

Past connection and isolation of *Rineloricaria* (Siluriformes: Loricariidae) across southeastern Brazilian coastal drainages: evidence for palaeodrainage connectivity and description of a new species



Correspondence:
Eduardo Mejia
edu.mejia@ufrj.br

Eduardo Mejia^{1,2} and Paulo A. Buckup²

Submitted February 18, 2026

Accepted May 27, 2026

Epub October 12, 2026

Associate Editor Marcelo Melo

Section Editor Bruno Melo

Editor-in-chief José Birindelli

Online version ISSN 1982-0224

Print version ISSN 1679-6225

Neotrop. Ichthyol.

vol. 24, no. 3, 2026

Pleistocene marine transgressions and regressions repeatedly inundated and exposed the Southeastern Brazilian coast, creating alternating phases of connectivity and isolation among adjacent river drainages, that may have structured freshwater fish diversification. We integrate mitochondrial (COI, CytB) and nuclear (RAG1) sequence data with multivariate morphometrics to evaluate the phylogeographic relationships among populations historically identified as *Rineloricaria steindachneri* across the lower Paraíba do Sul basin and neighboring coastal drainages of Espírito Santo; and to evaluate whether patterns are consistent with the hypothesis that Pleistocene sea-level oscillations were the primary driver of genetic diversification. Molecular analyses recovered two well-supported, allopatric lineages that are independently diagnosable by morphology: *R. steindachneri* restricted to the Paraíba do Sul basin, and *Rineloricaria capixaba* n. sp. occurring in the Itabapoana, Itapemirim, and Benevente basins. The two lineages show a mean COI divergence of 2.0%, with largely basin-specific mitochondrial haplotypes, including a COI haplotype shared between Itapemirim and Benevente. Nuclear haplotype sharing is consistent with incomplete lineage sorting. Our findings support a history of intermittent inter-basin connectivity during marine regressions followed by vicariant isolation associated with subsequent transgressions, yielding the present allopatric distributions.

Keywords: Integrative taxonomy, Phylogeography, Pleistocene, Population genetics, Sea-level oscillations.

¹ Instituto de Investigaciones de la Amazonía Peruana (IIAP), Av. José A. Quiñones km 2.5, Iquitos, Peru. (EM) edu.mejia@ufrj.br (corresponding author).

² Programa de Pós-Graduação em Ciências Biológicas (Zoologia), Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, 20940-040, Rio de Janeiro, RJ, Brazil. (PAB) buckup@acd.ufrj.br.

Transgressões e regressões marinhas durante o Pleistoceno expuseram e inundaram repetidamente sistemas de no Sudeste da costa do Brasil, promovendo fases alternadas de conectividade e isolamento entre bacias hidrográficas adjacentes que podem ter estruturado a diversificação de peixes de água doce. Integramos dados de sequências mitocondriais (COI, CytB) e nucleares (RAG1) com morfometria multivariada para avaliar as relações filogeográficas entre populações historicamente identificadas como *Rineloricaria steindachneri* no baixo rio Paraíba do Sul e em drenagens costeiras vizinhas do Espírito Santo, bem como testar se os padrões observados são consistentes com a hipótese de que oscilações pleistocênicas do nível do mar foram o principal fator de diversificação genética. As análises moleculares recuperaram duas linhagens alopatricas, bem suportadas e independentemente diagnosticáveis pela morfologia: *R. steindachneri*, restrita à bacia do Paraíba do Sul, e *Rineloricaria capixaba* n. sp., ocorrendo nas bacias dos rios Itabapoana, Itapemirim e Benevente. As linhagens apresentaram divergência média de 2,0% em COI, com haplótipos mitocondriais majoritariamente específicos por bacia, incluindo um haplótipo de COI compartilhado entre Itapemirim e Benevente. O compartilhamento limitado de haplótipos nucleares é consistente com sorting incompleto de linhagens. Nossos resultados sustentam um histórico de conectividade interbacias intermitente durante regressões marinhas, seguido por isolamento vicariante associado a transgressões subsequentes, resultando nas distribuições alopatricas atuais.

Palavras-chave: Filogeografia, Genética de populações, Oscilações do nível do mar, Pleistoceno, Taxonomia integrativa.

INTRODUCTION

Coastal drainages in southeastern Brazil consist of a series of isolated rivers that flow directly into the Atlantic Ocean. The palaeohydrological history of these drainages has been shaped primarily by sea-level fluctuations, which have driven vicariant events and dispersal processes affecting freshwater fish assemblages, with sporadic dispersal events connecting them to interior basins (Lima *et al.*, 2021; Pio, Carvalho, 2021; Souza *et al.*, 2023; Mejia *et al.*, 2025). During Pleistocene glacial periods, the Brazilian coast was impacted by lower sea levels, and exposed palaeodrainages connected currently isolated riverine basins (Thomaz *et al.*, 2018). These palaeodrainages may have served as dispersal routes for freshwater fishes, with periodic episodes of isolation and secondary contact among populations that promoted both speciation and lineage sympatry over time (Weitzman *et al.*, 1988; Buckup, 2011; Dias *et al.*, 2014; Thomaz, Knowles, 2018; Thomaz *et al.*, 2015, 2017, 2019; Shelley *et al.*, 2020; Pio, Carvalho, 2021).

To test phylogeographic hypotheses, we assessed the genetic diversity of a small group of whiptail armored catfish lineages related to *Rineloricaria steindachneri* (Regan, 1904). *Rineloricaria steindachneri* was described based on specimens previously identified as *Loricaria lima* by Steindachner (1881), with presumed geographic distribution including

at least six localities: “Rio Parahyba bei Juiz de Fora, Campos und Mendez, im Rio dos Macacos, Rio Preto, Rio Quenda, Rio das Velhas” (Steindachner, 1881). Regan (1904) described the species range as “Eastern Brazil, from the Rio Preto to the Rio Parahyba”. However, some of the river names listed by Steindachner may refer to more than one drainage or cannot be located in current maps, and the geographic limits of the species outside of the Paraíba do Sul River basin remain unclear. Therefore, specimens from different localities may correspond to more than one species.

In recent intensive ichthyological surveys conducted in the Paraíba do Sul River basin and adjacent coastal drainages, *R. steindachneri* was redescribed and regarded as geographically restricted to the lower Paraíba do Sul River (Mejia, Buckup, 2024). However, specimens of *Rineloricaria* from the Itapemirim, Itabapoana and Benevente rivers in southern Espírito Santo have long been identified as *R. steindachneri* in fish collections, even though no comprehensive taxonomic review has ever encompassed those basins, raising uncertainties about the correct delimitation of the species in this region. Therefore, the present study aims (1) to evaluate the phylogeographic relationships among *Rineloricaria* lineages related with *R. steindachneri* in the Paraíba do Sul, Itapemirim, Itabapoana, and Benevente River basins, (2) to test the hypothesis that Pleistocene sea-level oscillations were the primary cause of their genetic diversification, and (3) describe a newly discovered species of *Rineloricaria*. If connections among palaeodrainages during marine regressions facilitated dispersal among currently isolated rivers, the genetic relationships between populations are expected to reflect the boundaries of these palaeodrainages (Thomaz *et al.*, 2017). Conversely, if the proposed palaeodrainages do not accurately describe past connections between these basins, other biogeographic processes such as river capture may have played a more significant role (*e.g.*, Lima *et al.*, 2017). To investigate the origin of the genetic structure of *Rineloricaria* in the four drainages, we obtained nucleotide sequences of two mitochondrial genes (COI and CytB), as well as the nuclear gene RAG1. Additionally, we examined the morphology of *Rineloricaria* specimens from these hydrographic drainages to assess whether there is evidence of morphological differentiation supporting recognition of genetic lineages as distinct species.

