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A new species of whiptail catfish *Rineloricaria* (Siluriformes: Loricariidae) from Macaé and São João river basins, southeastern Brazil

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A new species of *Rineloricaria* from the São João and Macaé river basins in Rio de Janeiro State, southeastern Brazil is described. The new species is distinguished from most of its congeners by possessing of five series of lateral plates below the dorsal fin; mid-dorsal series consisting of four or five keeled plates extending posteriorly beyond the origin of the dorsal fin; pectoral girdle covered by plates; snout tip with naked area not reaching most anterior pore of infraorbital ramus of sensory canal; dorsal-fin spinelet present. The new species differs from *R. zawadzkii,* the most similar and geographically closest species, by having mid-ventral and lateral abdominal plates in contact (*vs*. separated by skin), the dorsal fin with a dark brown terminal band not reaching the edge, with inconspicuous dark dots along the lower edge (*vs*. band reaching the edge), and the caudal fin with a diffuse distal band with variegated white spots (*vs*. a well-defined distal band). The genetic distance based on *cytochrome c oxidase I* between the new species and the closest congeners supports its validity. The current distribution of the new species in the São João and Macaé basins is consistent with paleo-drainage connections influenced by sea-level fluctuations.

Keywords: DNA barcode, Freshwater fishes, Loricariinae, Sea-level variations, Suckermouth armored catfishes.

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Uma nova espécie de *Rineloricaria* das bacias dos rios São João e Macaé, no estado do Rio de Janeiro, sudeste do Brasil, é descrita. A nova espécie é distinguida da maioria das congêneres pela presença de cinco séries de placas laterais abaixo da nadadeira dorsal; série médio-dorsal composta por quatro ou cinco placas quilhadas que se estendem posteriormente além da origem da nadadeira dorsal; cintura peitoral coberta por placas; ponta do focinho com área nua que não alcança o poro mais anterior do ramo infraorbital do canal sensorial; *spinelet* presente. A nova espécie se diferencia de *R. zawadzkii*, a espécie mais semelhante e geograficamente mais próxima, por apresentar séries de placas abdominal, lateral e médio-ventral em contato (*vs*. separadas por uma área de pele), nadadeira dorsal com uma faixa terminal marrom escura que não chega à borda, tornando-se pouco conspícua com pontos escuros alinhados ao longo da borda inferior (*vs*. faixa chegando à borda inferior), e a nadadeira caudal com faixa distal difusa com manchas brancas variegadas (*vs*. faixa distal bem definida). A distância genética com base no *citocromo c oxidase I* entre a nova espécie e suas congêneres mais próximas suporta sua validade. A distribuição atual da nova espécie nas bacias dos rios São João e Macaé é consistente com as conexões de paleodrenagens influenciadas pelas flutuações do nível do mar.

Palavras-chave: Cascudos, Códigos de barra de DNA, Loricariinae, Peixes de água doce, Variações do nível do mar.

INTRODUCTION

The Neotropical genus *Rineloricaria* Bleeker, 1862 is widely distributed in Central and South America from southern Costa Rica to northern Argentina, inhabiting large rivers, streams, and ponds, from high mountain streams to large floodplain rivers (Reis, Cardoso, 2001; Fichberg, Chamon, 2008; Vera-Alcaraz *et al*., 2012). *Rineloricaria* is the most speciose genus of the subfamily Loricariinae with 74 valid species (Fricke *et al*., 2024). *Rineloricaria* is distinguished from other Loricariinae by the following characters: postorbital notch present; surface of lower lip with short round papillae; premaxilla with seven to 15 teeth on each ramus; dentary teeth strong, deeply bicuspidate; dorsal region with dark brown bars or blotches with the first one at the origin of dorsal fin; dorsal fin usually with the first element as a spinelet; abdomen with a polygonal preanal plate, usually bordered by three large trapezoidal plates (Fichberg, Chamon, 2008), and mid-dorsal series always present, varying from a short extension, reaching up to the origin of the dorsal-plate series to a longer extension, extending posteriorly below the dorsal fin (Mejia *et al*., 2023).

Currently, five valid species of *Rineloricaria* are documented in the Paraíba do Sul River and adjacent coastal basins: *R. nudipectoris* Mejia, Ferraro & Buckup, 2023, *R. paraibensis* Mejia & Buckup, 2024, and *R. zawadzkii* Costa-Silva, Silva & Oliveira, 2022, all described in the last three years. Additionally, *R. nigricauda* (Regan, 1904) and *R. steindachneri* (Regan, 1904) have been recently redescribed due to inadequate original descriptions (Mejia, Buckup, 2024). Previous studies have identified up to eight

morphotypes of *Rineloricaria* for the Paraíba do Sul and coastal basins of southeastern Brazil (Bizerril, 1999; Bizerril, Primo, 2001). As part of a long-term study on the *Rineloricaria* diversity in the coastal drainages in southeastern Brazil, we recognized an undescribed species endemic to the São João and Macaé river basins. This new species is formally described herein based on molecular and morphological data.

