65.0 mm SL (tissues 96870–96872), collected with the holotype; LBP 30315, 14, 21.2–65.0 mm SL, Itiquira, Ribeirão Comprido, 17°32'05"S, 54°25'36"W, L. F. C. Tencatt and M. N. Souza, 27 January and 19 February 2021; MNRJ 52507, 1, 70.8 mm SL, Itiquira, Ribeirão Comprido, 17°31'41"S, 54°25'30"W, L. F. C. Tencatt, M. N. Souza, and M. Santos, 20 October 2018; ZUFMS 6485, 3, 51.6–55.8 mm SL, Itiquira, córrego Ponte do Resolvido, 17°35'24"S, 54°20'30"W, L. F. C. Tencatt, M. N. Souza, and V. Carvalho G., 18 April 2019; ZUFMS 6486, 18, 52.6–69.7 mm SL, Itiquira, Ribeirão Comprido, 17°30'56"S, 54°25'03"W, L. F. C. Tencatt, M. N. Souza, and V. Carvalho G., 4 December 2019. Mato Grosso do Sul: MZUSP 125827, 4, 60.2–63.6 mm SL, Sonora, Córrego Lajeado, 17°35'47"S, 53°58'19"W, L. F. C. Tencatt, M. N. Souza, and M. Santos, 14 December 2018; NUP 22726, 6, 51.8–62.4 mm SL, Sonora, stream of the córrego Lajeado, 17°35'13"S, 53°57'58"W, L. F. C. Tencatt, M. N. Souza, V. Carvalho G., and M. Santos, 9 and 15 March 2019.

Diagnosis.—*Cyphocharax caboclo* belongs to the *C. saladensis* clade, along with *C. boiadeiro*, *C. saladensis*, and *C. vanderi*, that can be distinguished by having a truncate laterosensory system of the body, resulting in laterosensory pores only in the anterior scales of midlateral longitudinal series (except *C. vanderi* with complete lateral line) vs. a developed laterosensory system with complete lateral line in species of the other clades of *Cyphocharax* (i.e., *Curimatella alburna* clade, *Cy. gilbert* clade, *Cy. magdalenae* clade, and *Cy. spilurus* clade) and

Table 1. Morphometric data for holotype and 23 paratypes of *Cyphocharax caboclo*. Measurements other than the standard length are presented as proportions of either standard length or head length. Range includes holotype and all paratypes. SD = standard deviation.

	Holotype	Mean	Range	SD
Standard length (mm)	59.1	55.7	44.0–65.0	–
Percentages of standard length				
Greatest body depth	39.0	37.7	36.1–40.0	1.1
Snout to dorsal-fin origin	53.2	53.7	52.3–54.8	0.8
Snout to pectoral-fin origin	26.8	28.7	26.8–29.8	0.9
Snout to pelvic-fin origin	55.8	57.3	55.6–60.2	1.0
Snout to anal-fin origin	85.9	86.3	84.4–88.1	0.9
Dorsal-fin origin to hypural joint	55.8	53.8	51.2–56.0	1.1
Dorsal-fin origin to anal-fin origin	49.8	48.7	45.4–50.7	1.2
Dorsal-fin origin to pelvic-fin origin	38.3	37.2	35.3–39.6	1.2
Dorsal-fin origin to pectoral-fin origin	38.8	38.9	36.3–40.9	1.0
Caudal-peduncle depth	14.8	15.2	12.1–16.7	1.0
Pectoral-fin length	19.2	20.3	16.6–23.1	1.8
Pelvic-fin length	20.6	21.4	18.0–23.9	1.3
Dorsal-fin length	26.1	27.3	24.6–32.2	1.5
Head length	26.1	27.2	25.7–28.3	0.7
Percentages of head length				
Snout length	27.0	28.1	26.1–31.1	1.1
Orbital diameter	35.8	37.2	34.2–42.1	1.7
Postorbital length	41.7	39.1	36.2–41.7	1.5
Interorbital width	45.0	43.1	40.3–45.9	1.8

the three early-divergent species *Cy. abramoides*, *Cy. nigripinnis*, and *Cy. multilineatus*. Within the *C. saladensis* clade, *C. caboclo* is distinguished from *C. vanderi* by possessing incomplete or interrupted lateral line (vs. complete) and by the presence of an inconspicuous longitudinal black stripe along the lateral surface of the body (vs. absence). *Cyphocharax caboclo* differs from *C. saladensis* by the degree of development of laterosensory pores resulting in incomplete or interrupted lateral line with the range of 15–31 anterior scales of midlateral series with pores (vs. incomplete series with 8 or 9 pored scales), by the relative distance of the snout to anal-fin origin 84.4–88.1% of SL (vs. 76–84% of SL), and orbital diameter 34.2–42.1% of HL (vs. 27–34% of HL). *Cyphocharax caboclo* can be distinguished from *C. boiadeiro* by the presence of the interrupted lateral line not lengthened ontogenetically (vs. incomplete and ontogenetically lengthened lateral line). It also differs from *C. boiadeiro* by having two black blotches on the midlateral surface of the caudal peduncle. These blotches can be distinct from each other, with the morphology ranging from non-fused to fully fused, sometimes forming a single large blotch. The anterior blotch is predominantly elongated horizontally, and the posterior blotch is elongated vertically; when fused, the blotch forms a piriform or a T-shape format (Fig. 2A–E). This morphology is distinct from that of *C. boiadeiro* that has a single rounded or slightly elongated blotch, with the anterior half of the blotch slightly wider than the posterior half in some specimens; blotch never has a piriform or T-shape format (Fig. 2F–I).

Description.—Morphometric data summarized in Table 1. Body moderately elongate. Dorsal profile of head convex from tip of snout to dorsal-fin origin; slightly convex along dorsal-fin base; straight from base of last dorsal-fin ray to adipose-fin origin and slightly concave to origin of first dorsal caudal-fin procurent ray. Ventral profile of head convex from anterior margin of lower lip to isthmus. Ventral profile of body slightly convex from isthmus to pelvic-fin

origin, convex from that point to end of anal-fin base and concave to origin of first ventral caudal-fin procurent ray. Pre-pelvic region somewhat flattened transversely. Post-pelvic region of body transversely rounded. Head profile rounded anteriorly; overall profile of head pointed. Upper jaw longer than lower jaw. Mouth subterminal, horizontally aligned with ventral margin of orbits. Nostrils relatively close; anterior nostrils circular to ovoid, posterior nostrils crescent-shaped; aperture not closed by thin flap of skin separating nares. Adipose eyelid slightly developed anterior to orbit.