MATERIAL AND METHODS

Sampling and morphological identification. Specimens of *Rineloricaria* from the Itabapoana, Itapemirim, and Benevente River basins, available from the Ichthyological Collection of the Museu Nacional (MNRJ), Universidade Federal do Rio de Janeiro, and the fish collection of the Museu de Biologia Prof. Mello Leitão (MBML) of Instituto Nacional da Mata Atlântica were examined and compared with specimens from the Paraíba do Sul River basin previously identified as *R. steindachneri* in a redescription of this species (Mejia, Buckup, 2024). Standard morphological and morphometric data were collected from the specimens for further analysis and comparison with molecular results (see Morphological data and analysis section below). The morphometric data of *R. steindachneri* used in the present analyses was obtained from Mejia, Buckup (2024).

DNA extraction, amplification, and sequencing. Genomic DNA was extracted from muscle tissue preserved in anhydrous ethanol (see Tab. S1) using the salting-out method (Miller *et al.*, 1988). Three molecular markers were sequenced: the cytochrome oxidase subunit I (COI) and cytochrome b (CytB) genes from the mitochondrial genome, and the recombination activating gene 1 (RAG1) from the nuclear DNA. Partial sequences of these genes were amplified by Polymerase Chain Reaction (PCR) using primers FishF1 / FishR1 (Ward *et al.*, 2005) for the COI gene; primers CYTBFc / CYTB Rc (Lujan *et al.*, 2015) for the CytB gene, and primers RAG1Fa / RAG1R1186 for the RAG1 gene (Lujan *et al.*, 2015). The amplification reactions were performed in a volume of 25 μ l, each reaction containing 1 μ l of genomic DNA (approximately 50–100 ng), 1.5 units of Taq polymerase (Invitrogen Life Technologies São Paulo, Brazil), 1 $\times$ PCR Buffer, 5 mM MgCl₂, 0.2 μ M of each primer, and 0.25 mM of each dNTP; the remainder of the mixture consisted of distilled water. The thermal cycling conditions for COI included an initial denaturation at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 1 min, and extension at 72°C for 1 min 30 s. Final extension at 72°C for 6 min terminated the reactions. The PCR reactions for CytB and RAG1 started with denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 30 s at 57.8°C (CytB) or 58.3°C (RAG1), and 1 min at 72°C, with a final extension step at 72°C for 10 min followed by an indefinite hold at 4°C.

Quality of the amplified products was 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 chromatographs were manually edited and trimmed in BioEdit v. 7.1 (Hall, 1999). 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).

Genetic diversity, phylogenetic reconstruction and population structure. Pairwise genetic distances among COI sequences were computed in MEGA X (Kumar *et al.*, 2018) under the Kimura 2-parameter model (K2P; Kimura 1980). Genetic polymorphism of the COI dataset for *Rineloricaria* populations was quantified with the DNASP v. 5 (Librado, Rozas, 2009). For each morphotype, the following parameters were calculated: number of sequences, number of haplotypes (h), haplotype diversity (Hd), number of polymorphic sites (S), and nucleotide diversity (π), following Nei (1987). Population differentiation was assessed using F_{st} (Hudson *et al.*, 1992), and Dxy (average nucleotide divergence between populations), and gene flow was estimated as Nm derived from the F_{st} values. Neutrality tests (Tajima's D and Fu's F_s) were also performed on the COI sequences. Calculation of F statistic (Φ_{st}) and analysis of molecular variance (AMOVA) of the COI gene were performed in R (R Development Core Team, 2016) using the packages 'pegas' (Paradis, 2010) and 'adegenet' (Jombart, 2008). Groups in the AMOVA were defined considering (1) each population corresponding to the four drainages currently isolated by the sea (Paraíba do Sul, Itabapoana, Itapemirim, Benevente), (2) three palaeodrainages following Thomaz, Knowles (2018) (Benevente + Itapemirim, Itabapoana, Paraíba do Sul), and (3) two morphotypes (Benevente + Itapemirim + Itabapoana, Paraíba do Sul).

Phylogeographic relationships among populations were inferred using aligned sequences of the three loci (COI, CytB and RAG1) concatenated in sequences of 2712 bp, without gaps or missing data. A haplotype network was constructed using the Median-Joining method in PopArt (Leigh, Bryant, 2015), and a maximum likelihood (ML) phylogeographic tree was estimated using RAxML v. 8 (Stamatakis, 2014). The ML analysis was run using the GTRGAMMA model and 1000 bootstrap pseudoreplicates with *Rineloricaria zawadzkyi* Costa-Silva, Silva & Oliveira, 2022 as the outgroup (Tab. S1). A parsimony analysis using PAUP* v. 4.0a (Swofford, 2002) was also performed, with a heuristic search employing the TBR (tree-bisection-reconnection) branch swapping method. To identify apomorphic nucleotide states associated with single-nucleotide polymorphisms (SNPs) in the resulting tree, we used the “Describe Trees” option in PAUP*. The SNPs of the *Rineloricaria* morphotypes were then mapped on the ML tree using the sequence of *R. zawadzkyi* as the outgroup to polarize the character transformations. The SNPs were numbered sequentially according to their position in the concatenated matrix of genes, in the following order: COI, CytB and RAG1.

Reconstruction of palaeodrainages. To test the paleogeographic drainage hypothesis, we imported the palaeodrainage shapefiles as modeled by Thomaz, Knowles (2018) (DeepBlue repository: <http://dx.doi.org/10.7302/Z2HH6H8P>) into the QGIS software 3.16 (QGIS Development Team, 2017; Fig. 3). All layers were re-projected to WGS84 (EPSG:4326). Contemporary river courses were digitized as vector polylines directly over the palaeodrainage channels estimated by Thomaz, Knowles (2018: fig. 3). Modern shorelines (0 m) and Last Glacial Maximum shorelines (–125 m) were obtained from the Thomaz, Knowles (2018) shapefiles. Final map symbology, point locations of sampled specimens, and labeling were arranged in QGIS. We used the palaeodrainage reconstructions as a spatial hypothesis framework; these reconstructions have uncertainty and do not exclude river capture or ecological filtering.

Morphological data and analysis. Counts and measurements were taken on the left side of the specimens. Measurements were taken using digital calipers and recorded in 0.01 mm and are presented as percent of standard length (SL), except for subunits of the head, which are expressed as percent 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.

Specimens were initially assigned to operational morphotypes based on discrete morphological differences observed during taxonomic examination, mainly caudal-fin filament development, color pattern, and body plate ornamentation. These preliminary groupings were subsequently evaluated using morphometric and molecular analyses to test whether the observed variation was congruent with the proposed taxonomic hypotheses rather than with geography alone. To explore morphological differentiation among populations, a Principal Component Analysis (PCA) was performed in R using ‘factoextra’ (Kassambara, Mundt, 2017), ‘FactoMineR’ (Lê *et al.*, 2008), ‘ggplot2’

(Wickham, 2011), and ‘adeigenet’ (Jombart, 2008) packages. The specimens used in these analyses are indicated in parentheses in the list of examined material (see taxonomy section in Results). The PCA was conducted with size-adjusted multivariate morphometric data, with all measurements expressed as percent of standard length (SL) and head length (HL). Prior to the analyses, variables were centered and standardized to unit variance in R using the functions `scale()` and `prcomp()`, to minimize the influence of absolute body size and differences in measurement scale among variables. Additionally, to minimize possible effects of allometric components on comparisons among groups we only included adults and subadults so as to ensure that the groups are comprised of specimens with similar size ranges. To identify statistically significant differences in overall morphometric variation among the two morphotypes, we applied a permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) using the `adonis2` function in the ‘vegan’ package (Oksanen *et al.*, 2013) on a Euclidean distance matrix of the full dataset. To visualize the variance explained by each principal component and the clustering of individuals, a scatter plot of the first two principal components was generated using ‘ggplot2’ and ‘factoextra’ packages. We generated PCA eigenvalue plots (scree plots) and PC1–PC2 scatterplots to summarize the variance explained and visualize sample clustering. We then extracted the component loading scores to identify which measurements contributed most to the separation among groups. All figures were finalized in ‘ggplot2’. A formal description of the new species is provided in the Taxonomy section in Results.