MATERIAL AND METHODS

Meristic and morphometric data. Studied specimens are available in the ichthyological collections of the Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro (MNRJ), and Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP). The samples include muscle tissue (tis) preserved in anhydrous ethanol. Counts and measurements were taken on the left side using digital calipers in millimeters (mm) and are given as percentages of standard length (SL), except for subunits of the head, which are expressed as percentages of head length (HL). Meristic and morphometric data followed Isbrücker, Nijssen (1978), and Vera-Alcaraz *et al*. (2012). Nomenclature and counts for the lateral-plate series followed Schaefer (1997) with modifications proposed by Vera-Alcaraz *et al*. (2012). Identification of mid-dorsal series followed Mejia *et al*. (2023: fig. 1). Terminology for anterior, median, and posterior plates of the abdominal complex followed Isbrücker, Nijssen (1978). Counts of dorsal-fin rays do not include the spinelet as the first unbranched ray. In the description, values within parentheses represent the total number of specimens corresponding to the associated counts, and asterisks indicate counts for holotype. In the lists of examined specimens, (type-material) museum acronyms and catalog numbers are followed by the number of specimens preserved in ethanol indicated as "alc"; the range of standard length; the number of specimens measured for morphometric analyses, with the range of standard length in parentheses and the number of specimens used for molecular data indicated as "tis". Comparative material is listed by Mejia *et al.* (2023) and Mejia, Buckup (2024). Comparative data for *Rineloricaria altipinnis* (Breder, 1925), *R. cachivera* Urbano-Bonilla, Londoño-Burbano & Carvalho, 2023, *R. caracasensis* (Bleeker, 1862), *R. daraha* Rapp Py-Daniel & Fichberg, 2008, *R. eigenmanni* (Pellegrin, 1908), *R. heteroptera* Isbrücker & Nijssen, 1976, *R. jubata* (Boulenger, 1902), *R. konopickyi* (Steindachner, 1879), *R. melini* (Schindler, 1959), *R. quilombola* Chamon & Fichberg, 2022 and *R. teffeana* (Steindachner, 1879) were obtained from their original descriptions and/or high-resolution photographs of type-specimens available from Morris *et al.* (2006). Institutional abbreviations follow Sabaj (2020).

Molecular data. To support the morphological data, genomic DNA was extracted from muscle tissue preserved in anhydrous ethanol (Tab. **S1**) using the salting-out method (Miller *et al*., 1988). Partial sequences of the *mitochondrial cytochrome oxidase subunit I* (mt-co1) gene were amplified by Polymerase Chain Reaction (PCR) using primers FishF1 / FishR1 (Ward *et al*., 2005). The PCR protocol was as follows: denaturation at 94 °C/30 s, 35 cycles of 94 °C/45 s, 50 °C/30 s and 72 °C/45 s, and a final step of 72 °C/10 min. The amplified products were verified using 2% agarose gel electrophoresis. PCR

products were purified using Exo-SAP (Handy *et al*., 2011). Each PCR product was sequenced bidirectionally on an ABI3730xl (Applied Biosystems) automated sequencer at the Fundação Oswaldo Cruz (FIOCRUZ). The forward and reverse sequencing chromatograms were manually edited and trimmed in BioEdit (Hall, 1999). Barcode Index Number (BIN; Ratnasingham, Hebert, 2007, 2013) was generated for the final sequences using the algorithm RESL (Refined Individual Linkage) available in Barcode of Life Data Systems (BOLD Systems, http://boldsystems.org/). Genetic divergence among populations and between the geographically closest species (Mejia, Buckup, 2024), was performed under the Kimura 2-parameter model (K2P, Kimura, 1980) with MEGA X (Kumar *et al*., 2018). To identify fish lineages as separate species we used the suggested DNA barcoding threshold of 2% (Pereira *et al*., 2013). New nucleotide sequences and associated data (chromatograms, geospatial coordinates, and primer details) are available under their respective Sample ID and Process ID codes in BOLD Systems and GenBank databases (see Tab. **S1** for a full list of species and sequence accession codes).

RESULTS

Rineloricaria buckupi, new species

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(Figs. 1–3; Tab. 1)

Holotype. MNRJ 51116, 111.2 mm SL, Brazil, Rio de Janeiro, Macaé, Macaé River, bridge on RJ-168 highway, 22°19'39"S 41°58'55"W, 11 Nov 2017, P. A. Buckup, V. de Brito, E. Malanski & R. M. Dias.

Paratypes. All from Brazil, Rio de Janeiro State: Macaé River basin: MCP 55326, 1 alc, 105.0 mm SL, 1 tis, Macaé, Ouro River, Forested section on Bicuda road, 22°17'15"S 42°00'36"W, 25 Nov 2011, P. A. Buckup, C. Quijada & J. C. Pascoli. MCP 55327, 1 alc, 104.8 mm SL, 1 tis, Rio Bonito, Rio Bacaxá River, near the bridge on BR-101 highway, 22°41'36"S 42°33'13"W, 14 Aug 2023, P. A. Buckup, I. C. A. Souto-Santos & J. E. Mejia de Loayza. MNRJ 51002, 4 alc, 46.3–107.5 mm SL (1, 107.5 mm SL), 2 tis, Conceição de Macabu, Aduelas Creek (Macaé drainage), under the bridge, near a sign written "Projeto Caminhos de Darwin", 22°11'57"S 41°50'24"W, 17 Aug 2017, P. A. Buckup, D. F. Moraes Jr. & E. Malanski. MNRJ 55875, 1 alc, 155.9 mm SL, Casimiro de Abreu, bridge over the Macaé River near RJ-142 highway, 22°25'49"S 42°11'59"W, 20 Sep 2004, M. F. G. Brito. MNRJ 55876, 3 alc, 146.2–172.6 mm SL, Casimiro de Abreu, bridge over the Macaé River near RJ-142 highway, 22°25'49"S 42°11'59"W, 9 Jan 2005, M. F. G. Brito. MNRJ 55878, 4 alc, 132.6–141.1 mm SL, Casimiro de Abreu, bridge over the Macaé River near RJ-142 highway, 22°25'49"S 42°11'59"W, 9 Jan 2005, M. F. G. Brito. **São João River basin:** MNRJ 37445, 2 alc, 17.5–34.7 mm SL, Casimiro de Abreu, Aldeia Velha River, at bridge of BR-101 highway, 22°29'54"S 42°16'02"W, 21 Jul 2010, M. R. Britto, L. Villa-Verde & R. Bartolette. MNRJ 37475, 3 alc, 27.1–122.7

