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New records of *Aspidoras fuscoguttatus* Nijssen & Isbrücker, 1976 (Callichthyidae, Corydoradinae) from the São Francisco river basin, Brazil

Matheus Oliveira Freitas[1,2*], Adriano Hauer[1], Roger Henrique Dalcin[1,3], Luz Elena De la Ossa-Guerra[4], Roberto Ferreira Artoni[5], Rodrigo Leão de Moura[1], Vinícius Abilhoa[3,6]

1 Núcleo Rogério Valle de Produção Sustentável / Coordenação dos Programas de Pós-Graduação em Engenharia and Instituto de Biologia, Universidade Federal do Rio de Janeiro. Rio de Janeiro, RJ, Brazil • MOF: serranidae@gmail.com https://orcid.org/0000-0003-0122-0980. AH: adriano.hauer@hotmail.com • RHD: roger_dalcin@hotmail.com https://orcid.org/0000-0001-7896-7404 • RLM: moura@biologia.ufrj.br https://orcid.org/0000-0002-5597-6196

2 Instituto Meros do Brasil, Curitiba, PR, Brazil

3 Programa de Pós-graduação em Zoologia, Universidade Federal do Paraná, Curitiba, PR, Brazil • VA: vinicius.abilhoa@gmail.com https://orcid.org/0000-0002-9463-0200

4 Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular, Universidade Federal de São Carlos, São Carlos, SP, Brazil • LELOAG: 03ledg@gmail.com https://orcid.org/0000-0001-7407-9709.

5 Programa de Pós-Graduação em Biologia Evolutiva, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil • RVA: rfartoni@gmail.com https://orcid.org/0000-0002-2528-9846

6 Museu de História Natural Capão da Imbuia, Prefeitura de Curitiba, Curitiba, PR, Brazil

* Corresponding author

Abstract. *Aspidoras fuscoguttatus* Nijssen & Isbrücker, 1976 is a small, relatively common armored catfish considered endemic to the upper Paraná river basin. Here, based on recent collections, as well as morphological and molecular evidence, the known geographic distribution of this species is extended to the basin of the São Francisco River. Specimens were collected during biological surveys in two unnamed tributaries of the Paraopeba River, in the headwaters of the São Francisco River. The new records may be a consequence of headwater stream-captures between São Francisco and upper Paraná drainages. Continued surveys are needed to assess and mitigate natural and human impacts, including biological exchanges between basins.

Keywords. Armored catfishes, biogeography, Brumadinho, DNA barcode, endemicity, geographic distribution, freshwater fishes

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Introduction

The teleost fish family Callichthyidae is widely distributed throughout the Neotropics and includes subfamilies Callichthyinae, with five genera and 17 species, and Corydoradinae, with three genera and 207 species (Fricke et al. 2023). Species of Corydoradinae are characterized by their small size (<9 cm), proportionally higher bodies, and smaller maxillary barbels (Britto 2003).

The genus *Aspidoras* Ihering, 1907 includes 18 valid species distributed in the Araguaia, Upper Paraná, lower São Francisco, Paraguay, Parnaíba, Tapajós, Tocantins, and Xingu river basins, including associated coastal drainages (Britto and Lima 2003; Reis 2003; Tencatt et al. 2022). These fish occur largely in sandy

bottom stretches of small streams where they feed on invertebrates and plant fragments (Araujo and Garutti 2003; Casatti et al. 2009). This genus is distinguished from other corydoradine genera by the following diagnostic features: there is a small laminar expansion at the base of branched rays on the pectoral fin, and there are two cranial fontanels, the posterior one in the parieto-supraoccipital and the anterior one between the frontals (Reis and Van Der Sleen 2018; Tencatt et al. 2022).

Aspidoras fuscoguttatus Nijssen & Isbrücker, 1976 was recently redescribed by Tencatt et al. (2022), and its distribution was reported as limited to the upper Paraná River. Here, based on recent collections followed by morphological and genetic analyses, we record it for the first time from two unnamed tributaries of the Paraopeba River, in the headwaters of the São Francisco River basin. The new records of *A. fuscoguttatus* may represent evidence for headwater capture between the Upper Paraná and São Francisco river basins (Langeani et al. 2007). The need for continued assessments and monitoring in highly threatened catchments is highlighted.