Taxonomy. We used an integrative taxonomic approach (*sensu* Padial *et al.*, 2010) to delimitate species, formally recognizing lineages that are independently diagnosed by morphologic and molecular characters. In the lists of examined specimens (comparative and 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 within the lot with the range of their standard lengths, the number of specimens measured for morphometric analyses, with the range of their standard lengths in parentheses; the number of specimens used for molecular data indicated as “tis”, and, when available, the number of cleared and stained specimens indicated as “c&s”. To avoid unnecessary proliferation of paratypes, only lots of specimens that were measured, photographed or representative of adult morphology were formally designated as paratypes of the newly described species, the remaining specimens were listed as additional non-type material.

Specimens examined for comparative purposes are listed in Mejia *et al.* (2023, 2025), and Mejia, Buckup (2024). Comparative data for species not directly examined were obtained from published species descriptions and/or high-resolution photographs of type specimens available in the All Catfish Species Inventory Image Base (Morris *et al.*, 2006). Institutional abbreviations follow Sabaj (2020). In species descriptions, values within parentheses denote the number of specimens corresponding to the associated counts, and an asterisk indicates values corresponding to the holotype.

RESULTS

Genetic diversity, phylogenetic reconstruction and population structure. Analysis of COI gene polymorphism revealed notable differences between two *Rineloricaria* morphotypes from the Paraíba do Sul, Itabapoana, Itapemirim, and Benevente River basins. Genetic distances based on the COI gene reflect divergence by geographical origin. Average pairwise distances between the Paraíba do Sul population and the other basins ranged from 0.019 to 0.022 (Tab. 1). The lowest pairwise distance was observed between Itapemirim and Benevente populations (0.002). When populations were grouped according to the operational morphotypes defined above, the average genetic distance between morphotypes was 0.020 (2.0%).

The Paraíba do Sul morphotype exhibited lower haplotype ($H_d = 0.476$) and nucleotide diversity ($\pi = 0.00073$) than the Itabapoana+Itapemirim+Benevente morphotype ($H_d = 0.867$; $\pi = 0.00501$; Tab. 2). Pairwise F_{st} values (0.861) and estimated gene flow ($N_m = 0.08$) indicated strong genetic differentiation between morphotypes (Tab. 1). Neutrality tests (Tajima's D and Fu's F_s) did not deviate significantly from neutral expectations in either morphotype (Tab. 1).

TABLE 1 | Mean K2P genetic distances based on COI sequences among populations (below diagonal) and within populations (diagonal) of *Rineloricaria*. Standard error estimates are presented above the diagonal. Note that the mean distances between populations of the lower Paraíba do Sul River basin and those of the other coastal rivers in Southern Espírito Santo are much greater than those among the latter basins.

	Basin	1	2	3	4
1	Paraíba do Sul	0.001	0.005	0.005	0.005
2	Itabapoana	0.019	0.002	0.003	0.003
3	Itapemirim	0.022	0.009	0.003	0.001
4	Benevente	0.022	0.007	0.002	0

TABLE 2 | Genetic diversity, population differentiation metrics, and neutrality statistics based on COI gene for two *Rineloricaria* morphotypes. Morphotype M1 from Paraíba do Sul River basin; Morphotype M2 from Itabapoana, Itapemirim, and Benevente rivers. Neutrality tested with Tajima's D and Fu's F_s ; "ns" = not significant at $P \geq 0.05$.

Parameter	<i>Rineloricaria steindachneri</i> (M1)	<i>Rineloricaria capixaba</i> (M2)	Between Morphotypes
Number of sequences	7	15	22
Number of haplotypes (h)	2	6	8
Haplotype diversity (H_d)	0.476	0.867	0.892
Number of polymorphic sites (S)	1	11	20
Nucleotide diversity (π)	0.00073	0.00501	0.01174
Average nucleotide divergence (Dxy)	-	-	0.02066
Fixation index (F_{st})	-	-	0.861
Estimated gene flow (N_m)	-	-	0.08
Tajima's D	0.012	-0.885	-
Fu's F_s	0.589	0.501	-

Neotropical Ichthyology, 24(3):e260024, 2026



FIGURE 1 | Maximum-likelihood tree among 22 specimens of *Rineloricaria* with nucleotide substitutions optimized under parsimony. Letter codes within circles indicate the two species recognized in this study. Nucleotide substitutions are numbered according to the concatenated matrix of genes (COI: 1–654, CytB: 655–1704, RAG1: 1705–2712). The concatenated matrix included *R. zawadzki* as outgroup. Polymorphic positions, where chromatograms showed overlapping peaks, are indicated by their IUPAC codes (Y = C/T; S = G/C). Palaeodrainages P-numbers follow Thomaz, Knowles (2018).