FIGURE 1 | *Rineloricaria buckupi*, holotype, MNRJ 51116, 111.2 mm SL, Brazil, Macaé, Macaé River; lateral, dorsal, and ventral views.

mm SL (1, 122.7 mm SL), 3 tis, Silva Jardim, Aldeia Velha River, under bridge on Aldeia Velha road, surroundings the RPPN Fazenda Bom Retiro, 22°28'03"S 42°17'51"W, 17 Jul 2010, M. R. Britto & F. Pupo. MNRJ 42777, 1 alc, 72.4 mm SL,1 tis, Silva Jardim, Imbau River, 2 km upstream from BR-101 highway, 22°37'10"S 42°28'02"W, 26 Feb 2014, P. A. Buckup, G. Beltrao & G. A. Ferraro. MNRJ 43012, 1 alc, 48.6 mm SL, 1 tis, Casimiro de Abreu, Lontras River, along BR-101 highway, 22°28'45"S 42°09'03"W, 27 Feb 2014, P. A. Buckup, G. Beltrão & G. A. Ferraro. MNRJ 52425, 1 alc, 46.3 mm SL, 1 tis, Silva Jardim, Maratuã River (tributary left bank of São João River) bridge on road between BR-101 highway and Bananeiras, 22°30'07"S 42°21'40"W, 2 Sep 2019, P. A. Buckup, I. C. A. Souto-Santos & G. A. Ferraro. MNRJ 54658, 6 alc, 55.3–101.8 mm SL (1, 101.8 mm SL), 2 tis, Rio Bonito, Bacaxá River, near bridge on BR-101 highway, 22°41'36"S 42°33'13"W, 14 Aug 2023, P. A. Buckup, I. C. A. Souto-Santos & J. E. Mejia de Loayza. MNRJ 54674, 10 alc, 37.2–99.3 mm SL (1, 99.3 mm SL), 3 tis, Casimiro de Abreu, Lontras River, on secondary road between Casimiro de Abreu and Professor Souza, 22°29'42"S 42°09'01"W, 14 Aug 2023, P. A. Buckup, I. C. A. Souto-Santos & J. E. Mejia de Loayza.

Diagnosis. *Rineloricaria buckupi* can be distinguished from most congeners except for *R. aequalicuspis* Reis & Cardoso, 2001, *R. altipinnis*, *R. anhaguapitan* Ghazzi, 2008, *R. anitae* Ghazzi, 2008, *R. baliola* Rodriguez & Reis, 2008, *R. cacerensis* (Miranda Ribeiro, 1912), *R. cachivera*, *R. caracasensis*, *R. capitonia* Ghazzi, 2008, *R. daraha*, *R. eigenmanni*, *R. fallax* (Steindachner, 1915), *R. formosa* Isbrücker & Nijssen, 1979, *R. hasemani* Isbrücker