Dorsal fin pointed, distal margin straight; first and second branched rays longest. Dorsal-fin rays iii,9* (24), first unbranched ray very short. Distal margin of pectoral fin slightly rounded. Tip of adpressed pectoral fin reaching three or four scales short of vertical through pelvic-fin origin. Pectoral-fin rays i,12 (3), ii,12* (3), i,13 (14), ii,13 (1), i,14 (2). Pelvic-fin profile pointed. Tip of adpressed pelvic fin reaching three or four scales short of anal-fin origin. Pelvic-fin rays ii,7 (1), i,8 (4), ii,8 (8), i,9 (3), ii,9* (8). Caudal fin forked. Adipose fin present. Anal fin emarginate, anteriormost branched rays twice length of ultimate ray. Anal-fin rays iii,7* (24), first ray very short.

Lateral line longitudinal scales from supracleithrum to hypural joint 26 (3), 27* (18), or 28* (3). Laterosensory pores on anterior scales of longitudinal series 15 (1), 16 (2), 17* (2), 18 (2), 19 (2), 20 (3), 21 (1), 22 (1), 23 (3), 24 (1), 25 (2), 28 (1), 29 (1), 30 (1), or 31 (1). Number of pored scales not increasing with standard length. Variation of laterosensory pores available in morphological dataset (Supplemental File: Morphological Data Matrix; see Data Accessibility). Continuous series of scales posterior to hypural joint 3* (6) or 4 (18). Scales in transverse series from dorsal-fin origin to lateral line 4.5 (1) or 5* (23). Scales in transverse series from lateral line to anal-fin origin 4* (23) or 5 (1). Scales between anus and anal-fin origin 1* (23) or 2 (1). Middorsal series of scales from

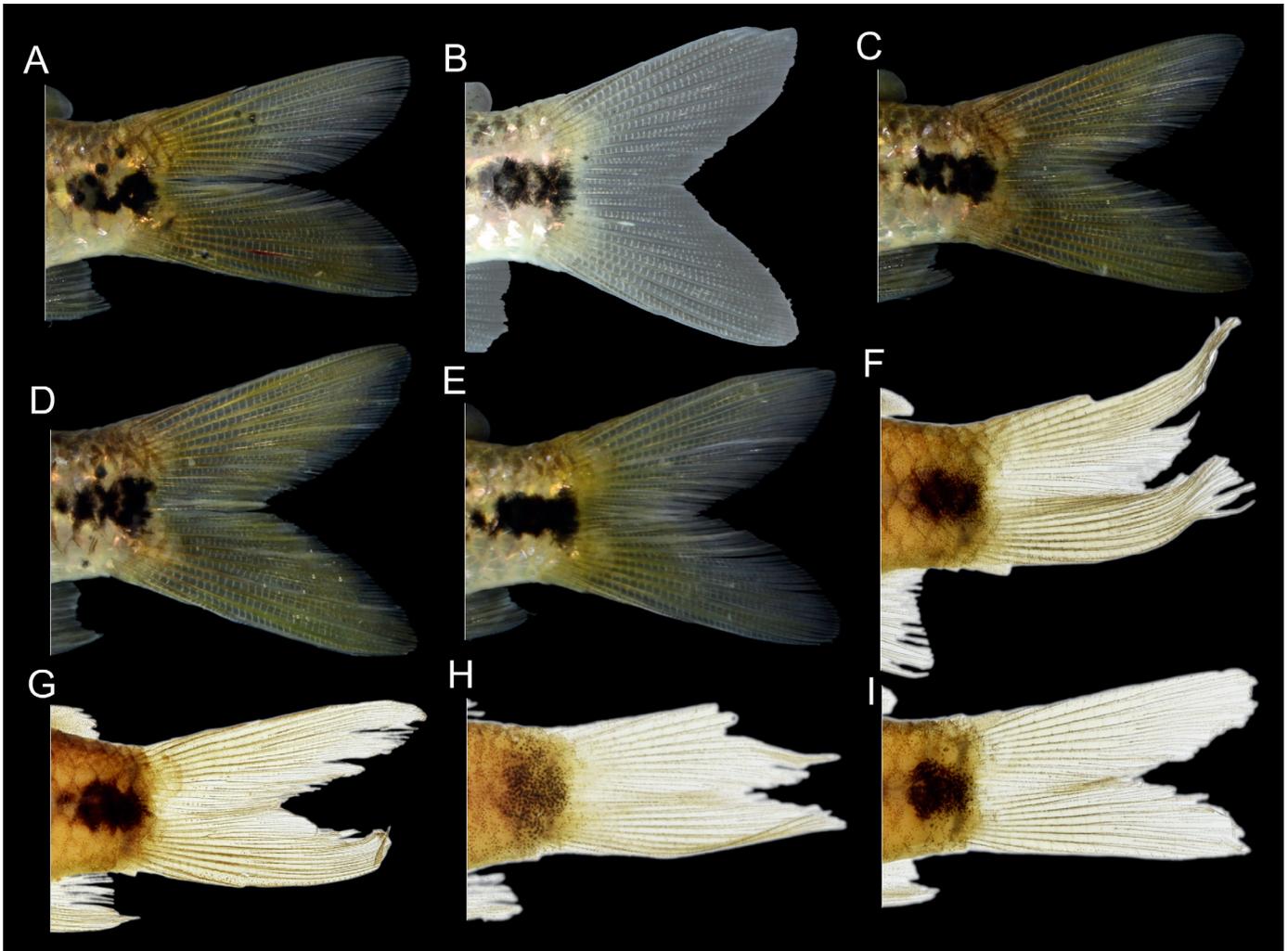


Fig. 2. Color pattern variation in the caudal peduncle of *Cyphocharax caboclo*, five uncatalogued topotypes ~40–50 mm SL (A–E), and *Cyphocharax boiadeiro*, four paratypes ~20–50 mm SL (F–I).

tip of supraoccipital to dorsal-fin origin 9 (9), 10* (12), or 11 (3). Circumpeduncular scales 12* (1), 13 (7), 14 (8), or 15 (8). Adherent scales over basal portions of pelvic fin present; scales primarily covering last unbranched fin-ray.

Total vertebrae 29 (3) or 30* (20).