Methods

The São Francisco river basin (639,219 km²) flows through central Brazil across the states of Minas Gerais, Bahia, Pernambuco, Alagoas, and Sergipe (ANA 2002). The source of the Paraopeba River is in the Veloso Mountain Range, between the Vertentes and Espinhaço ranges, at about 1,400 m above sea level. The Paraopeba River flows into the Três Marias Reservoir before reaching the main channel of the São Francisco River. This 500 km long river has a drainage area of about 13,600 km², and its main tributaries are the Maranhão, Betim, Macacos, and Sarzedo streams (on the right bank), and the Camapuã, Manso, Juatuba, Águas Claras, and Florestal streams (on the left bank). The Paraopeba River is one of the main tributaries of the São Francisco River and has been continually and negatively affected by agricultural and mining activities, as well as by urban and industrial sewage (mainly from the Betim stream) and has been recently affected by a large-scale rupture of a tailings dam at the at the Córrego do Feijão iron-ore mine (Parente et al. 2022).

Specimens were obtained from two unnamed tributary streams of the Paraopeba River during the monitoring program of the project "Study on Aquatic Biota and Riparian Communities on the Paraopeba River" (Fig. 1). Electrofishing and drag nets were used to sample fishes in August 2019 and 2022 at two localities in the municipality of Brumadinho: (1) a stream inside Corrégo do Feijão Mine (20°07′45″S, 044°08′20″W) and (2) a creek near the city of Brumadinho (20°05′18″S, 044°12′31″W). Both streams were unaffected by the Córrego do Feijão dam rupture in January 2019 (Fig. 2). Fourteen *A. fuscoguttatus* specimens were collected. Specimens for genetic analyses ($n = 4$) were fixed in 96% PA alcohol and maintained refrigerated at –10 °C. The other specimens were fixed in 10% formalin and stored in 70% alcohol. Vouchers are deposited in the Museu de História Natural Capão da Imbuia (MHNCI) ichthyological collection. Measurements were made in millimeters to the nearly 0.1 mm using digital calipers, following Reis (1997). Measurements are presented both as percentage of standard length (SL) or percentages of head length (HL) (Table 1).

Total DNA was extracted using the saline method and was checked for quality using 1% agarose gel electrophoresis and Nanovue (Bruford et al. 1992). About 625 pb of the mitochondrial gene cytochrome c oxidase subunit I (COI) were amplified using universal primers for fish, FishF1 and FishR1 (Ward et al. 2005). A polymerase chain reaction (PCR) was carried out using the GoTaq® DNA Polymerase (Promega) protocol, with 10 µL buffer (1× (1.5 mM MgCl2)2), 1 µL PCR nucleotide mix (10 mM), 1 µL of each primer (10 mM), 0.25 µL GoTaq® DNA polymerase (1.25 u), 2 µL DNA template (50 ng/µL), and ultrapure water to make up a total volume of 50 uL. The thermal profile consisted of an initial denaturation step of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 45 s at 52 °C, and 1 min at

Figure 1. *Aspidoras fuscoguttatus* specimen from Brumadinho, Paraopeba drainage, Minas Gerais, Brazil. Scale bar: 1 cm.