& Nijssen, 1979, *R. heteroptera*, *R. isaaci* Rodriguez & Miquelarena, 2008, *R. jaraguensis* (Steindachner, 1909), *R. jubata*, *R. konopickyi*, *R. latirostris* (Boulenger, 1900), *R. maacki* Ingenito, Ghazzi, Duboc & Abilhoa, 2008, *R. malabarbai* Rodriguez & Reis, 2008, *R. maquinensis* Reis & Cardoso, 2001, *R. melini*, *R. microlepidogaster* (Regan, 1904), *R. morrowi* Fowler, 1940, *R. nudipectoris*, *R. osvaldoi* Fichberg & Chamon, 2008, *R. pentamaculata* Langeani & de Araujo, 1994, *R. phoxocephala* (Eigenmann & Eigenmann, 1889), *R. platyura* (Müller & Troschel, 1849), *R. quilombola*, *R. reisi* Ghazzi, 2008, *R. rodriquezae* Costa-Silva, Oliveira & Silva, 2021, *R. rupestris* (Schultz, 1944), *R. steindachneri*, *R. stewarti* (Eigenmann, 1909), *R. teffeana*, *R. tropeira* Ghazzi, 2008, *R. zaina* Ghazzi, 2008 and *R. zawadzkii* by having five lateral series of plates below the dorsal fin (*vs*. four lateral series of plates below the dorsal fin), and mid-dorsal series extending below and posterior to dorsal fin (*vs*. mid-dorsal series not extending beyond the origin of dorsal fin). *Rineloricaria buckupi* differs from *R. aequalicuspis*, *R. anhaguapitan*, *R. baliola*, *R. capitonia*, *R. latirostris*, *R. maacki*, *R. malabarbai*, *R. maquinensis*, *R. microlepidogaster*, *R. nudipectoris*, *R. reisi*, and *R. tropeira* by the extensive ventral covering of the pectoral girdle by plates (*vs*. absence of plates on most of the ventral surface of the pectoral girdle). It differs from *R. cacerensis*, *R. fallax*, *R. formosa*, *R. hasemani*, *R. jubata*, *R. melini*, *R. morrowi*, *R. quilombola*, *R. teffeana*, and *R. zaina* by the mid-dorsal series consisting of four or five keeled plates (*vs*. mid-dorsal series consisting of six to twelve keeled plates extending beyond the origin of the dorsal-fin base). It differs from *R. isaaci*, *R. jaraguensis*, *R. pentamaculata*, *R. rupestris*, *R. stewarti*, and *R. zawadzkii* by the mid-ventral and lateral abdominal plates series in contact with each other (*vs*. series of mid-ventral and lateral abdominal plates separated by an area of skin). It differs from *R. altipinnis, R. caracasensis*, *R. eigenmanni*, *R. heteroptera*, *R. konopickyi*, *R. osvaldoi*, *R. phoxocephala*, and *R. platyura* by breeding males lacking hypertrophied odontodes on the dorsum of the head and predorsal region (*vs*. hypertrophied odontodes present on the dorsum of the head and predorsal region in breeding males). It differs from *R. anitae* and *R. daraha* by having three longitudinal series of plates in the median complex of abdominal plates (*vs*. several irregularly organized abdominal plates on the median complex in *R. daraha*; or five to six longitudinal series of abdominal plates on the median complex in *R. anitae*). It is distinguished from *R. cachivera* and *R. rodriquezae*, by its narrow head width 63.8–70.5% HL (*vs*. 73.5–93.5% HL in *R. cachivera* and 71.6–81.2% HL in *R. rodriquezae*). It is distinguished from *R. steindachneri* by wider cleithrum 15.9–18.1% SL and pelvic-fin margin reaching or surpassing anal-fin origin (*vs*. narrow cleithral width 13.4–15.8% SL and pelvic-fin margin never reaching the anal-fin origin).

Description. Morphometric data of holotype and paratypes in Tab. 1. Body strongly depressed at caudal peduncle, tapering posteriorly from pectoral fin. In lateral view, dorsal profile slightly convex from tip of the snout to origin of dorsal fin and relatively concave at dorsal base; straight from base of dorsal fin to caudal peduncle. Head length larger than cleithral width. In dorsal view, head elongated with triangular anterior profile. Snout tip with small naked area, not reaching the anteriormost pore of infraorbital ramus of sensory canal. Eye elliptical, with large, deep postorbital notch; iris operculum present. Lower lip covered by irregularly sized papillae randomly distributed around mouth. Short maxillary barbel; teeth bicuspid; dentary teeth larger than those of premaxilla; 6(5), 7*(10) teeth on premaxilla and dentary teeth 6(2), 7*(13) on dentary.

Two predorsal plates between parieto-supraoccipital and nuchal plate with two low, inconspicuous ridges. Five (dorsal, mid-dorsal, median, mid-ventral, ventral) lateral plate series below dorsal fin. Dorsal series 20*(2), 21(5), 22(8) plates. Mid-dorsal series weakly keeled 4*(5), 5(10) plates. Median series 27*(3), 28(10), 29(2) plates. Lateral line complete. Mid-ventral plate series 15*(6), 16(6), 17(3) plates. Median and mid-ventral series with weak lateral ridge; these keels coalesce in last 11th(5), 12th*(8), 13th(2) plate of median series. Ventral series with 22*(5), 23(10). Lateral abdominal series with 7(11), 8*(4) plates between pectoral and pelvic-fin origin; number of plates on opposite sides of body sometimes different. Adjacent lateral and medial abdominal plates series in contact with each other. Abdomen entirely covered by plates, from opercular region to urogenital papilla. Posterior abdominal plate complex with large well-developed preanal plate anteriorly bordered by three polygonal plates, bordered by five plates. Middle abdominal plate complex, between lateral abdominal plates, with two or three longitudinal series posteriorly and four to six series anteriorly. Anterior complex of abdominal plates irregular and smaller anteriorly; anterior margin of complex with minute polygonal plates.

Dorsal-fin spinelet present. Unbranched dorsal-fin ray (dorsal spine) length shorter than head length (Tab. 1). Dorsal-fin origin slightly posterior to pelvic-fin origin; dorsal-fin rays i,7*(15); tip of dorsal fin when adpressed surpassing anal-fin origin, reaching eighth plate posterior to fin origin. Pectoral-fin rays i,6*(15); distal pectoral-fin margin slightly convex, when adpressed, surpassing pelvic-fin origin. Pelvic-fin rays i,5*(15); pelvic-fin margin reaching anal-fin origin; distal pelvic-fin margin convex; third branched ray slightly longer than unbranched pelvic-fin ray. Anal-fin rays i,5*(15); posterior anal-fin margin rounded; second branched ray longest. Caudal fin emarginated with i,10,i*(15) rays. Upper unbranched ray slightly longer than lower one, extended as short filament.