Color in alcohol.—Ground coloration of body brownish yellow (Fig. 3). Upper lip, snout, and dorsal portion of head and opercle with small, dark brown or black chromatophores; lower jaw with dark brown or black chromatophores, more so on lower lip margin. Margins of scales along midlateral and dorsolateral surfaces of body outlined by series of small brown or black chromatophores forming a reticulate pattern, more evident on middle portion of flanks; such pattern diffuse in some specimens. Scales of dorsal portion of body with dark brown or black chromatophores, more concentrated on anterior and posterior margins; scales almost entirely covered by dark chromatophores in some specimens. Series of scales below lateral line with dark brown or black chromatophores, more so on anterior margins. Dark pigmentation absent or diffuse on scales of ventrolateral surface of body between pectoral- and pelvic-fin origins. Ventral margin of flanks posterior to pelvic-fin origin generally lacking or with few scattered chromatophores.

Region posterior to anal-fin origin with dark chromatophores, more evident around anal-fin base in some specimens. Deep-lying longitudinal dark stripe under midlateral scale series running along midlateral surface of body, from supracleithrum to anterior margin of caudal peduncle. Stripe more evident in central region of flanks under dorsal fin. Stripe fainter on region anterior to dorsal-fin origin and posterior to adipose-fin origin. Stripe interrupted in some specimens (Fig. 3). Posterior portion of caudal peduncle with two longitudinally aligned moderate-sized black blotches, anterior blotch from rounded to horizontally ellipsoid, and posterior blotch from rounded to vertically ellipsoid. Caudal-peduncle dark blotches separated (Fig. 3A), closely set (Fig. 3B), partially fused (Fig. 3C), or fused (Figs. 3D–E). Fused pattern presenting piriform (Fig. 3D) or T-shaped (Fig. 3E) larger, horizontally elongated blotch. Rays of dorsal, anal, and caudal fins outlined by dark brown or black chromatophores; such pattern absent or diffuse in pectoral and pelvic fins; proximal margin of dorsal fin with dark chromatophores on membranes. Adipose fin dusky, entirely covered by dark brown or black chromatophores.

Color in life.—Overall color pattern similar to preserved specimens but with lighter ground coloration (Fig. 3). Body

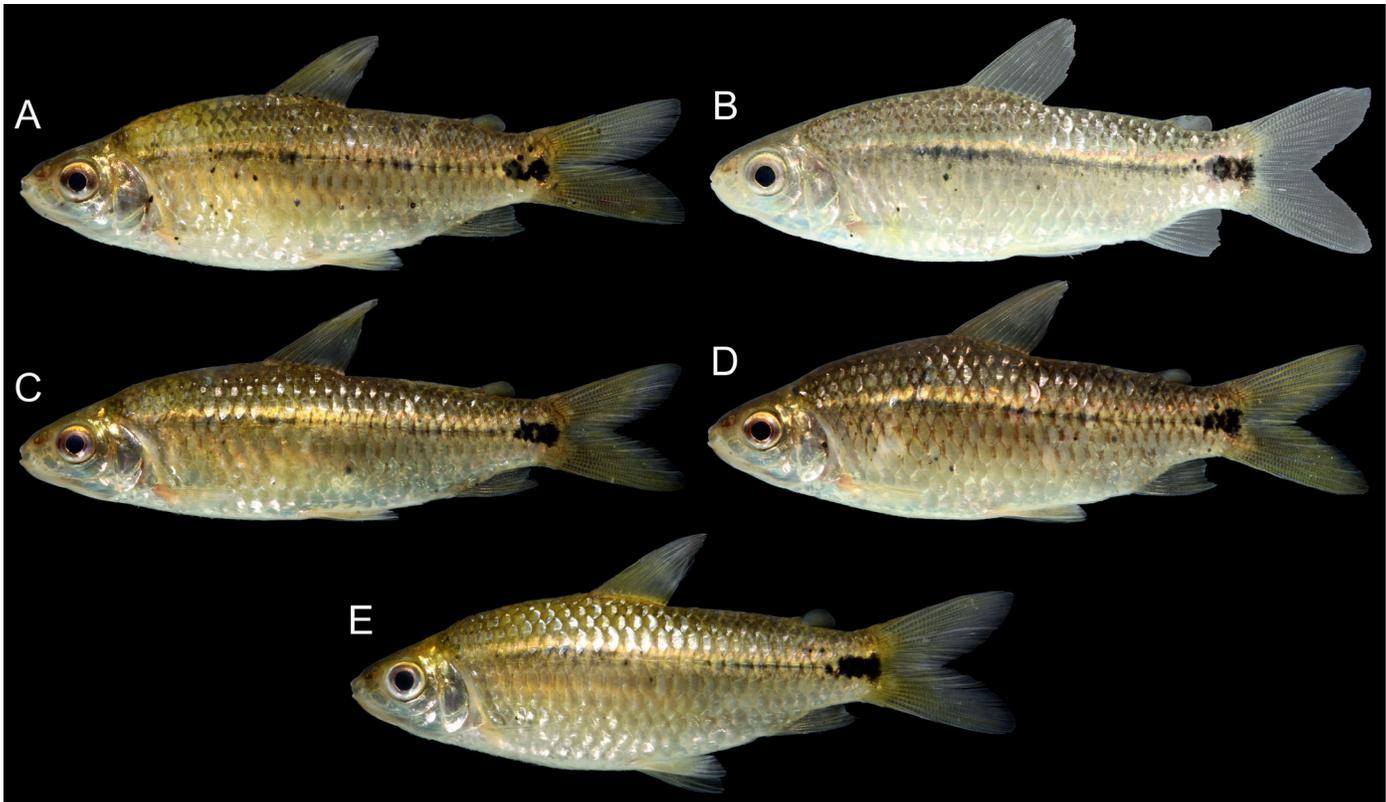


Fig. 3. Topotypes of *Cyphocharax caboclo* photographed alive, showing color pattern variation in lateral view (A–E). Smaller black dots randomly scattered on the body apparently caused by unknown parasites. Uncatalogued specimens ~40–50 mm SL. Photographs by L. F. C. Tencatt.

generally grayish white ventrally and brownish olive dorsally (Fig. 3A, C–E). Some specimens with ground coloration grayish white ventrally and grayish olive on latero-dorsal portions of body (Fig. 3B). Regions around midlateral longitudinal stripe generally with lighter and brighter yellow to beige. Dark longitudinal stripe generally inconspicuous.

Sexual dimorphism.—Secondary sexual characters not found.

Distribution.—*Cyphocharax caboclo* is currently known from tributaries of the upper Rio Correntes, itself a tributary of the upper Rio Paraguai basin in the border region between Mato Grosso and Mato Grosso do Sul states of central Brazil (Fig. 4).