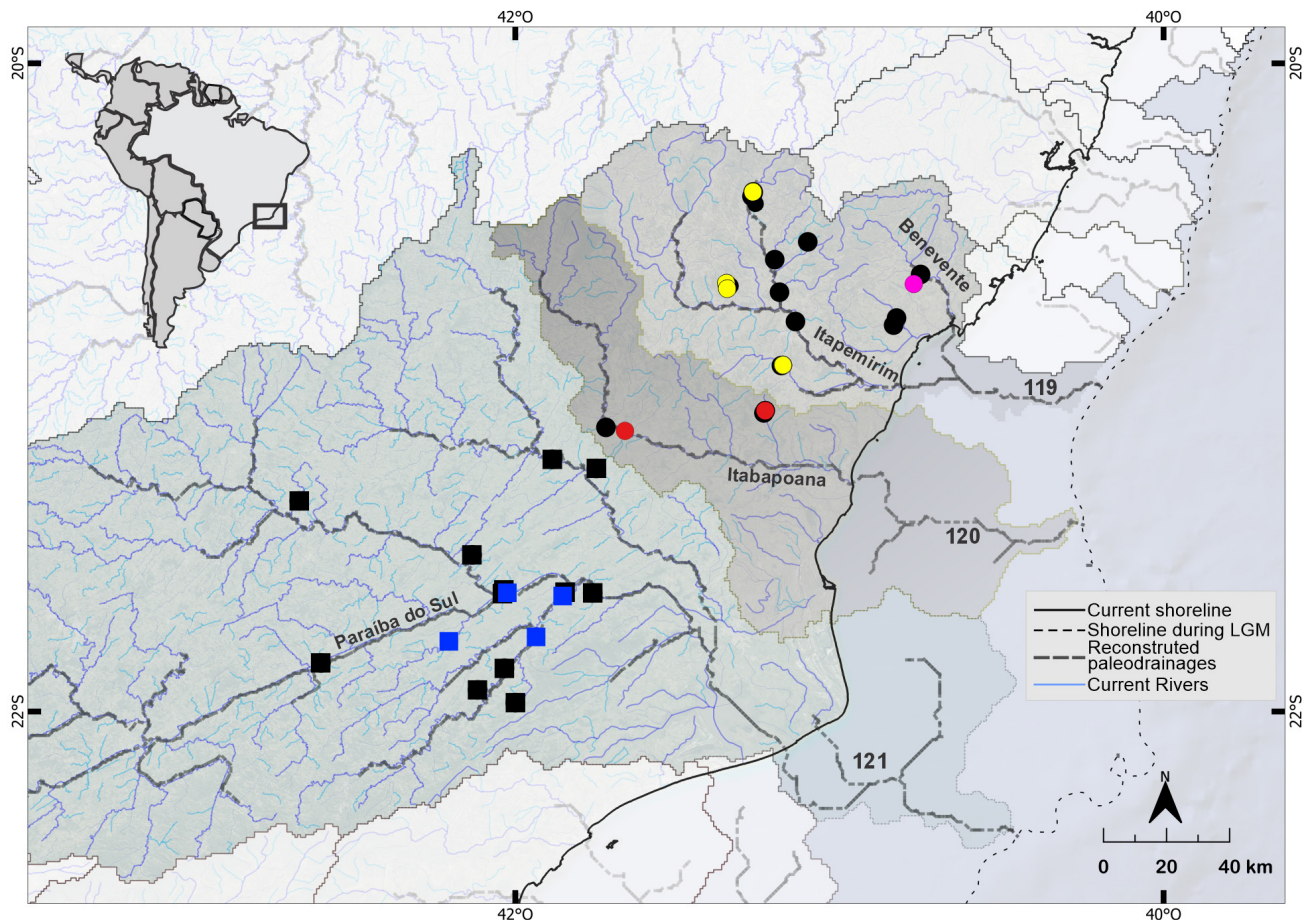


FIGURE 2 | Geographic distribution of *Rineloricaria steindachneri* and *R. capixaba*. Color indicates river basins: Paraíba do Sul basin (blue), Itabapoana basin (red), Itapemirim basin (yellow), and Benevente basin (purple). Symbols indicate species: squares represent *R. steindachneri* and circles represent *R. capixaba*. Black symbols indicate localities from which molecular data were not obtained. Each symbol may represent more than one sample.

Median-joining networks for COI and CytB genes closely mirrored the ML tree, with mitochondrial haplotypes exhibiting stronger geographic clustering than the nuclear RAG1 haplotypes (Fig. 3). Eight COI and 11 CytB haplotypes were detected, each confined to a single river basin except for one COI haplotype shared between the Benevente and Itapemirim basins (Fig. 3). In contrast, only three RAG1 haplotypes were detected, two of which were found in both Paraíba do Sul and Itapemirim specimens.

AMOVA (Tab. 3) corroborated the strong geographic structure inferred from pairwise K2P distances, haplotype networks, and the ML tree. A highly significant genetic structure was recovered under all grouping schemes (Tab. 3). The strongest structure was observed when populations were grouped according to the palaeodrainage hypothesis, separating the Paraíba do Sul, Itabapoana, and Benevente + Itapemirim drainages ($\Phi_{ST} = 0.9783$). A high differentiation was also recovered when the four contemporary drainages were treated separately ($\Phi_{ST} = 0.9728$), whereas grouping samples into two morphotypes resulted in a slightly lower, although still very high, structure value ($\Phi_{ST} = 0.9397$). These results indicate strong genetic differentiation among coastal basins, while also supporting a closer historical relationship between the Benevente and Itapemirim drainages.

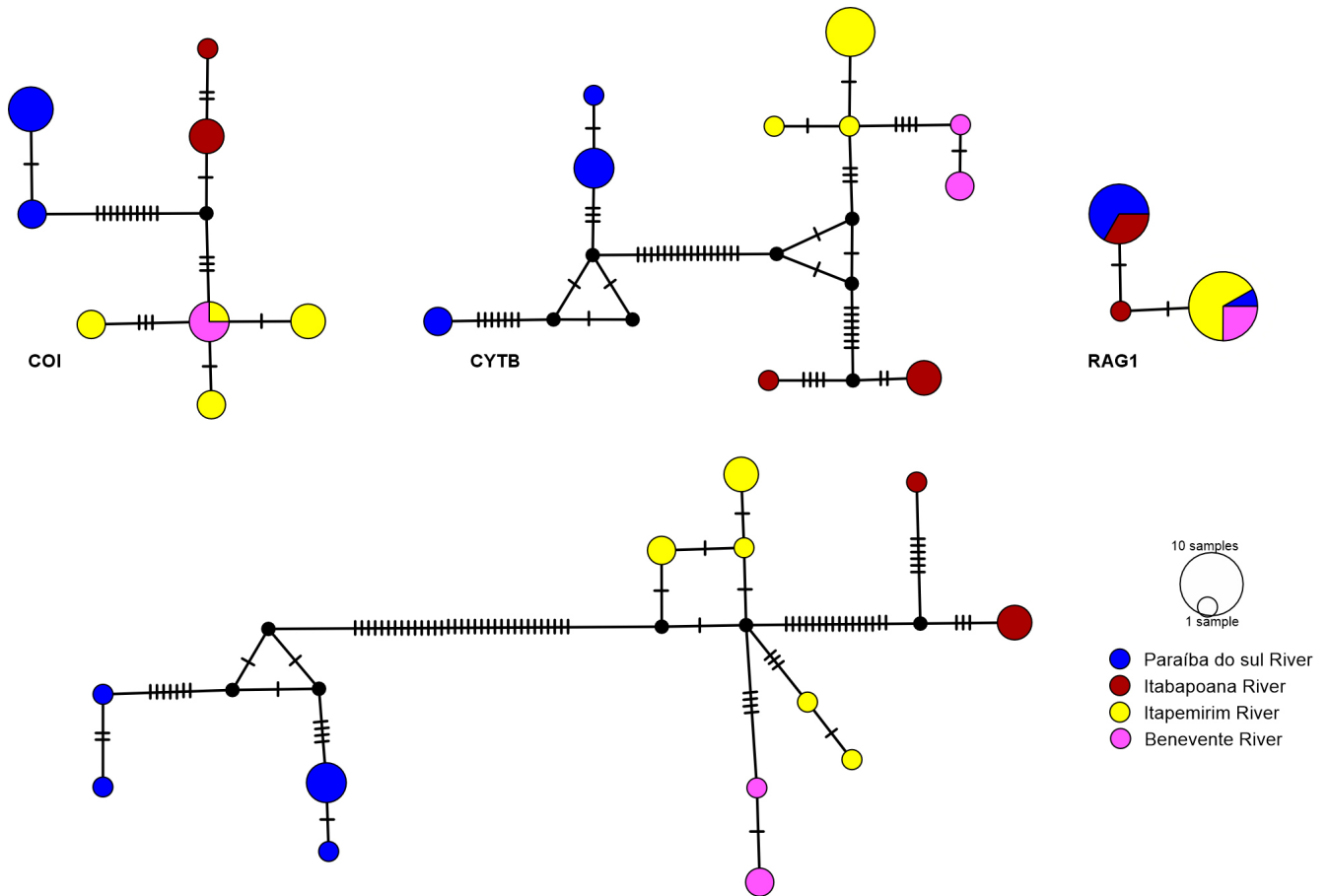


FIGURE 3 | Haplotype network of *Rineloricaria* samples generated with median-joining approach, with geographic locations identified by colors.

TABLE 3 | Molecular Variance Analysis (AMOVA) and genetic diversity in *Rineloricaria* populations. * = Significant Φ_{ST} values ($P < 0.05$).

Population grouping	Φ_{ST}	Variance between groups	Variance within groups
(1) Four separated populations (Paraiba do Sul, Itabapoana, Itapemirim, and Benevente)	0.9728*	1.4188e-04	3.9711e-06
(2) Three populations (Paraiba do Sul, Itabapoana, Benevente+Itapemirim)	0.9783*	1.6521e-04	3.6631e-06
(3) Two populations (Paraiba do Sul vs. Benevente + Itapemirim + Itabapoana)	0.9397*	2.1252e-04	1.3629e-05

Morphological differentiation. Results of the Principal Component Analysis (PCA) confirmed the morphological and geographic separation between the two *Rineloricaria* morphotypes (morphotype 1: Paraíba do Sul; morphotype 2: Itabapoana + Itapemirim + Benevente). The first three principal components together explained 75.0 % of the multivariate diversity (PC1 = 55.9%, PC2 = 11.7%, PC3 = 7.4%; Fig. 4B). Specimens of each morphotype formed distinct clusters along the PC1–PC2 axes, without overlap (Fig. 4A). PC1 was driven primarily by distances correlated with overall body-length and caudal-peduncle width, and PC2 was mostly influenced by unbranched fin-ray counts and eye diameter. Group membership explained 76.6% of the variance in the Euclidean distance matrix ($R^2 = 0.7658$, $F_{1,31} = 101.37$, $P = 0.001$). The variables with the strongest contributions to each axis are highlighted in Tab. 4. In addition to the multivariate separation revealed by PCA, the two morphotypes differ in diagnostic characters detailed in the Taxonomy section below.