Coloration in alcohol. Background color of dorsal surface of head and body light brown. Ventral surface pale yellow. Sensory system pores on head and lateral medial plates notably darker. Five dark brown transverse bars on dorsal surface; first at dorsal-fin origin, second at tip of reclined dorsal fin, following bars located on caudal peduncle; third and fourth bars sometimes fused, forming four bars in some specimens (Fig. 2). Dorsal fin with dark brown terminal band not reaching border; terminal band with chromatophores concentrated on three unbranched rays on superior edge, becoming inconspicuous with dark dots aligned across inferior fin rays. Pectoral, pelvic, and anal fin covered with dark dots aligned across fin rays forming bands. Caudal fin with two vertical dark bands: broader solid one basally, one at distal margin, with variegated white spots. All fin membrane hyaline (Figs. 2–3).

Sexual dimorphism. Mature males with abundant hypertrophied odontodes on the lateral margins of the head. Dorsal region of pectoral-fin rays covered almost entirely with thin, long odontodes with curved tips. Unbranched pectoral-fin ray thick, strongly curved, with short, densely arranged odontodes (Fig. 4).

Geographical distribution. *Rineloricaria buckupi* is so far known from five tributaries of São João River (Aldeia Velha River, Bacaxá River, Capivari River, Lontra River,

TABLE 1 | Morphometric data of holotype and paratypes of *Rineloricaria buckupi*. Range includes the holotype. N = number of specimens; SD = standard deviation.

	Holotype	N	Range	Mean	SD
Standard length (mm)	111.23	15	99.3–172.6	–	–
Percent of standard length					
Head length	23.6	15	22.8–24.9	23.8	0.7
Predorsal length	33.4	15	32.6–35.1	33.7	0.7
Postdorsal length	66.5	15	64.4–67.3	66.4	0.8
Prepectoral length	18.9	15	18.0 –20.6	19.0	0.7
Postpectoral length	82.7	15	81.2–84.7	83.0	1.1
Prepelvic length	30.1	15	30.1–35.0	32.0	1.4
Postpelvic length	70.1	15	66.3–71.4	69.4	1.2
Preanal length	45.9	15	45.9–50.4	47.8	1.4
Postanal length	54.0	15	50.1–54.4	52.9	1.3
Unbranched dorsal-fin ray	21.1	15	18.2–21.1	20.0	0.8
Unbranched pectoral-fin ray	15.8	15	13.2–17.4	15.7	1.2
Unbranched pelvic-fin ray	14.7	15	13.5–16.1	14.7	0.8
Unbranched anal-fin ray	17.8	15	15.1–18.3	16.9	0.9
Pectoral-pelvic fin distance	12.4	15	12.4–15.8	13.9	0.9
Abdominal length	16.9	15	16.0–18.1	17.2	0.7
Cleithral width	16.0	15	15.9–18.1	16.7	0.6
Depth at dorsal-fin origin	9.3	15	8.9–11.0	10.1	0.7
Width at anal-fin origin	10.8	15	10.8–12.8	11.7	0.6
Caudal peduncle depth	1.6	15	1.5–1.7	1.6	0.1
Caudal peduncle width	3.2	15	2.8–3.4	3.2	0.1
Percent of head length					
Snout length	55.2	15	50.7–55.2	52.3	1.2
Eye diameter	22.1	15	11.9–17.0	15.2	1.2
Maximum orbital diameter	14.7	15	18.3–22.3	20.4	1.3
Interorbital width	19.5	15	17.4–21.8	19.4	1.5
Internarial width	7.4	15	6.1–8.0	7.4	0.5
Head depth	35.6	15	33.8–40.1	36.9	1.8
Head width	67.8	15	63.8–70.5	66.7	2.2
Free maxillary barbel	9.0	14	5.3–11.8	7.7	1.9
Ventrorostral length	6.1	15	4.3–9.2	6.3	1.1
Lower lip length	17.2	15	11.2–18.0	14.3	2.1

FIGURE 2 | *Rineloricaria buckupi*, paratypes. Note the remarkable dark brown transverse bars on dorsal surface. **A.** MNRJ 54658, 77.9 mm SL; five bars on dorsal surface. **B.** MNRJ 54658, 101.8 mm SL; notice third and fourth bars fused.