Ecological notes.—*Cyphocharax caboclo* is endemic to the upper portion of the Rio Correntes basin, upstream from a subterranean stretch of natural karst tunnel (17°36'49"S, 54°50'14"W; SEMA, 2005), and is the only curimatid recorded in that basin so far (LFCT, pers. obs.). The new species was found inhabiting a wide range of mesohabitats, always in lentic sites, from marginal swampy areas to the main channel of small rivers (Fig. 5). At the type locality (Ribeirão Comprido and córrego Ponte do Resolvido), the species was generally found in association with submerged vegetation and mud/sand substrate (Fig. 5A–C). In the córrego Lajeado and its tributary, the species was found in similar mesohabitat with sandy substrate (Fig. 5D). *Cyphocharax caboclo* is syntopic with a new species of *Aequidens*, a new *Astyanax*, *Characidium chicoi*, *Characidium* aff. *zebra*, a possibly new *Cnesterodon*, *Eigenmannia correntes*, a new *Hemigrammus*, a new *Hypostomus*, *Melanorivulus* cf. *dapazi*,

Moenkhausia lopesi, and a new genus and species of heptapterid. In addition to the type locality, we observed in tributaries of the upper Rio Correntes the presence of another new species of *Astyanax*, two new species of *Hypostomus*, a new species of *Ancistrus*, and a new species of *Leporinus* (LFCT, pers. obs.).

Conservation status.—*Cyphocharax caboclo* is known from four tributaries of the upper Rio Correntes basin, which is clearly impacted by human activity, largely from agriculture (e.g., sugar cane, soybean, corn, and eucalyptus crops), resulting in severe deforestation and siltation (Fig. 5). It is important to note that the type locality has been impacted by the installation of a small hydroelectric power plant in the main channel of the Rio Correntes (17°32'12"S, 54°25'59"W). Additional hydroelectric projects are planned for the region (see ANA, 2019), and thus, we expect that this relatively restricted species will be further negatively impacted in the near future. The extent of occurrence of *Cyphocharax caboclo* was estimated at about 200 km², and according to the International Union for Conservation of Nature categories and criteria (IUCN Standards and Petitions Subcommittee, 2019), *Cyphocharax caboclo* is herein suggested to be categorized as Near Threatened (NT–B1biii).

Etymology.—The specific epithet “*caboclo*” apparently derives from the Tupi, one of the most iconic Brazilian indigenous languages, *caaboc* (or *caá-boc*), which means “the one removed from the woods.” The term is widely used in Brazil to designate a person born and raised in rural areas, generally

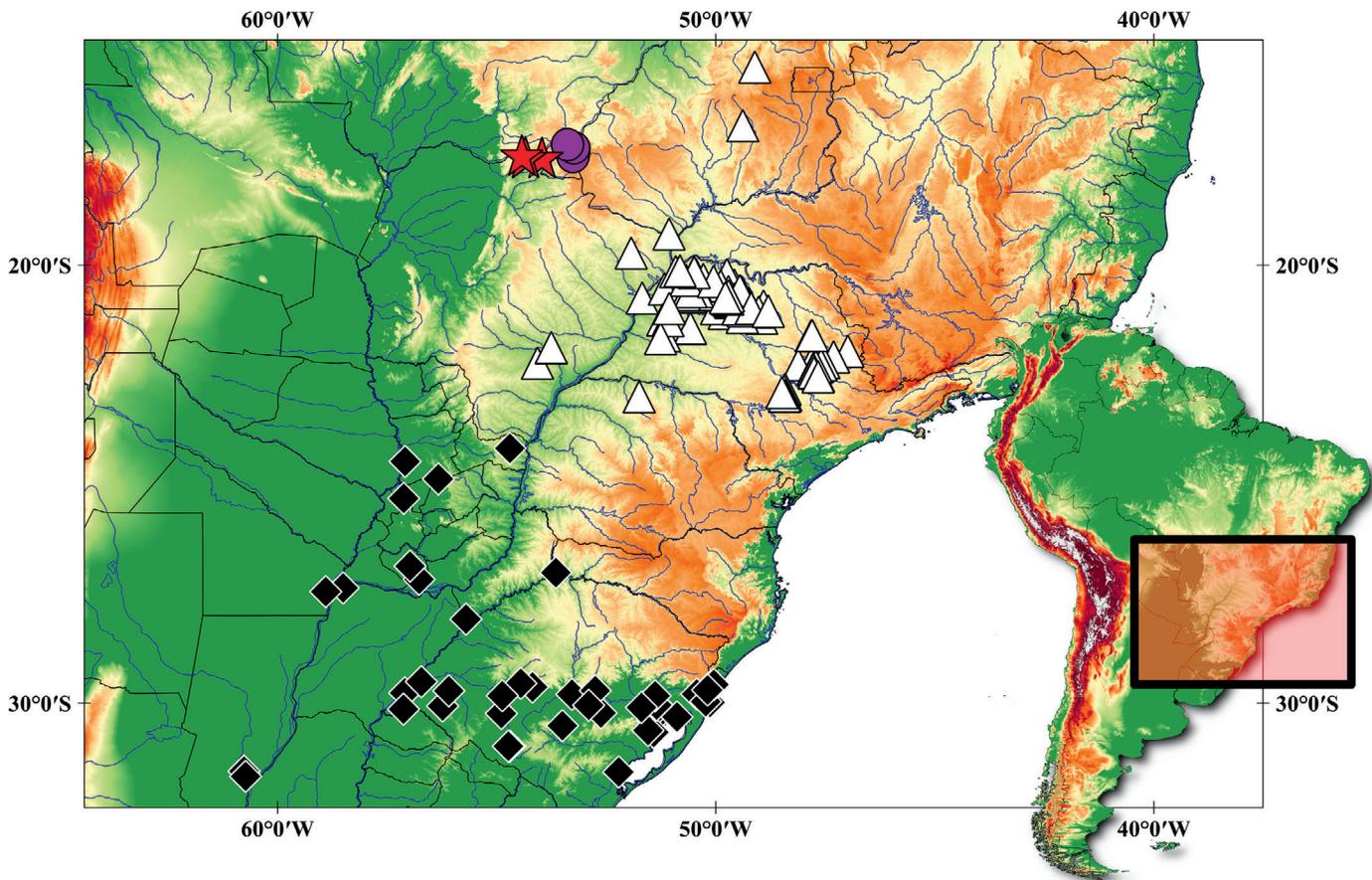


Fig. 4. Map of central-southern South America showing the geographic distribution of species of the *Cyphocharax saladensis* clade. Red stars denote the type locality and additional records of the species in the Rio Correntes, upper Rio Paraguai. Remaining symbols represent museum records of *C. boiadeiro* in the upper Rio Araguaia (purple circles), *C. saladensis* in the lower Rio Paraná-Paraguai, Uruguay, and coastal rivers of southern Brazil (black diamonds), and *C. vanderi* in the upper Rio Paraná (white triangles). Each symbol may represent more than one locality.

connoting simplicity and kindness. The name honors the “caboclos” from all over Brazil. A noun in apposition.