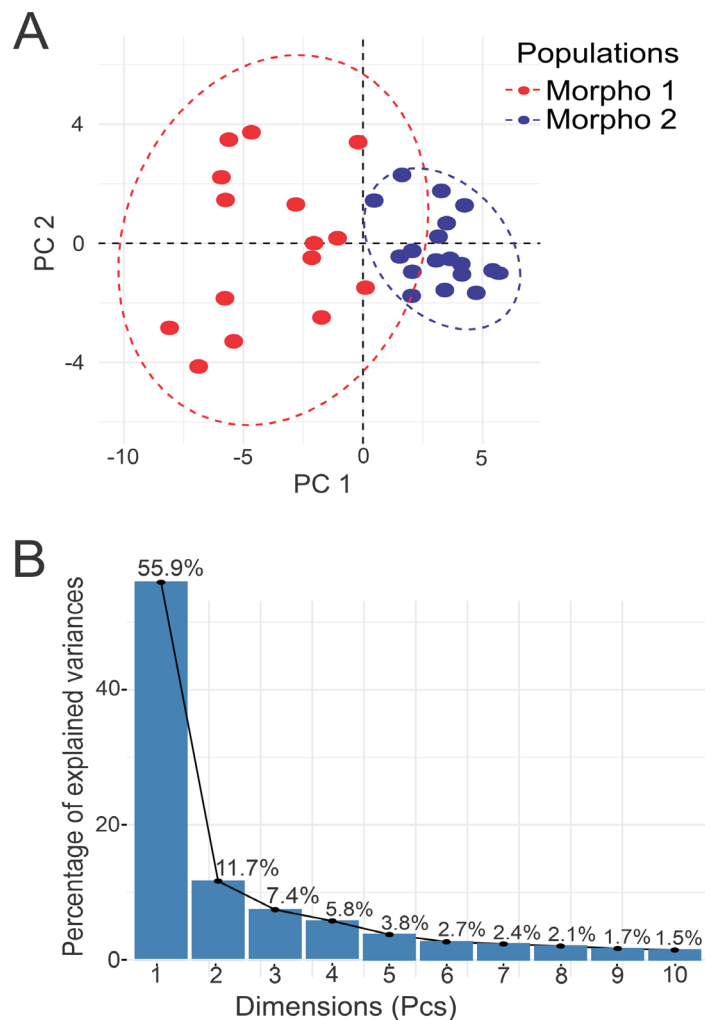


FIGURE 4 | Morphometric differentiation of *Rineloricaria* morphotypes. **A.** Scatterplot of individual scores on the first two principal components (PC1 vs. PC2) for morphotype 1 (*R. steindachneri*, Paraíba do Sul; red), morphotype 2 (*R. capixaba*, Itabapoana + Itapemirim + Benevente; blue). Ellipses represent 95% confidence intervals for each group. **B.** Eigenvalues plot showing the percentage of variance explained by the first ten principal components.

TABLE 4 | Variable loadings for the first three axes of the Principal Component Analysis of *Rineloricaria* morphometric data, highlighting the highest eigenvector values.

	Characters	Axis 1	Axis 2	Axis 3
1	Head length	-0.234	-0.010	0.073
2	Predorsal length	-0.239	-0.003	0.025
3	Postdorsal length	0.236	-0.004	0.038
4	Prepectoral length	-0.224	0.118	0.021
5	Postpectoral length	0.183	-0.209	0.172
6	Prepelvic length	-0.226	-0.102	-0.062
7	Postpelvic length	0.232	0.017	0.113
8	Preanal length	-0.232	-0.124	0.033
9	Postanal length	0.236	0.066	0.021
10	Unbranched dorsal-fin ray	-0.186	0.146	0.278
11	Unbranched pectoral-fin ray	-0.141	0.284	0.329
12	Unbranched pelvic-fin ray	-0.145	0.303	0.290
13	Unbranched anal-fin ray	-0.124	0.358	0.155
14	Thoracic length	-0.171	-0.250	-0.063
15	Abdominal length	-0.187	-0.170	0.221
16	Cleithral width	-0.228	0.055	0.069
17	Depth at dorsal-fin origin	-0.217	-0.011	-0.175
18	Width at anal-fin origin	-0.218	-0.147	-0.023
19	Caudal peduncle depth	-0.232	-0.113	0.010
20	Caudal peduncle width	-0.234	0.014	0.025
21	Snout length	-0.091	0.247	-0.243
22	Eye diameter	0.082	0.387	0.018
23	Maximum orbital diameter	0.148	0.232	0.121
24	Interorbital width	-0.040	0.096	-0.170
25	Internarial width	0.043	0.107	-0.174
26	Head depth	-0.152	0.046	-0.408
27	Head width	-0.164	0.152	0.046
28	Free maxillary barbel	0.005	0.321	-0.303
29	Ventrorostral length	0.181	0.035	0.217
30	Lower lip length	0.035	0.214	-0.348

Taxonomic account

Rineloricaria capixaba, new species

(Figs. 5–6A, B; Tab. 5)

urn:lsid:zoobank.org:act:4089CE38-79E5-454A-828A-E1292D556093

Rineloricaria steindachneri (non Regan, 1904). —Sarmiento-Soares, Martins-Pinheiro, 2013:13 (checklist). —
Vieira-Guimarães *et al.*, 2024:15 (checklist).

Rineloricaria sp. —Vieira *et al.*, 2014:79 (checklist).

Holotype. MBML 10371, 171.1 mm SL, Brazil, Espírito Santo State, Conceição do Castelo, Itapemirim River basin, Castelo River upstream of Viçosa Small Hydroelectric Powerplant, 20° 23' 57" S 41° 15' 58" W, 7 Jul 2015, E. H. de Barros.



FIGURE 5 | *Rineloricaria capixaba*, holotype, MBML 10371, 171.1 mm SL, male, Brazil, Espírito Santo State, Conceição do Castelo municipality, Itapemirim River. Lateral, dorsal, and ventral views.