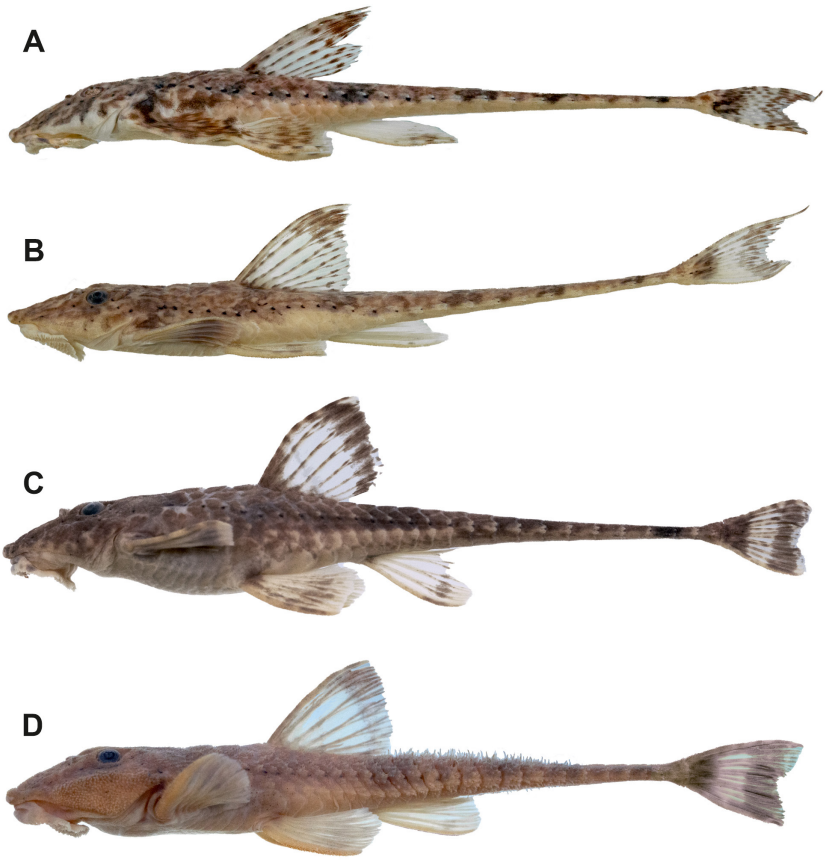

FIGURE 3 | Comparison of chromatophores distribution in the bands on dorsal and caudal fin in *Rineloricaria buckupi*, MNRJ 51116, holotype, 111.2 mm SL (**A**), MNRJ 51002, paratype, 107.5 mm SL (**B**), and *R. zawadzkii*, MNRJ 37497, 122.1 mm SL (**C**), MNRJ 28475, 127.9 mm SL (**D**).

and Maratuã River) and two tributaries of the lower Macaé River (Ouro River and Aduelas Creek). São João and Macaé are coastal rivers flowing directly to the Atlantic Ocean, State of Rio de Janeiro, southeastern Brazil (Fig. 5). Specimens were collected from shallow streams (*ca*. 1–2 m deep) with variable current speeds and associated with sandy or muddy bottom with marginal submerged vegetation. *Rineloricaria buckupi* was recorded in syntopy with *R. zawadzkii* in the São João and Macaé basins. However, the new species appears to be restricted to the middle and lower Macaé River basin, with its farthest record at 22°25'49.20"S 42°11'59.58"W (64 km from its delta in the Atlantic Ocean), and does not extend to the upper region, where *R. nudipectoris* and *R. zawadzkii* are frequently recorded.

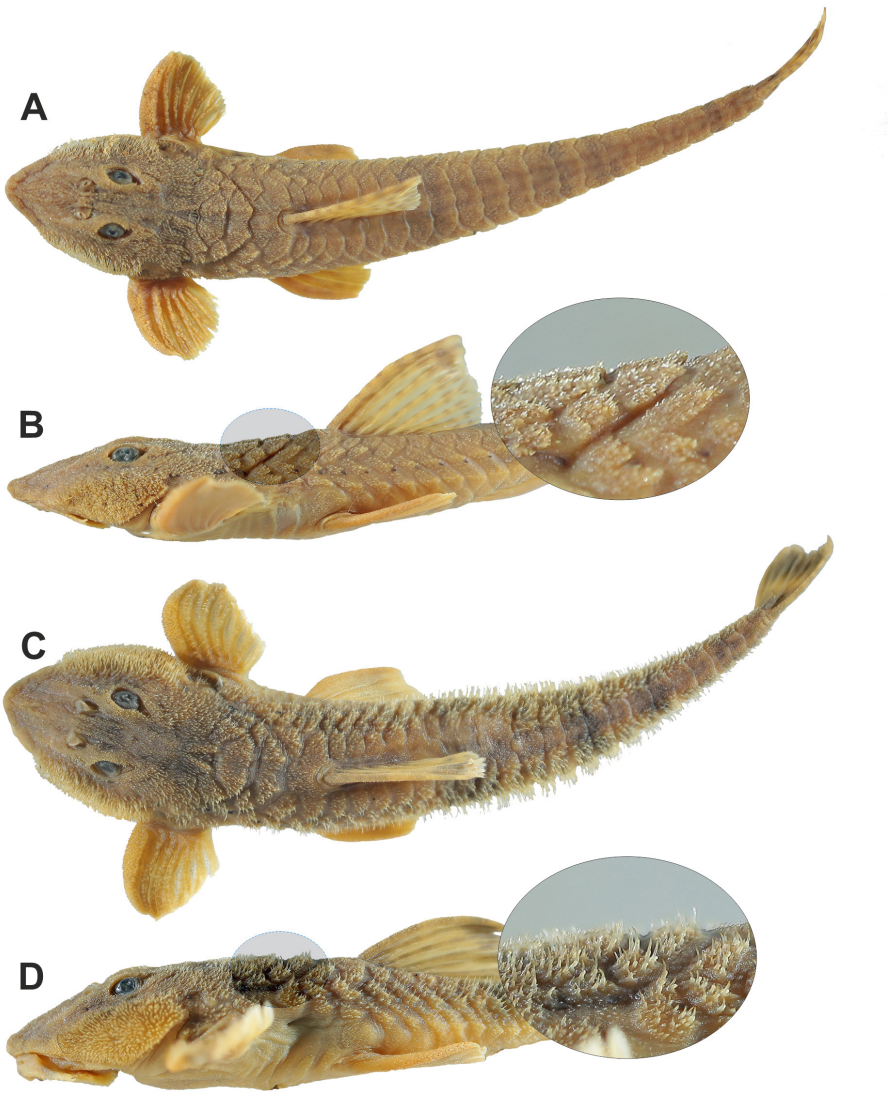

FIGURE 4 | Comparison of breeding males of *Rineloricaria buckupi*, MNRJ 55876, paratype, 147.7 mm SL (**A**, **B**), and *R. zawadzkii*, MNRJ 28475, 137.3 mm SL (**C, D**). Note the presence of hypertrophied odontodes on the lateral margins of the head and the thick unbranched pectoral-fin ray in *R. buckupi*, as well as hypertrophied odontodes on the dorsum of the head and predorsal region in *R. zawadzkii*.