Molecular results and phylogenetic relationships.—The first matrix included 218 terminals, six genes, and 5,358 bp with 2,130 variable sites. The second matrix included ten terminals, the *COI* gene, and 657 bp with 59 variable sites (Supplemental File: Molecular Data Matrix; see Data Accessibility). Newly generated sequences from *Cyphocharax caboclo* were deposited in GenBank under accession numbers MZ753806 and MZ753807. DAMBE did not indicate any saturation for both matrices in either transitions or transversions in both asymmetrical (Iss.cAsym) and symmetrical (Iss.cSym) topologies. The second matrix for the “*C. saladensis* clade” included *COI* sequences of four specimens of *C. vanderi*, two specimens of *C. saladensis*, two specimens of *C. boiadeiro*, and two specimens of *C. caboclo*. The best-fitting model found for this matrix was Kimura-2-parameters (K2P) under the Bayesian information criterion. The overall K2P distance among specimens of the *C. saladensis* clade was 0.041 ± 0.005 . The pairwise K2P distance between *C. caboclo* and *C. vanderi* was 0.040 ± 0.010 , between *C. caboclo* and *C. saladensis* was 0.042 ± 0.051 , and between *C. caboclo* and *C. boiadeiro* was 0.035 ± 0.009 (Table 2).

The ML topology of the first matrix including all Curimatidae suggests the presence of a well-supported clade (100% bootstrap) herein named the “*Cyphocharax saladensis*

clade” (Fig. 6). This clade appears consistently as sister to the other species of the *C. spilurus* clade (*C. gillii*, *C. oenas*, *C. spiluroopsis*, and *C. spilurus*) except for *C. sanctigabrielis*, which is sister to both clades (Fig. 6). The *C. saladensis* clade includes *C. vanderi* (upper Rio Paraná) as sister to a subclade that has *C. saladensis* (lower Rio Paraná, Rio Uruguai, and coastal rivers of southern Brazil) sister to *C. caboclo* (upper Rio Paraguai) and *C. boiadeiro* (upper Rio Araguaia). Bootstrap support values for the node subtending the three latter species are relatively moderate: 62%; and for the clade with *C. caboclo* and *C. boiadeiro* are relatively high: 94% (Figs. 6–7). Genetic distances among taxa of the clade are demonstrated by branch lengths from the ML analysis (Fig. 7) and fully specified in Table 2.

DISCUSSION

Phylogenetic relationships.—Phylogenetic relationships of curimatids have been improved recently using molecular data (Melo et al., 2016a, 2018; Dorini et al., 2020). The phylogeny provided support for the recognition of the species-rich CSLC (Melo et al., 2018), which includes *C. abramoides*, *C. multilineatus*, *C. nigripinnis*, all species of *Steindachnerina*, *Curimatella lepidura*, and four smaller subclades: the *Cyphocharax gilbert* clade, the *C. magdalenae* clade, the *C. spilurus* clade, and the *Curimatella alburna* clade (Melo et al., 2018). The incorporation of *Cyphocharax caboclo* into

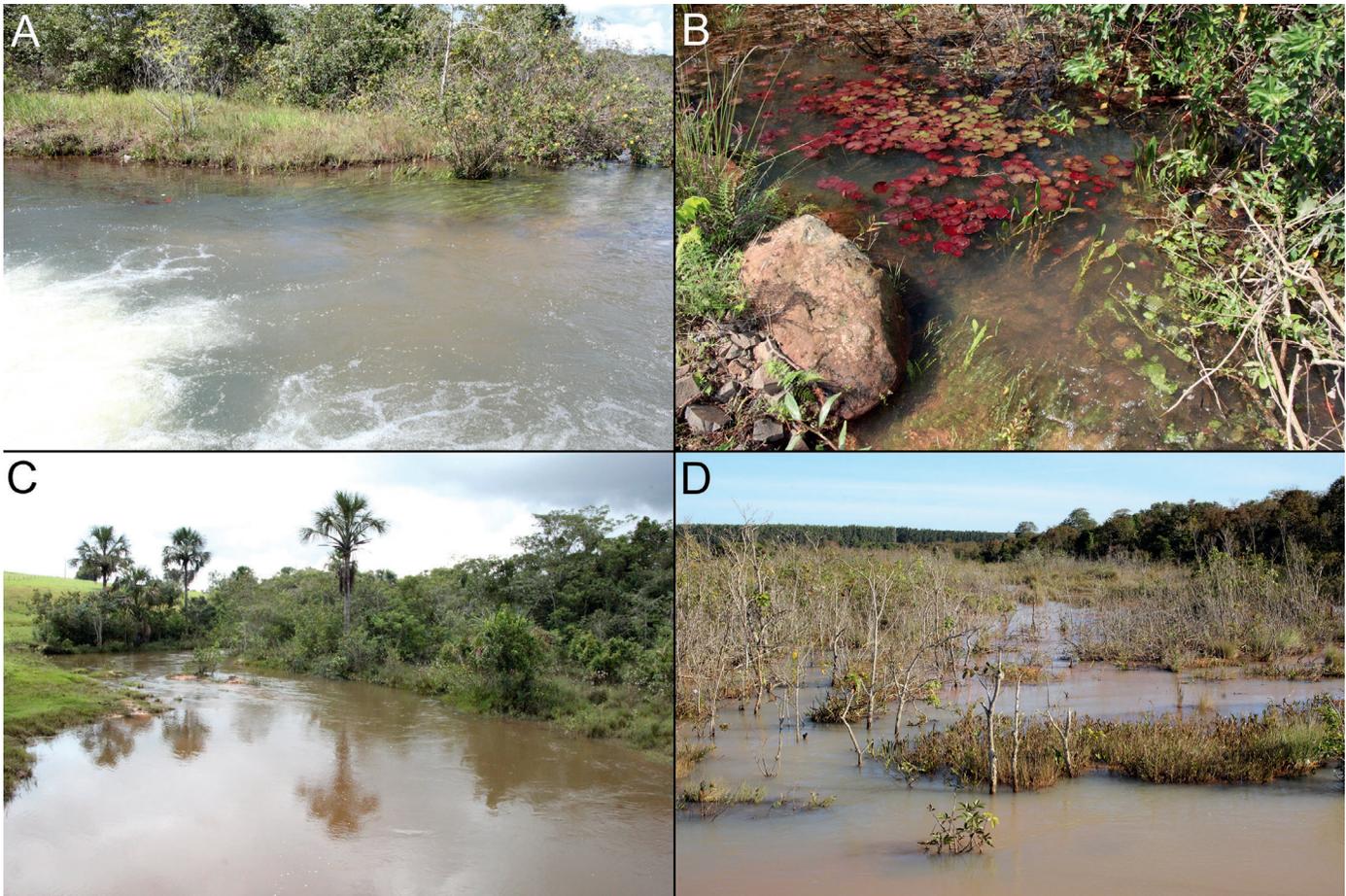


Fig. 5. Habitats of *Cyphocharax caboclo* in the Rio Correntes: (A–B) type locality, the Ribeirão Comprido; (C) córrego Ponte do Resolvido; (D) tributary of the córrego Lajeado. Photographs by L. F. C. Tencatt (A–C) and H. Gimênes (D).