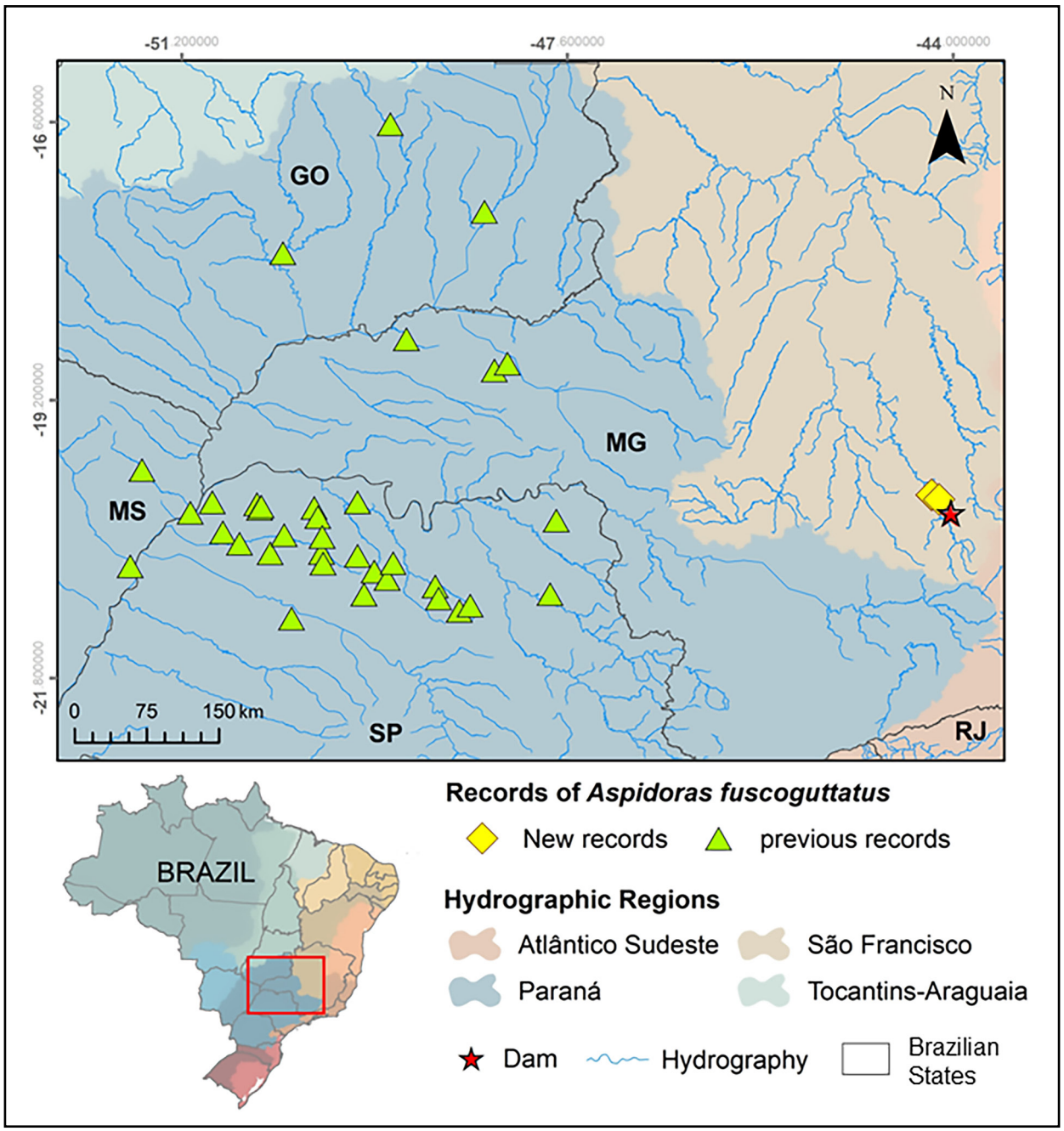

Figure 2. Map showing records of *A. fuscoguttatus* in the Paraná river basin. Sources: green triangles = SpeciesLink and Tencatt et al. 2022; yellow diamonds = new records from tributaries of the Paraopeba River in the São Francisco river basin. Symbols may represent more than one locality. The red star represents site of the Córrego do Feijão Mine tailings dam collapse in January 2019.

72 °C, with a final extension step of 10 min at 72 °C. The PCR products were purified using the Macherey-Nagel™ NucleoSpin™ Gel and PCR Clean-up Kit, and were sequenced by Macrogen Inc.

All sequences were manually checked, and the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for comparisons with sequences deposited in the NCBI database (https://www.ncbi.nlm.nih.gov/genbank/), numbers OR742315, OR742316, OR742317, and OR742318. The MAFFT algorithm was used for sequence alignment (Katoh et al. 2022). All sequences are provided in the Appendix, Table A1. A phylogenetic tree using the sequences and showing the position of *A. fuscoguttatus* within Callichthyidae was constructed using the RAxML v. 7.2.8 software (Stamatakis 2014), employing 2000 replicates. *Corydoras nattereri* Steindachner, 1876 and *Corydoras paleatus* (Jenyns, 1842) were used as the outgroup. A haplotype network was constructed using PopART v. 1.7 (Leigh and Bryant 2015; Clement et al. 2002) using all data available from other populations. Finally, the kimura 2-parameter (K2P) distances were calculated using MEGA v. 11 (Tamura et al. 2021), comparing the three populations of *A. fuscogutattus* from upper Paraná and São Francisco river drainages.