Etymology. The specific name *buckupi* (noun, masculine, singular genitive), is a patronym for Paulo A. Buckup, in recognition of his valuable teachings as an advisor to numerous students, including the authors of this paper. Paulo has done outstanding work and made numerous contributions to Neotropical ichthyology, including advances in the systematics of *Rineloricaria* over the past 25 years.

Conservation status. *Rineloricaria buckupi* is endemic to the São João and Macaé river basins in Rio de Janeiro State. Populations of this species are distributed across seven rivers tributaries of two unconnected basins (São João and Macaé, Fig. 5). Currently, no imminent threats to the species have been identified. Therefore, *Rineloricaria buckupi* is provisionally categorized as Least Concern (LC) according to the IUCN categories and criteria (IUCN Standards and Petitions Committee, 2024).

Molecular analysis. DNA barcodes based on the partial sequence of mt-co1 mitochondrial gene were generated for 16 specimens of *Rineloricaria buckupi*. Each river basin hosts a unique haplotype for the mt-co1 gene, with all specimens within the same basin sharing this specific haplotype; these haplotypes differ from each other by six mutations. The algorithm RESL (Refined Single Linkage) available in Barcode of Life Data Systems corroborated the separation of *R. buckupi* from the other geographically closest species included in this study. *Rineloricaria buckupi* received the Barcode Index Number BOLD:ADA2018.

The average genetic distance for the DNA barcode (mt-co1) between *Rineloricaria buckupi* and the closest described species *R. zawadzkii* was 4.0%. The new species also has a relatively high genetic distance from its other geographically closest congeners: *R. nigricauda*, *R. nudipectoris*, *R. paraibensis*, and *R. steindachneri*, with all values higher than 6.4% (Tab. **2**). On the other hand, pairwise intraspecific distances in the new species were 0.0% within specimens from the same population and 0.9% between specimens from different populations (Tab. **S2**).