Paratypes. All from Brazil, Espírito Santo State. Itabapoana River basin: MNRJ 23005, 1 alc, 93.6 mm SL, Mimoso do Sul, Córrego do Farol (tributary of Preto River), BR-101, downstream from São José das Torres, 21°04'43"S 41°13'59"W, 6 Aug 2001, P. A. Buckup, A. T. Aranda & F. A. G. Melo. MNRJ 51332, 1 alc (177.52 mm SL), Bom Jesus do Itabapoana, Itabapoana River, 21°07'24"S 41°43'14"W, 8 Oct 2010, I. Verissimo. MNRJ 54075, 1 alc (107.3 mm SL), 3 tis, Mimoso do Sul, Ribeirão das Flores (tributary of Preto River), 160 m W of BR-101 highway, near exit to São José das Torres, 21°04'18"S 41°13'44"W, 12 Oct 2022, P. A. Buckup, I. C. A. Souto-Santos & J. E. Mejia de Loayza. Itapemirim River basin: MBML 3202, 2 alc, (2, 1.73–166.12 mm SL), Castelo, Itapemirim River basin, on ES-475 road, 20°36'20"S 41°11'59"W, no date, J. L. Helmer. MBML 3923, 1 alc (173.4 mm SL), Cachoeiro de Itapemirim, Itapemirim River upstream of the mouth of Córrego Jacaré, 20°47'48"S 41°08'09"W, 14 Oct 2010, M. P. Gomes. MBML 7358, 2 alc (2, 159.9–166.1 mm SL), Conceição do Castelo, Castelo River at the site of Viçosa Small Hydroelectric Powerplant, Itapemirim River basin, 20°23'48"S 41°15'58"W, 27 Set 2013, E. H. Barros & R. L. Teixeira. MBML 10364, 2 alc, 136.7–139.3, (1, 139.3 mm SL), Conceição do Castelo, Castelo River upstream of Viçosa Small Hydroelectric Powerplant, 20°24'32"S 41°16'10"W, 6 Jul 2005, E. H. Barros. MBML 11215, 1 alc (100.9 mm SL), Castelo, Ribeirão Bateia, tributary of Prata River, Parque Estadual do Forno Grande, 20°33'03"S 41°05'52"W, 24 Nov 2014, K. R. Santos. MNRJ 36652, 2 alc, 69.5–128.1 mm SL, (1, 128.1 mm SL), 1 tis, Cachoeiro de Itapemirim, Ribeirão Floresta, Burarama, 20°41'04"S 41°20'39"W, 1 Oct 2009, L. Sarmento-Soares & R. Martins-Pinheiro. MNRJ 54102, 1 alc (107.3 mm SL), 2 tis, Atílio Vivacqua, Muqui do Norte River, secondary road east of Atílio Vivacqua, tributary of Muqui River, 20°55'53"S 41°10'25"W, 13 Oct 2022, P. A. Buckup, I. C. A. Souto-Santos & J. E. Mejia de Loayza. MNRJ 54152, 2 alc, 111.1–129.5 mm SL, (1, 129.5 mm SL), 2 tis, Cachoeiro de Itapemirim, Ribeirão Floresta, bridge upstream of Burarama, 20°40'42"S 41°20'50"W, 13 Oct 2022, P. A. Buckup, I. C. A. Souto-Santos & J. E. Mejia de Loayza. Benevente River basin: MNRJ 54181, 6 alc, 37.5–114.9 mm SL, (1, 114.9 mm SL), 3 tis, Alfredo Chaves, channelized stream, near Cachoeira de Cima, upstream from the Alfredense Temple, 20°40'51"S 40°46'15"W, 15 Oct 2022, P. A. Buckup, I. C. A. Souto-Santos & J. E. Mejia de Loayza.

Non-types. All from Brazil, Espírito Santo State. Itabapoana River basin: MBML 3724, 1 alc, 134.37 mm SL, Mimoso do Sul, Ribeirão da Flores, tributary of Preto River, on José Alves Toledo road, 21°04'18"S 41°13'44"W, 22 Oct 2010, L. M. Sarmento-Soares, R. F. Martins-Pinheiro, M. M. C. Roldi, M. M. Lopes & M. M. Martinelli. MNRJ 55819, 1 alc, 154.2 mm SL, 1 tis, Bom Jesus do Itabapoana, Itabapoana River, about 100 m upstream of bridge on Santo Eduardo road, 21°08'03"S 41°39'42"W, 30 Oct 2024, T. F. Barros & R. G. Pereira. Itapemirim River basin: MNRJ 54158, 4 alc, 28.1–62.9 mm SL, 3 tis, Conceição do Castelo, Castelo River between the access bridge to Viçosa Small Hydroelectric Powerplant and upstream waterfall (site of an abandoned old Small Hydroelectric Powerplant), 20°23'48"S 41°16'00"W, 13 Oct 2022, P. A. Buckup, I. C. A. Souto-Santos & J. E. Mejia de Loayza. MBML 9628, 1 alc, 92.3 mm SL, Conceição do Castelo, Castelo River downstream of Viçosa Small Hydroelectric Powerplant, 20°24'37"S 41°16'12"W, 3 Nov 2014, E. H. Barros. Iconha River basin: MBML 8432, 1 alc, 120.9 mm SL, Iconha, Itapoama River near BR-101 highway, 20°48'28"S

40° 49'59"W, 16 May 2014, T. A. Volpi, M. M. Lopes, K. B. S. de Paula & E. L. Muhl. MBML 12205, 2 alc, 93.5–113.1 mm SL, Iconha, Iconha River, upstream near ES-375 highway, 20° 47'09"S 40° 49'26"W, 14 Dec 2016, G. I. Almeida & A. Anastácio.

Diagnosis. *Rineloricaria capixaba* is distinguished from its congeners except *R. aequalicuspis* Reis & Cardoso, 2001, *R. altipinnis* (Breder, 1925), *R. anhuapitan* Ghazzi, 2008, *R. anitae* Ghazzi, 2008, *R. baliola* Rodriguez & Reis, 2008, *R. buckupi* Mejia, Ferraro & Souto-Santos, 2025, *R. cacerensis* (Miranda Ribeiro, 1912), *R. cachivera* Urbano-Bonilla, Londoño-Burbano & Carvalho, 2023, *R. capitonia* Ghazzi, 2008, *R. caracasensis* (Bleeker, 1862), *R. daraha* Rapp Py-Daniel & Fichberg, 2008, *R. eigenmanni* (Pellegrin, 1908), *R. fallax* (Steindachner, 1915), *R. formosa* Isbrücker & Nijssen, 1979, *R. hasemani* Isbrücker & Nijssen, 1979, *R. heteroptera* Isbrücker & Nijssen, 1976, *R. isaaci* Rodriguez & Miquelarena, 2008, *R. jaraguensis* (Steindachner, 1909), *R. jubata* (Boulenger, 1902), *R. konopickyi* (Steindachner, 1879), *R. latirostris* (Boulenger, 1900), *R. maacki* Ingenito, Ghazzi, Duboc & Abilhoa, 2008, *R. malabarbai* Rodriguez & Reis, 2008, *R. maquinensis* Reis & Cardoso, 2001, *R. melini* (Schindler, 1959), *R. microlepidogaster* (Regan, 1904), *R. morrow* Fowler, 1940, *R. nudipectoris* Mejia, Ferraro & Buckup, 2023, *R. osvaldoi* Fichberg & Chamon, 2008, *R. pentamaculata* Langeani & de Araujo, 1994, *R. phoxocephala* (Eigenmann & Eigenmann, 1889), *R. platyura* (Müller & Troschel, 1849), *R. quilombola* Chamon & Fichberg, 2022, *R. reisi* Ghazzi, 2008, *R. rodriguezae* Costa-Silva, Oliveira & Silva, 2021, *R. rupestris* (Schultz, 1944), *R. teffeana* (Steindachner, 1879), *R. steindachneri*, *R. stewartia* (Eigenmann, 1909), *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). It differs from *R. aequalicuspis*, *R. anhuapitan*, *R. baliola*, *R. capitonia*, *R. latirostris*, *R. maacki*, *R. malabarbai*, *R. maquinensis*, *R. microlepidogaster*, *R. nudipectoris*, *R. reisi*, and *R. tropeira* by the extensive plate covering of the ventral surface of the pectoral girdle (*vs.* absence of plates on most of ventral surface of 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 five or six inconspicuously keeled plates (*vs.* mid-dorsal series consisting of six to twelve keeled plates extended posteriorly to approximately posterior end of dorsal-fin base). It differs from *R. isaaci*, *R. jaraguensis*, *R. pentamaculata*, *R. rupestris*, *R. stewarti*, and *R. zawadzkii* by presenting 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 the lack of hypertrophied odontodes on the dorsum of the head and predorsal region of breeding males (*vs.* hypertrophied odontodes on the dorsum of the head and predorsal region present in breeding males). It differs from *R. anitae* and *R. daraha* by having three or four 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. rodriguezae* by its narrow head (head width 61.1–70.8% *vs.* 73.5–93.5% in *R. cachivera* and 71.6–81.2% HL in *R. rodriguezae*). It is distinguished from *R. buckupi* by the smaller eye diameter (14.4–17.1% *vs.* 20.1–22.3% HL). The new species (Figs. 6C–E) differs from *R. steindachneri* (Figs. 6A–B), the most



FIGURE 6 | Morphological variation of *Rineloricaria steindachneri* (A. MNRJ 54703, 112.7 mm SL; B. MNRJ 54725, 107.9 mm SL), and *R. capixaba* from the Itabapoana River basin (C. MBML 3724, 134.4 mm SL), the Itapemirim River basin (D. MBML 10364, 139.3 mm SL) and the Benevente River basin (E. MBML 54181, 114.8 mm SL).

similar and geographically closest species, by having less pronounced keeled pre-dorsal and mid-dorsal plate series (*vs.* strongly keeled), by a conspicuous black marginal band on the distal portion of dorsal and caudal-fin rays (*vs.* faint black marginal band formed by conspicuous dark dots), and by the lack of a caudal-fin filament (*vs.* upper caudal-fin filament present). A genetic distance comparison with *Rineloricaria steindachneri* is presented in Tab. 1.