The updated distribution map is based on our records and data provided by Tencatt et al. (2022), which was retrieved from the SpeciesLink database (CRIA 2023).

Table 1. Morphometric and meristic data of *Aspidoras fuscoguttatus* specimens from Tencatt et al. (2022), including the holotype, and the seven specimens reported here. SD = standard deviation; * = % of standard length (SL); ** = % of head length (HL).

Morphometric data	**Tencatt et al. (2022) Paraná river basin**		**Present study São Francisco river basin**	
	Range (*n* = 21)	**Mean ± SD**	**Range (*n* = 7)**	**Mean±SD**
Standard length (mm)	26.7–37.2	29.9 ± 2.9	23.32–31.65	28.55 ± 3.28
Body depth*	24.0–31.4	28.2 ± 1.7	22.54–24.19	23.44 ± 0.59
Predorsal distance*	41.4–46.0	44.1 ± 1.1	37.85–42.23	39.90 ± 1.75
Prepelvic distance*	43.5–49.8	46.9 ± 1.7	44.13–47.08	45.16 ± 1.05
Preanal distance*	72.1–78.7	75.5 ± 1.9	71.63–75.78	73.06 ± 1.37
Preadipose distance*	82.3–87.7	85.1 ± 1.6	76.27–82.07	78.96 ± 2.0
Dorsal spine length*	12.4–16.2	14.7 ± 1.2	9.28–14.24	12.55 ± 1.6
Pectoral spine length*	12.4–19.6	16.4 ± 1.8	14.50–17.41	15.68 ± 1.07
Adipose-fin spine length*	8.4–11.6	10.2 ± 0.9	8.62–10.34	9.54 ± 0.52
Caudal peduncle depth*	13.8–15.9	14.8 ± 0.7	13.71–15.82	14.81 ± 0.86
Dorsal-fin base length*	11.5–15.6	14.0 ± 1.0	12.31–13.88	13.45 ± 1.01
Adipose distance length*	23.4–31.2	27.4 ± 2.1	21.08–26.93	24.28 ± 2.17
Maximum cleithral width*	24.3–29.6	26.6 ± 1.2	16.75–19.64	18.30 ± 1.05
Head length	32.2–35.6	33.6 ± 0.7	32.67–34.38	33.37 ± 0.52
Maxillary barbel length**	16.9–24.2	20.9 ± 2.2	15.08–21.71	18.02 ± 2.56
Head depth**	69.7–79.8	74.8 ± 2.9	59.74–67.45	64.88 ± 2.60
Least interorbital distance**	36.3–43.8	40.1 ± 1.7	35.52–37.35	36.46 ± 0.74
Horizontal orbit diameter**	16.7–20.2	18.5 ± 1.1	12.44–14.32	13.13 ± 0.74
Snout length**	43.8–51.0	47.2 ± 2.0	38.96–43.71	40.93 ± 1.55
Least internarial distance**	14.7–22.6	19.3 ± 1.9	14.45–17.38	16.40 ± 1.00
Meristic data	**Tencatt et al. (2022) Paraná river basin**		**Present study São Francisco river basin**	
	Range (*n* = 21)	**Mean ± SD**	**Range (*n* = 7)**	**Mean ± SD**
Dorsal-fin rays	II,8	—	II,8	—
Pectoral-fin rays	I,8–9	—	II,8	—
Pelvic-fin rays	I,5	—	I,5	—
Anal-fin rays	ii,5–6	—	ii,5-6	—
Caudal-fin rays	i,11–12,i	—	i,12,i	—
Dorsolateral body plates	25–28	—	26–27	—
Ventrolateral body plates	23–25	—	24	—
Dorsolateral body plates along dorsal-fin base	6–7	—	6	—
Dorsolateral body plates between adipose-fin spine and caudal-fin base	6–10	—	10	—

Results

***Aspidoras fuscoguttatus* Nijssen & Isbrücker 1976**
Figures 1–3

Materials examined. BRAZIL – **Minas Gerais** • Brumadinho municipality, unnamed tributary stream of the Paraopeba River, São Francisco river basin; 20° 07′45″S, 044°08′20″W; 19.VIII.2019; M.O. Freitas, A. Hauer & T.T. Occhi leg.; drag nets; 3 spec., in formalin, MHNCI 12832 • Same municipality, unnamed tributary stream of the Paraopeba River, São Francisco river basin; 20°05′18″S, 044°12′31″W; 19.VIII.2022; M.O. Freitas, A. Hauer & R.H. Dalcin leg.; electrofishing; 4 spec. in ethanol, 7 spec. in formalin, MHNCI 12833.