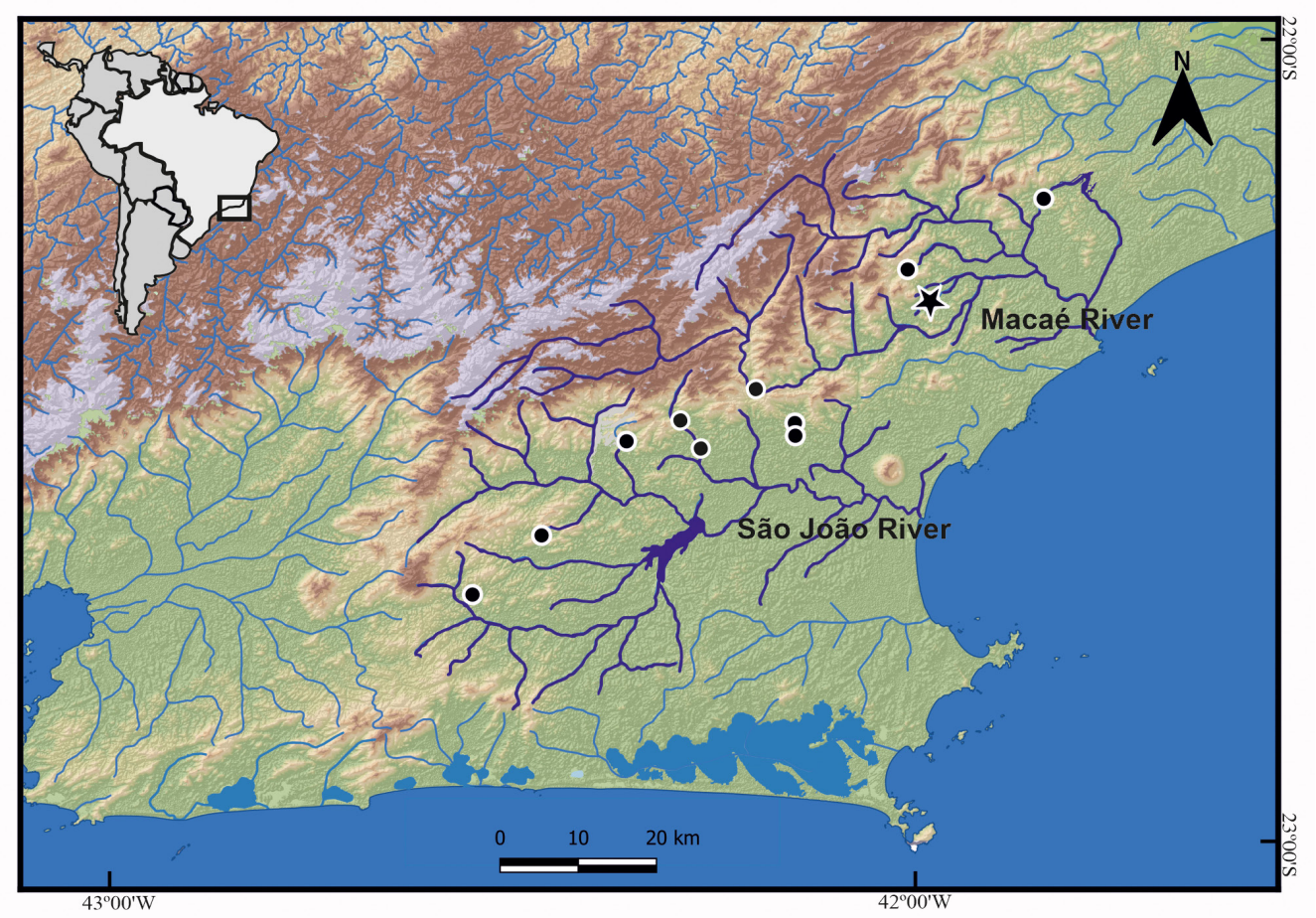

FIGURE 5 | Partial map of southeastern Brazil (as indicated by the black square) showing the geographic distribution of *Rineloricaria buckupi*. Star represents the type-locality; black circles may represent more than one collection event in the same locality.

TABLE 2 | Pairwise genetic distance values for *mitochondrial cytochrome oxidase c subunit 1* (mt-co1) gene between *Rineloricaria buckupi* and closest described species using a Kimura 2 Parameter model. Standard error estimates are shown above the diagonal and were obtained by a bootstrap procedure (100 pseudoreplicates).

		1	2	3	4	5	6
1	*Rineloricaria buckupi*		0.009	0.009	0.009	0.011	0.01
2	*Rineloricaria zawadzkii*	0.04		0.009	0.009	0.012	0.01
3	*Rineloricaria nigricauda*	0.074	0.071		0.01	0.01	0.008
4	*Rineloricaria steindachneri*	0.065	0.057	0.082		0.012	0.01
5	*Rineloricaria nudipectoris*	0.072	0.077	0.068	0.091		0.012
6	*Rineloricaria paraibensis*	0.064	0.066	0.07	0.066	0.082	

DISCUSSION

The new species is assigned to the genus *Rineloricaria* based on the combination of morphological features proposed for the genus: postorbital notch present; surface of the lower lip with short round papillae; premaxilla with seven teeth on each ramus; dentary teeth strong, deeply bicuspidate; dorsal region with dark brown bars, dorsal fin with the first element as a spinelet; abdomen with a polygonal preanal plate, bordered by three large trapezoidal plates and mid-dorsal lateral plate series present (Fichberg, Chamon, 2008; Mejia *et al*., 2023). *Rineloricaria buckupi* is restricted to secondary tributaries of the São João and Macaé river basins, where it occurs in syntopy with *R. zawadzkii.*

The new species can be differentiated from *Rineloricaria zawadzkii*, the genetically closest species (Tab. 2), by the possession of mid-ventral and lateral abdominal plate series in contact with each other, and a dorsal fin with a dark brown terminal band not reaching the edge, becoming inconspicuous with dark dots aligned along the lower edge (Fig. 3; *vs*. series of mid-ventral and lateral abdominal plates separated by a wide post-pectoral naked area, and dorsal and caudal fins with a broad dark-brown terminal band reaching the edge). Additionally, the main diagnostic character of *Rineloricaria zawadzkii* is a strong secondary sexual dimorphism, with breeding males possessing hypertrophied odontodes covering the lateral and dorsal regions of the head and body (Figs. 4C, D; see Costa-Silva *et al*., 2022: fig. 1). In contrast, breeding males of *Rineloricaria buckupi* possess hypertrophied odontodes on the lateral margins of the head but lack them on the dorsum of the head and the predorsal region (Figs. 4A, B).

The average genetic distance between *Rineloricaria buckupi* and the closest described species (Tab. 2) is considerably higher than the thresholds suggested for delimiting fish species (2–3%; Ward, 2009; Pereira *et al*., 2013). In contrast, intraspecific genetic distances within populations are low, while genetic distances between Macaé and São João populations are relatively high (Tab. **S2**). The Macaé and São João basins are adjacent yet independent drainages emptying separately in the Atlantic Ocean. A reconstruction of the paleodrainages connections inferred for a sea level retreat of -125 m during the maximum glacial period of the Pleistocene (Thomaz, Knowles, 2018) suggests that these basins have been in the same paleodrainage (the Macaé-São João paleo-river), which would explain the current distribution of the new species and the observed genetic structure, which reflects a lack of gene flow due to the geographic isolation of these basins. The discovery of another undescribed species of whiptail catfish close to one of the largest metropolitan areas in Brazil further illustrates the incomplete knowledge about fish diversity. It highlights the need to document this unknown diversity before it faces potential extinction due to increasing human occupation.

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AUTHORS' CONTRIBUTION

Eduardo Mejia: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing-original draft, Writing-review and editing.

Gustavo A. Ferraro: Data curation, Formal analysis, Investigation, Methodology, Writing-review and editing.

Igor C. A. Souto-Santos: Data curation, Formal analysis, Investigation, Methodology, Writing-review and editing.

ETHICAL STATEMENT

This research was based on specimens previously preserved in official biological collections, and no living animals were handled in the laboratory.

COMPETING INTERESTS

The authors declare no competing interests.

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