Description. Morphometric data of holotype and paratypes in Tab. 5. Body depressed at caudal peduncle, tapering posteriorly from dorsal fin. In lateral view, dorsal profile slightly convex from tip of snout to origin of dorsal fin, concave at dorsal-fin base insertion, straight from base of dorsal fin to base of caudal fin. Head elongated in dorsal view, with triangular profile. Body widest at pectoral-fin origin, progressively narrowing until end of caudal peduncle. Snout tip with small area of skin without odontodes, not reaching 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. Maxillary barbel short. Teeth bicuspidate; dentary teeth larger than those of premaxilla; premaxilla with 6*(7), 7(7); dentary teeth with 6(4), 7*(10).

TABLE 5 | Morphometric data of holotype and paratypes of *Rineloricaria capixaba*. Range includes the holotype. N = Number of specimens measured; SD = Standard deviation.

	Holotype	N	Range	Mean	SD
Standard length (mm)	171.1	–	93.6–177.5	–	–
Percent of standard length					
Head length	25.1	15	21.8–25.1	23.6	1.1
Predorsal length	35.1	15	31.3–35.7	33.8	1.5
Postdorsal length	65.0	15	63.9–68.6	66.2	1.3
Prepectoral length	19.9	15	17.2–20.2	19.0	0.8
Postpectoral length	81.9	15	81.4–84.6	82.8	1.0
Prepelvic length	34.9	15	30.9–35.6	33.2	1.7
Postpelvic length	66.8	15	66.3–71.4	68.2	1.5
Preanal length	50.0	15	43.4–51.8	48.3	2.1
Postanal length	50.1	15	49.1–54.8	52.0	1.6
Unbranched dorsal-fin ray	20.5	15	16.3–21.3	19.4	1.6
Unbranched pectoral-fin ray	15.4	15	13.1–16.8	15.3	1.1
Unbranched pelvic-fin ray	13.6	15	12.2–15.6	14.1	1.0
Unbranched anal-fin ray	15.3	15	14.3–18.2	16.2	1.1
Pectoral-pelvic fin distance	15.8	15	13.2–17.0	14.9	1.3
Abdominal length	17.5	15	15.4–19.4	17.3	1.0
Cleithral width	17.0	15	15.2–18.1	16.7	0.9
Depth at dorsal-fin origin	9.9	15	8.1–10.7	9.7	0.7
Width at anal-fin origin	12.5	15	10.1–14.0	12.2	1.0
Caudal peduncle depth	1.9	15	1.3–2.1	1.7	0.2
Caudal peduncle width	3.5	15	2.7 – 3.7	3.4	0.3
Percent of head length					
Snout length	52.7	15	51.2–54.5	52.9	0.9
Eye diameter	13.7	15	12.2–17.1	14.7	1.5
Maximum orbital diameter	18.7	15	17.8–22.1	19.9	1.4
Interorbital width	19.8	15	18.8–21.7	20.3	0.9
Internarial width	7.7	15	6.3–8.7	7.6	0.6
Head depth	35.9	15	34.1–39.8	36.8	1.4
Head width	64.8	15	61.7–69.5	65.5	2.1
Free maxillary barbel	4.9	15	4.6–10.5	6.7	1.7
Ventro-rostral length	6.3	15	5.1–7.7	6.3	0.8
Lower-lip length	16.6	15	12.8–19.5	16.9	1.9

Two predorsal plates between parieto-supraoccipital and nuchal plate with two low ridges. Five (dorsal, mid-dorsal, median, mid-ventral, ventral) lateral series of plates below dorsal fin. Lateral line complete. Plates of dorsal series 21(6), 22*(8), 23(1). Plates of mid-dorsal series 5(7), 6*(8), weakly keeled. Plates of median series 28*(14), 29 (1). Plates of mid-ventral series 15(2), 16(5), 17*(8). Median and mid-ventral series with slightly pronounced keels; keels coalescent in 10th(2), 11th*(7), 12th(5), 13th(1) plate of median series. Ventral series with 21(1), 22(5), 23*(9) plates. Lateral abdominal series with 6(7), 7*(8) 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; these polygonal plates bordered anteriorly 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 small polygonal plates.

Dorsal-fin spinelet present. Dorsal-fin rays i,7*(15); unbranched ray (dorsal spine) length shorter than head length (Tab. 5). Dorsal-fin origin slightly posterior to pelvic-fin origin; tip of dorsal fin when adpressed surpassing anal-fin origin, reaching seventh 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; second and third branched rays slightly longer than unbranched ray. Anal-fin rays i,5*(15); posterior anal-fin margin rounded. Caudal fin slightly emarginated with i,10,i*(15) rays; upper unbranched ray slightly longer than lower one; caudal-fin filament absent.

Coloration in alcohol. Background color of dorsal surface of head and body light brown, with four (or five) transverse dark brown bars; first at dorsal-fin origin, three (or four) on the dorsum of caudal peduncle. Dorsal fin with conspicuous dark brown band across distal region, reaching margin of fin; bands with chromatophores more densely distributed on two anterior branched rays, becoming inconspicuous along posterior rays. Pectoral, pelvic, and anal fin with dark dots aligned across fin rays forming weak bands. Caudal fin with two vertical dark bands: one basal, one distal at margin of fin; both bands equally broad, solidly pigmented. Ventral surface pale yellow (Figs. 5, 6C–E).

Sexual dimorphism. Mature males with abundant hypertrophied odontodes on lateral margins of head. Dorsal surface of pectoral-fin rays of mature males covered almost entirely with thin, long odontodes with curved tips. Unbranched pectoral-fin ray thick, strongly curved, with short, densely arranged odontodes (Fig. 5).

Geographical distribution. *Rineloricaria capixaba* is known from the Itabapoana, Itapemirim, Iconha and Benevente river basins, Espírito Santo state, Brazil (Fig. 2). Specimens were collected in moderately large streams (1–2 m deep) with variable current speeds, typically associated with sandy or muddy bottoms and marginal submerged vegetation. *Rineloricaria capixaba* is restricted to the southeastern region of the Espírito

Santo state, south of the Jucu River basin. Based on extensive sampling of northern streams of Espírito Santo documented in the MBML and MNRJ ichthyological collections, species of *Rineloricaria* does not occur northward of the Benevente River basin.

Conservation status. *Rineloricaria capixaba* is restricted to coastal river basins in the southern region of the Espírito Santo state, Brazil. The Extent of Occurrence of *Rineloricaria capixaba* is estimated to be approximately 3249 km², determined through the minimum convex polygon method. No significant threats to the species have been identified. Therefore, *Rineloricaria capixaba* may be classified in the Least Concern (LC) category according to the IUCN criteria for evaluation of risks of extinction (IUCN Standards and Petitions Committee, 2024).

Etymology. The specific epithet, *capixaba* is used as an adjective. This is a Brazilian Portuguese word derived from the Tupi-Guarani native American language. It originally meant “plantation land” but the term is currently used as the demonym to designate inhabitants of the Brazilian State of Espírito Santo, where the new species is distributed.