Identification. All 14 collected specimens of this small, armoured catfish species present branched rays with small, laminar expansions bearing irregular margins that form pointed structures on the inner part of their pectoral-fin base margin; an apparently a character exclusive to the genus *Aspidoras* (Tencatt et al. 2022). Additionally, all specimens present an extremely reduced to moderately developed pectoral-fin spine, which contrasts with the relatively well-developed pectoral-fin spine of *Corydoras* Lacepède, 1803 and *Scleromystax* Günther, 1864 (Tencatt et al. 2022). Morphology, meristics, and color pattern were consistent with those described by Tencatt et al. (2022) for *A. fuscoguttatus*; these include a moderately to well-developed inner infraorbital laminar expansion and a relatively wide frontal bone (width equal to or slightly larger than half of the entire length of the bone), contrasting with the narrow frontal bone that is typical of most congeners. The Paraopeba river drainage specimens also present a

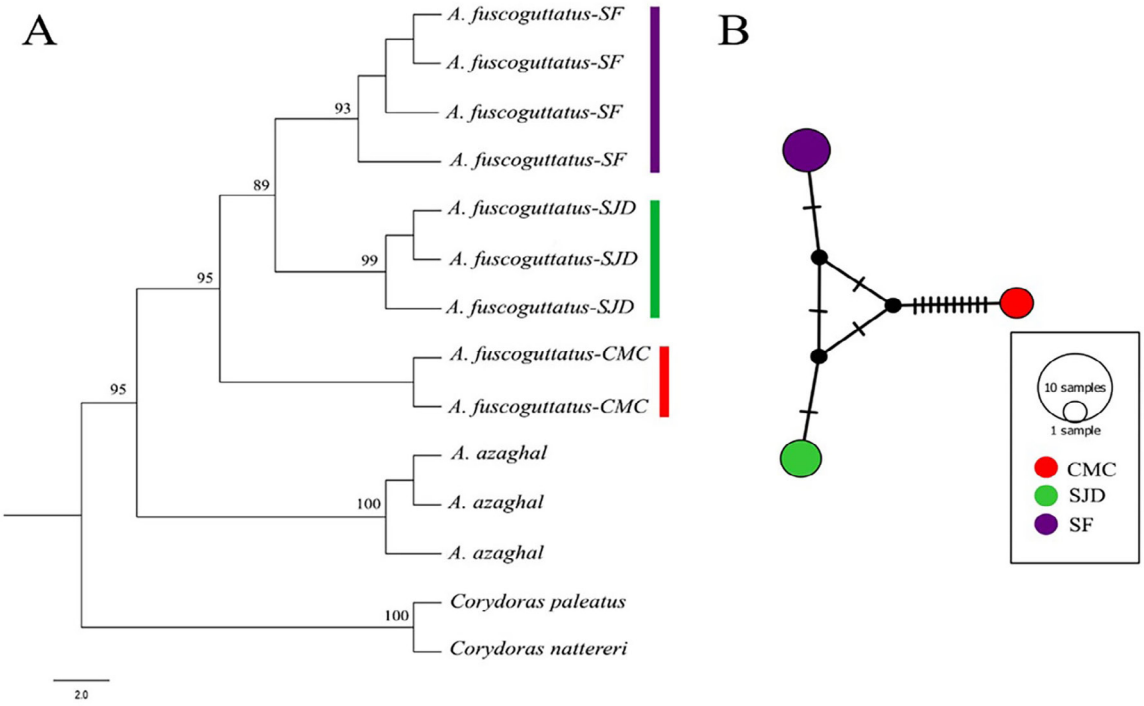

Figure 3. A. Phylogenetic tree showing *A. fuscoguttatus* and related species. Numbers above the phylogram branches represent bootstrap support at each node. **B.** Haplotype network of the three *A. fuscoguttatus* populations. CMC = Córrego Milho Cozido (upper Paraná basin = red), SJD = Rio São José dos Dourados (upper Paraná basin = green), and SF = São Francisco river basin (Paraopeba tributaries = purple).