the phylogeny allows us to split the *C. spilurus* clade into three smaller subgroups: *C. sanctigabrielis* alone, an endemic to the upper Rio Negro (Melo and Vari, 2014); the *C. spilurus* clade with *C. gillii*, *C. oenas*, *C. spiluropsis*, and *C. spilurus*; and the *C. saladensis* clade with *C. boiadeiro*, *C. caboclo*, *C. saladensis*, and *C. vanderi*. This novel proposal is justified by significant morphological discrepancies among clades, most notably the modifications in the laterosensory system of the body and the phenotypic variability of the pigmentation pattern.

Most species of the *Cyphocharax saladensis* clade possess a truncate laterosensory system resulting in laterosensory pores only in the anterior scales of the midlateral longitudinal series. This condition can be interpreted as a synapomorphy uniting *C. saladensis*, *C. boiadeiro*, and *C. caboclo*, whereas the complete lateral line series can be interpreted as a plesiomorphy present in *C. vanderi* (Britski, 1980), in most

of the remaining species of *Cyphocharax*, and in all curimatid genera except for *Curimatopsis* (Fig. 6; see Vari, 1992). Other species of *Cyphocharax* also possess modifications in the laterosensory system. *Cyphocharax vexillapinnus* from the upper and middle Amazon (Napo, Itaya, Solimões) has no more than eight pored scales (Vari, 1992), although the presence of a conspicuous dark spot in the middle portion of the anterior dorsal-fin rays suggests a close relationship with *C. notatus*. *Cyphocharax aninha* from the Rio Paru in southeastern Guiana Shield has no more than nine pored scales, a truncate laterosensory system, and a distinct, vertically elongated dark spot over the caudal peduncle (Wosiacki and Miranda, 2013). *Cyphocharax punctatus* from the Marowijne basin (Suriname and French Guyana) also has the number of pored scales along the longitudinal series increasing with standard length and 8–20 anterior pored scales (Vari and Nijssen, 1986; Vari, 1992). In addition, *C.*

Table 2. Pairwise Kimura-2-parameters distance within and between species of the *Cyphocharax saladensis* clade (mean \pm standard deviation). Intraspecific divergence appears in the diagonal for each species.

	<i>C. vanderi</i>	<i>C. saladensis</i>	<i>C. caboclo</i>	<i>C. boiadeiro</i>
<i>C. vanderi</i>	0.001 \pm 0.001			
<i>C. saladensis</i>	0.046 \pm 0.009	0.000 \pm 0.000		
<i>C. caboclo</i>	0.040 \pm 0.010	0.042 \pm 0.051	0.013 \pm 0.008	
<i>C. boiadeiro</i>	0.062 \pm 0.010	0.051 \pm 0.009	0.035 \pm 0.009	0.002 \pm 0.002