laterally compressed head with a convex dorsal profile; a moderately developed and slightly pointed snout; ventral profile of body slightly convex from the isthmus to pelvic-fin origin; pelvic-fin rays numbering i,5; anal-fin rays numbering ii,5(6) and ii,6(1); caudal-fin rays numbering i,12,i; dorsolateral body plates numbering 26(6), 27(1); 24 ventrolateral body plates; 6 dorsolateral body plates along dorsal-fin base; 10 dorsolateral body plates between adipose-fin spine and caudal-fin base (Table 1). In addition, our specimens presented small spots on the dorsal fin (absent in congeners), well-defined dark brown or black blotches on top of the head, and flank with several dark brown or black markings. A dark brown stripe extending from the anteroventral portion of the orbit to the upper lip, which is typical of other *Aspidoras* species, was inconspicuous in our specimens.

Confirmation of species-level identification was based on the COI molecular marker, with the BLAST tool indicating 99% similarity with known sequences. Phylogenetic analysis placed the population from the Paraopeba river basin as a sister to that from the upper Paraná river basin São José dos Dourados River (SJD), thereby confirming the validity our identification of the Paraopeba specimens as *A. fuscoguttatus* (Fig. 3). In addition, the three populations seem to be structured, as the lowest genetic distance is found between the Paraopeba (São Francisco) and Upper Paraná (SJD) populations (Table 2); they had no shared haplotypes (Fig. 3B).

Ecological notes. The *A. fuscoguttatus* specimens from the tributaries of the Paraopeba River were captured syntopically with specimens of *Astyanax lacustris* (Lütken, 1875), *Psalidodon fasciatus* (Cuvier, 1819), *Knodus moenkhausii* (Eigenmann & Kennedy, 1903), *Phalloceros uai* Lucinda, 2008, and *Geophagus brasiliensis* (Quoy & Gaimard, 1824), all of which have been previously reported from this basin. Sample sites were third-order streams with a mean width of 4.5 m, a depth of about 50 cm, and moderate amounts of marginal vegetation (Fig. 4). Microhabitats included backwater and rapids and areas with sparse stones and sandy bottom, with relatively low water flow. The land adjacent to the sampled streams was heavily deforested.

Discussion

The Neotropics encompass some of the world's most diverse and complex fish assemblages and have been directly affected by the human impacts on natural freshwater ecosystems. These impacts have dramatically

Table 2. Kimura 2-parameter (K2P) distances with standard errors in parenthesis obtained from computations of several populations of *Aspidoras fuscoguttatus*. Population abbreviations: CMC = Córrego Milho Cozido (upper Paraná basin), SJD = Rio São José dos Dourados (upper Paraná basin), and SF = São Francisco river basin (tributaries of the Paraopeba River).

	SF	SJD
Paraopeba (SF)		
Upper Paraná (SJD)	0.52% (± 0.003)	
Upper Paraná (CMC)	2.13% (± 0.006)	2.12% (± 0.006)

Figure 4. Sampling sites of *Aspidoras fuscoguttatus* in two tributaries of the Paraopeba River, in the São Francisco river basin. **A.** Stream inside the Corrégo do Feijão Mine (20°07′45″S, 044°8′20″W). **B.** Creek near the city of Brumadinho (20°05′18″S, 044°12′31″W).

reduced biodiversity and critically threaten natural resources and services both nowadays and in the future (Albert et al. 2021). This scenario highlights the importance of multidisciplinary approaches for conservation (Ottoni et al. 2023). Data on the ecology, biology, and fish systematics are still lacking from vast areas of the São Francisco river basin, especially in its headwaters. For instance, approximately 320 fish species are known to occur in the São Francisco basin, of which several have been described only relatively recently (e.g. Penido et al. 2021). Such information gaps hamper the development of conservation and restoration initiatives in these highly threatened sites, which are heavily deforested and impacted by mining and agro-industrial activities. Despite the size and importance of the Paraopeba river drainage, which is considered a priority area for biodiversity conservation in the state of Minas Gerais (Drumond et al. 2005), previous fish surveys have only been occasional. Contributions from Alves and Vono (1998), Alves and Leal (2010), and Alves (2012) revealed a relatively high fish richness in this drainage, as well as the presence of migratory species (e.g *Prochilodus argenteus* Spix & Agassiz, 1829 and *Prochilodus costatus* Valenciennes, 1850). Only two other species of the family Callichthyidae, *Callichthys callichthys* (Linnaeus, 1758) and *Hoplosternum littorale* (Hancock, 1828), have been previously recorded from the Paraopeba river drainage (Alves 2012).

The previously known distribution of *A. fuscoguttatus* comprised only the upper Paraná river basin, with records from the states of Goiás, Mato Grosso do Sul, Minas Gerais, and São Paulo (Tencatt et al. 2022). Our new records extend the known distribution of this species to the São Francisco river basin and comprise the first known occurrences of the subfamily Corydoradinae in the Paraopeba river drainage All individuals were sampled in small, highly impacted water bodies, at sites dominated by sandy or rocky bottoms and with sparse riparian vegetation, the typical habitat for small, armored catfishes (Araujo and Garutti 2003), including *A. fuscoguttatus* in the upper Parana river basin (Tencatt et al. 2022).

The fish fauna of the upper Paraná and São Francisco river basins share several species belonging to Curimatidae, Characidae and Anostomidae, among other families (Araujo and Garutti 2003; Langeani et al. 2007; Aquino et al. 2009). Species shared between adjacent basins may result from recent colonization originated at geomorphologically unstable headwaters (Ribeiro et. al. 2006). Indeed, the duration, frequency, and direction of these so-called "captures" of headwater streams can be ultimately related with the faunal similarity among adjacent basins and comprise one of the key processes underlying the biogeography of freshwaters (Albert and Reis 2011). In addition, headwater captures promote adaptive radiation in freshwater faunas, as speciation may follow the re-establishment of dispersion barriers (Menezes et al. 2008). For instance, Costa (2010) showed that endemism rates within the family Rivulidae in the upper Paraná, São Francisco, and Tocantins basins are the outcome of vicariant events that likely originated from such punctuated biophysical connections.

As a concluding remark, we reinforce the importance of further fish surveys in regions that are supposedly well assessed, using morphological and genetic analyses to ensure that adequate baselines are built. As found in this study, more analyses are needed to explore the genetic diversity of *A. fuscoguttatus*. Herein, a 0.52% of genetic distance was recorded between the Paraopeba and one of the upper Paraná (SJD) populations, whereas a genetic distance of about 2.1% was recorded between the upper Paraná Córrego Milho Cozido (CMC) population and the other two. Gene flow and migration studies would help to understand and prioritize areas for conservation. This general suggestion is especially relevant for areas under threat from chronic anthropogenic disturbance, such as dams, industrial, agricultural, and urban discharges, and mining activity, especially in the Paraopeba river basin (Parente et al. 2022). Although we did not record *A. fuscoguttatus* in the channel of the Paraopeba River, which was directly affected by the dam rupture at the Córrego do Feijão iron-ore mine, three of our specimens were sampled within the working area of the mine, in an area not affected by the dam rupture. We emphasize the importance of improving baseline datas and carrying out long-term monitoring by public or private

organizations to better understand potential population and community shifts in the Paraopeba River and its tributaries, as well as to guide the much-needed conservation and restoration initiatives.

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Author Contributions

Conceptualization: MOF, RHD, VA. Formal analysis: RHD, LEOG, RFA, MOF. Funding acquisition: MOF, RLM, RFA. Investigation: MOF, RHD, AH, VA, LEOG, RLM, RFA. Writing – original draft: MOF, RHD, VA. Writing – review and editing: MOF, RHD, AH, VA, LEOG, RLM, RFA.

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Appendix

Table A1. Sources of sequences used in the phylogenetic tree (Fig. 3).

Species	Genbank accession no.	Locality	Source
Aspidoras fuscoguttatus	JN988689-90	São José dos Dourados River, upper Paraná Basin, Brazil	Pereira et al. 2013
	MK464058-59	Milho Cozido stream (site), upper Paraná River tributary, DF, Brazil	Bagley et al. 2019
	OR742315-18	Paraopeba River, São Francisco river basin, Brazil	This study
Aspiradoras azaghal	MZ959001, MZ959021, MZ959024	Igarapé do Pontal basin tributary, lower Xingu river basin, Brazil.	Tencatt et al. 2020
Corydoras paleatus	JX111730	Tapalque stream, Cuenca del Plata, Buenos Aires, Argentina	Rosso et al. 2012
Corydoras nattereri	KT874583	Aldeia Velha stream, Rio de Janeiro, Brazil	Moreira et al. 2016