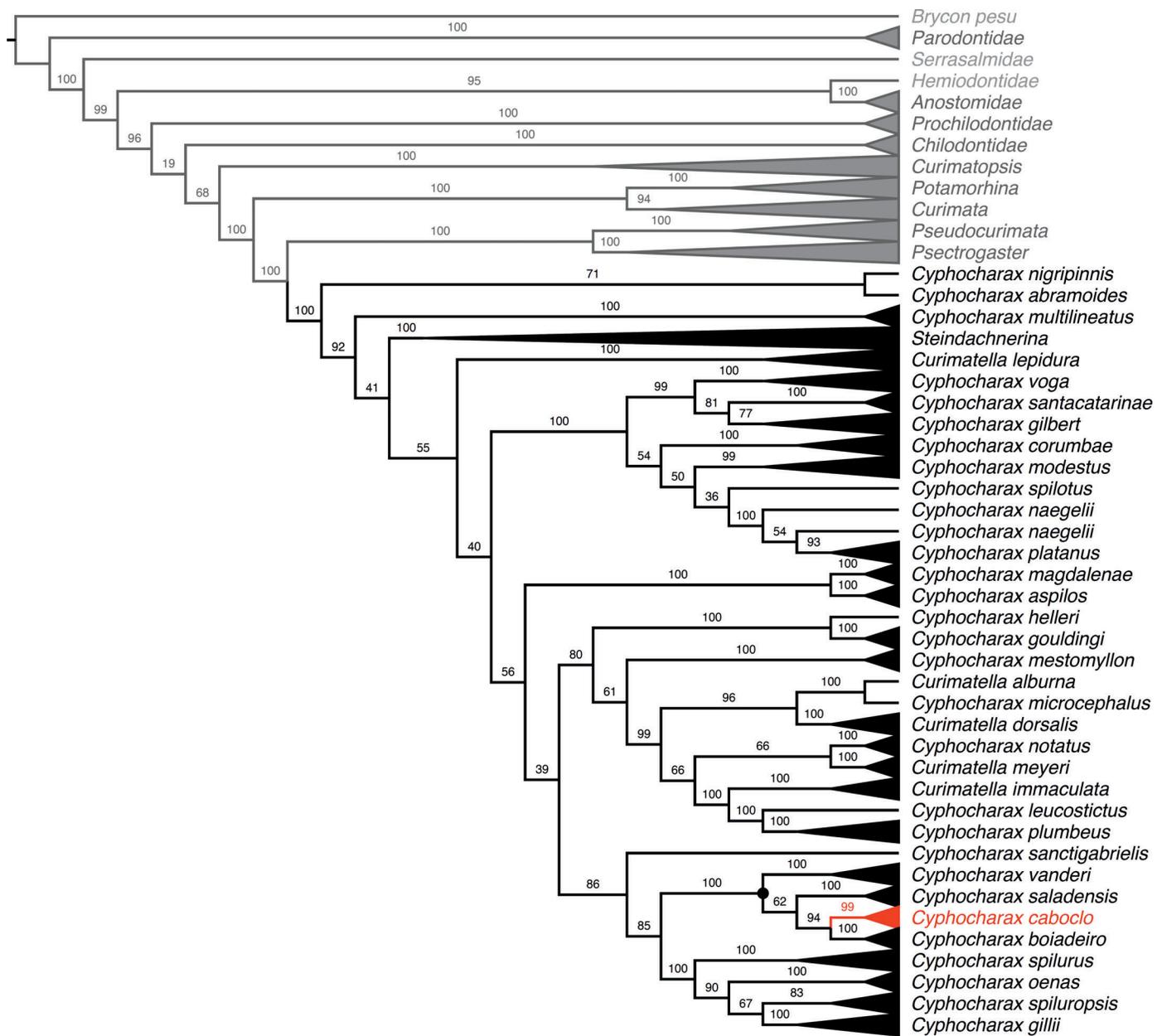


Fig. 6. Maximum-likelihood tree of Curimatidae using the multilocus dataset (218 taxa, six loci, 5,358 bp) depicting the *Cyphocharax saladensis* clade (black dot) and the position of *C. caboclo* as sister to *C. boiadeiro*. Numbers at left of nodes represent bootstrap support. See Data Accessibility for tree file.

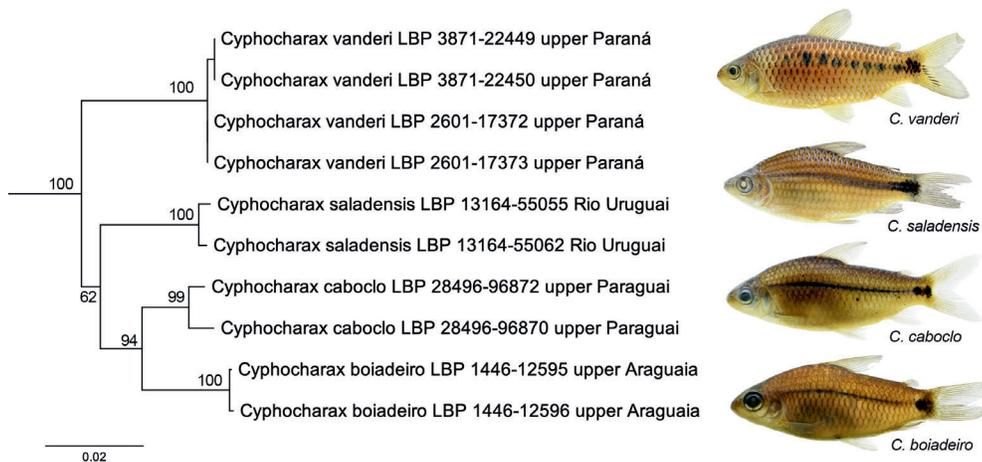


Fig. 7. Pruned maximum-likelihood tree showing internal relationships of the *Cyphocharax saladensis* clade. Numbers at left of nodes represent bootstrap support. Codes at right of tip names represent museum catalog numbers and voucher specimens. Photographs: *C. vanderi*, LBP 8116, 69.6 mm SL; *C. saladensis*, LBP 13164, 51.9 mm SL; *C. caboclo*, MNRJ 52506, 59.1 mm SL, holotype; *C. boiadeiro*, LIRP 14133, 42.9 mm SL, holotype. Photographs by B. F. Melo.

gangamon from Rio Tapajós presents a lengthening of the series from incomplete to a complete system when adults (Vari, 1992). Incorporation of these species in the phylogeny will determine whether they represent related taxa or instances of morphological convergence.

In addition to the evolution of the laterosensory system of the body, phenotypic variability of the pigmentation pattern is an important character for the morphological evolution of the *Cyphocharax saladensis* clade. Sister to the three species, *C. vanderi* from the upper Paraná basin possesses 4–6 large and conspicuous dark spots on the lateral surface of the body (Britski, 1980). Three other unsampled species have similar modifications of the body pigmentation. *Cyphocharax punctatus* from the Marowijne basin possesses 4–5 large spots over the body (Vari and Nijssen, 1986; Vari, 1992). This condition was previously hypothesized to be synapomorphic for *C. vanderi* plus *C. punctatus*, but without an explicit phylogenetic analysis (Vari, 1992); such hypothesis remains to be tested with the inclusion of *C. punctatus*. In addition, *Cyphocharax albula* from the Rio das Velhas of the São Francisco basin presents a similar pigmentation pattern but with additional and smaller dark spots (7–11) over the longitudinal series of the body (Lütken, 1874; Dutra et al., 2016). Lastly, *C. jagunco* from the Rio Jequitinhonha in the eastern Brazilian Shield possesses 10–17 dark spots in the same body region (Dutra et al., 2016). Although no study has investigated their phylogenetic relationships, the shared presence of irregular dark spots suggests their close relationship with *C. vanderi*. Next steps involve gathering samples, sequencing, aligning base pairs, and generating a phylogeny to better understand the phylogenetic significance of the dark spots over the midlateral surface of the body.

The remaining three species of the *Cyphocharax saladensis* clade do not possess dark spots over the midlateral surface of the body. Instead, *C. saladensis*, *C. boiadeiro*, and *C. caboclo* share the presence of a single thin midlateral dark stripe (Meinken, 1933; Vari, 1992; Melo, 2017), which is plesiomorphically absent in *C. vanderi* (Britski, 1980; Vari, 1992). Dark stripe(s) along the body midline vary substantially across species of the genus. For example, *C. corumbae* from Rio Paranaíba (upper Rio Paraná) has a relatively thicker stripe, beginning at the supracleithrum and extending into the posterior margin of the middle caudal-fin rays (Pavanelli and Britski, 1999). In adults of *C. laticlavus* from the Ecuadorian Amazon, the stripe begins at the vertical line through the dorsal-fin base, extends into the lateral surface of the caudal peduncle, and connects with the dark spot of the caudal peduncle, giving an appearance of a single dark stripe (Vari and Blackledge, 1996). The condition observed in the three most-derived species of the *C. saladensis* clade, however, is different: the thin dark stripe of *C. saladensis* connects with the dark spot of the caudal peduncle, but does not form an evident, single dark stripe, whereas the stripe of *C. boiadeiro* and *C. caboclo* remains unconnected with the dark spot (Fig. 7).

Historical biogeography and conservation.—Members of the *Cyphocharax spilurus* clade occur predominantly across Greater Amazonia (Amazon, Orinoco, and Guianas), except *C. gillii* from the Rio Paraguai basin (Vari, 1992). The presence of *C. gillii* in the Rio Paraguai basin can be explained by geomorphological events of mega river captures between the Rio Paraguai and the upper Rio Madeira during the Paleogene

(Lundberg et al., 1998; Tagliacollo et al., 2015) and more recently during the Neogene (Hubert et al., 2007; Carvalho and Albert, 2011; Ribeiro et al., 2013; Melo et al., 2016b). However, the occurrence of both *C. gillii* and *C. saladensis* in the Rio Paraguai basin suggests that the dispersal events from the Amazon to Paraguai basins occurred in multiple lineages of the CSLC and, more specifically, in two distinct clades: the most recent common ancestor (MRCA) of *C. gillii* and *C. spiluroopsis*, and the MRCA of the *C. saladensis* clade (Fig. 6).

Species of the *Cyphocharax saladensis* clade occur in drainages of the La Plata basin such as Paraná, Paraguai, Uruguay, and coastal rivers of southern Brazil (Vari, 1992), except *C. boiadeiro*, endemic to the upper Rio Araguaia above the Couto de Magalhães waterfall (Melo, 2017). A biogeographic study has performed ancestral area reconstructions using a time-calibrated phylogeny for the family Curimatidae (Melo et al., 2021). Results of this study indicate that the MRCA of the *C. saladensis* clade originated in the Paraguai basin during the early/middle Miocene (~23–11 Ma), that *C. vanderi* diversified into the upper Rio Paraná during the late Miocene (~11.3 Ma), and that *C. saladensis* and *C. boiadeiro* split from each other contemporaneously during the late Miocene at approximately 9.8 Ma (Melo et al., 2021).

This evidence indicates the timing of the split of *Cyphocharax boiadeiro* (Araguaia) and *C. saladensis* (lower Paraná, Uruguay, and southern Brazil) but does not explain how *C. boiadeiro* reached the Rio Araguaia. The discovery of *C. caboclo* living in the uplands of the upper Rio Paraguai fills this knowledge gap. We hypothesize that the evolutionary history of the entire clade was mediated by four events of river capture (Fig. 8): 1) Western Amazon–La Plata leading to the establishment of the MRCA of the entire clade in the La Plata region; 2) La Plata–upper Rio Paraná, which mediated the colonization of *C. vanderi* in the upper Paraná; 3) La Plata–upper Rio Paraguai, promoting the colonization of the MRCA of *C. caboclo* plus *C. boiadeiro* in that region; 4) upper Rio Paraguai–upper Rio Araguaia and the northward dispersal of *C. boiadeiro* (Fig. 8). This scenario best explains the evolutionary history of the group and hypothesizes a headwater capture event with subsequent allopatric speciation of *C. caboclo* and *C. boiadeiro*, one in the Correntes and another in the Araguaia basins. This additionally explains the modern distribution of *C. boiadeiro*, the single species of the clade living outside the La Plata system.

The headwaters of the Rio Araguaia and the headwaters of the Rio Correntes and Rio Taquari collectively represent one of the smallest regions of endemism for Neotropical freshwater fishes (Dagosta et al., 2020). In this region there is a concentration of endemic species, notably Characidae, Callichthyidae, and Rivulidae (Lima and Moreira, 2003; Dagosta et al., 2020), with *Cyphocharax caboclo* and *C. boiadeiro* being two examples. Other species descriptions have augmented the number of endemics for both drainage systems (Lima and Moreira, 2003; Campos-da-Paz and Queiroz, 2017; Oliveira et al., 2017; Ribeiro-Silva et al., 2020; Dagosta and de Pinna, 2021). The upper Rio Correntes alone presents at least ten undescribed species, many of which are likely endemic to that basin (see Ecological notes).

Hosting a rich and unique biota (Oliveira and Marquis, 2002; Diniz et al., 2010; Werneck, 2011), the Cerrado biome is well known for being one of the hotspots of biodiversity (Myers et al., 2000; Cardoso da Silva and Bates, 2002). The continuous discovery of species endemic to this region

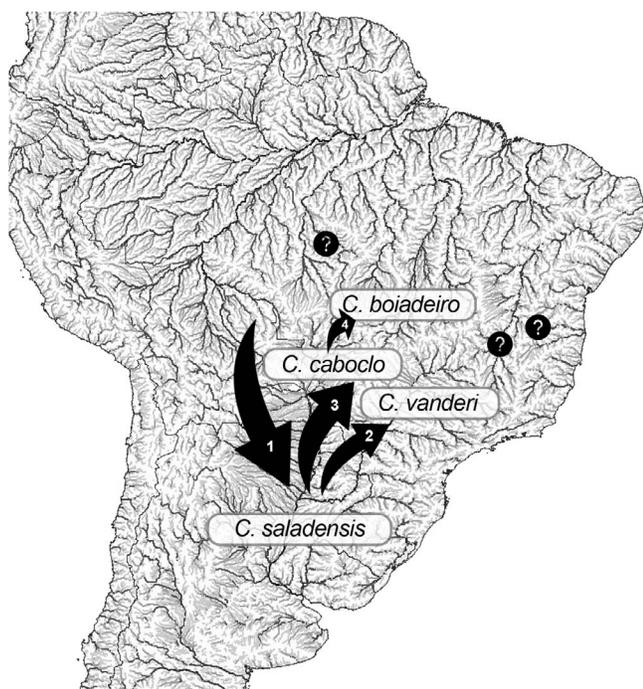


Fig. 8. Hypothesis of historical biogeography for species of the *Cyphocharax saladensis* clade highlighting four major river capture events: 1 = Amazon–La Plata; 2 = La Plata–upper Paraná; 3 = La Plata–upper Paraguai; 4 = upper Paraguai–upper Araguaia. Question marks represent unknown relationships and biogeographic histories for putative related species discussed in the text: *C. gangamon* Rio Tapajós, *C. albula* Rio São Francisco, and *C. jagunco* Rio Jequitinhonha.

highlights the importance of prioritizing conservation efforts in the Cerrado, which has suffered severe anthropogenic impacts (Klink and Machado, 2005). There are 47 species of freshwater fishes formally listed as threatened (VU, EN, and CR categories) in the Cerrado (ICMBIO, 2018), and our estimates suggest one more NT species to the region (see Conservation status section). This intensifies the need for additional research across the upper Correntes, Taquari, and Araguaia systems in order to increase sampling efforts, describe the fish diversity, evaluate their threatened status, and propose management programs focused on this extremely important biogeographic region.

MATERIAL EXAMINED

Material Examined is available as supplemental material (see Data Accessibility).

DATA ACCESSIBILITY

Supplemental material is available at <https://www.ichthyologyandherpetology.org/i2021057>. Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY

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