DISCUSSION

Our analysis revealed clear genetic and morphological distinction between the two *Rineloricaria* morphotypes: *R. steindachneri* and the newly described *R. capixaba*. These results confirming our earlier delimitation of the former species (Mejia, Buckup, 2024), which restricted *R. steindachneri* to the lower Paraíba do Sul basin, while the geographic distribution of *R. capixaba* is assigned to the coastal Itabapoana, Itapemirim, Iconha, and Benevente river basins of southern Espírito Santo. Mitochondrial COI data demonstrate strong differentiation ($F_{st} = 0.861$; $N_m = 0.08$) and an average K2P divergence of ~2.0%, supporting their recognition as distinct evolutionary lineages and matching the 2–3% threshold commonly used to delimit freshwater fish species (Ward *et al.*, 2009; Pereira *et al.*, 2013; Ribolli *et al.*, 2017).

Neutrality tests (Tajima’s D and Fu’s F_s) were not significant, in both species for the COI gene, so we cannot reject the standard neutral model; in other words, we found no evidence of recent selection or demographic change in this marker given our sample sizes ($n = 7$ and $n = 15$). Furthermore, *R. capixaba* shows higher haplotype diversity than *R. steindachneri* (Tab. 2), which is expected for a lineage distributed across three isolated basins, each with its own isolated haplotype, compared with the single-basin range of *R. steindachneri* (Fig. 2).

Our results reveal pronounced population structuring in *R. capixaba*. AMOVA results, the multiloci tree (COI + CytB + RAG1), and the median-joining haplotype networks demonstrate strong phylogeographic partitioning, with basin-specific mitochondrial haplotypes (Fig. 3). However, the persistence of a shared RAG1 haplotype in the Paraíba do Sul species indicates that nuclear haplotypes are not fully sorted, a pattern expected under relatively recent isolation (*i.e.*, slower coalescence of nuclear loci). The distribution of lineages of freshwater fishes in the coastal region of Brazil has been influenced by geomorphologic (river-capture events) and Pleistocene sea-level

oscillations (Weitzman *et al.*, 1988; Ribeiro, 2006; Dias *et al.*, 2014; Thomaz, Knowles, 2018; Oliveira-Silva *et al.*, 2023). Our results are partially consistent with the hypothesis that marine regressions intermittently exposed palaeodrainage (at depths of up to –130 m below current sea level during the Last Glacial Maximum), allowing brief inter-basin connections, followed by subsequent transgressions that fragmented rivers drainages and isolated headwater populations (Thomaz, Knowles, 2018). The lowering of sea level during glaciations may have led to connection among coastal rivers facilitating brief dispersal pulses (Thomaz, Knowles, 2018; Oliveira-Silva *et al.*, 2023).

Our findings indicate that alternating marine regressions and transgressions have played a key role in the present geographic distribution of populations of *R. capixaba*. A comparison between the current limits of coastal river basins with the extent of palaeodrainages proposed by Thomaz, Knowles (2018) suggests that the Itapemirim and Benevente rivers belonged to the same palaeodrainage (Palaeodrainage 119) during the Last Glacial Maximum, whereas the Itabapoana was isolated in a separate system (Palaeodrainage 120). Thus, the shared nuclear haplotype between the Itapemirim and Benevente basins likely reflects their past connection, while the relationship between the Itabapoana population and those in these northern basins is more likely to be the result of headwater-capture events rather than direct dispersal through paleochannels. River-capture has been identified as one of the main reasons for the sharing of ichthyofauna between adjacent drainages in Brazilian coastal basins (Ribeiro, 2006; Buckup, 2011; Camelier *et al.*, 2018). Even though no explicit capture event has been documented between the Itapemirim and Itabapoana rivers, their geographic proximity and low watershed divide make this a plausible hypothesis (Piol *et al.*, 2019). Comparable scenarios have been proposed to account for parapatric distributions in the region, such as the armored catfish *Loricariichthys melanurus* Reis, Vieira & Pereira, 2021 (Reis *et al.*, 2021) and the characiform *Oligosarcus acutirostris* Menezes, 1990 (Souza *et al.*, 2023).

Comparisons between modern coastal basins configurations and the palaeodrainage reconstructions of Thomaz, Knowles (2018) indicate that the phylogeographic structure of *R. capixaba* is consistent with alternating phases of connectivity and isolation during the Pleistocene. The affinity between the Itapemirim and Benevente populations matches their inferred shared palaeodrainage under lowered sea levels, whereas the distinctiveness of Itabapoana is more plausibly explained by persistent isolation and/or headwater capture. Together, these patterns support a scenario in which marine regressions intermittently exposed palaeodrainage connections enabling short-lived dispersal, followed by transgressions and drainage rearrangements that promoted vicariant divergence in southeastern Brazilian coastal rivers.

We conclude that mitochondrial and nuclear DNA sequence data, together with multivariate morphometrics, support the recognition of two allopatric and independently diagnosable species within populations formerly identified as *R. steindachneri*. Accordingly, *R. steindachneri* is restricted to the lower Paraíba do Sul River basin, whereas the northern coastal populations are described herein as *R. capixaba*, occurring in the Itabapoana, Itapemirim, Iconha, and Benevente basins of southern Espírito Santo.

ACKNOWLEDGMENTS

The authors thank Thiago S. Soares and Raiane G. Souza (Museu de Biologia Prof. Mello Leitão – MBML, Instituto Nacional da Mata Atlântica) for access to comparative material of *Rineloricaria* from coastal drainage of Espírito Santo. We thank to Luisa Sarmiento-Soares (Universidade Federal do Espírito Santo) for photographs of specimens from Espírito Santo. We are grateful to Marcelo E. Britto, Cristiano R. Moreira, and Anna Salles (MNRJ) for curatorial and technical support. To Rosana Mazzoni for support that facilitated this study. Gustavo A. Ferraro, Igor C. A. Souto-Santos, and Manuela Dopazo (MNRJ) provided important help during fieldwork and laboratory activities.

REFERENCES

- **Anderson MJ.** A new method for non-parametric multivariate analysis of variance. *Austral Eco.* 2001; 26(1):32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>
- **Buckup PA.** The eastern Brazilian shield. In: Albert JS, Reis RE, editors. *Historical biogeography of Neotropical freshwater fishes*. Berkley: University of California Press; 2011. p.203–10.
- **Camelier P, Menezes NA, Costa-Silva GJ, Oliveira C.** Molecular and morphological data of the freshwater fish *Glandulocauda melanopleura* (Characiformes: Characidae) provide evidences of river captures and local differentiation in the Brazilian Atlantic Forest. *PLoS ONE.* 2018; 13(3):e0194247. <https://doi.org/10.1371/journal.pone.0194247>
- **Dias MS, Oberdorff T, Hugueny B, Leprieur F, Jézéquel C, Cornu JF et al.** Global imprint of historical connectivity on freshwater fish biodiversity. *Ecol Lett.* 2014; 17(9):1130–40. <https://doi.org/10.1111/ele.12319>
- **Hall TA.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symp.* 1999; 41:95–98.
- **Handy SM, Deeds JR, Ivanova NV, Hebert PDN, Hanner RH, Ormos A et al.** A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. *J AOAC Int.* 2011; 94(1):201–10. <https://doi.org/10.1093/jaoac/94.1.201>
- **Hudson RR, Slatkin M, Maddison WP.** Estimation of levels of gene flow from DNA sequence data. *Genetics.* 1992; 32(2):583–89. <https://doi.org/10.1093/genetics/132.2.583>
- **International Union for Conservation of Nature (IUCN). Standards and Petitions Subcommittee.** Guidelines for using the IUCN Red List categories and criteria, Version 16 [Internet]. Gland; 2024. Available from: <https://www.iucnredlist.org/resources/redlistguidelines>
- **Isbrücker I, Nijssen H.** Two new species and a new genus of Neotropical mailed catfishes of the subfamily Loricariinae Swainson, 1838 (Pisces, Siluriformes, Loricariidae). *Beaufortia.* 1978; 27(339):177–206. <https://repository.naturalis.nl/pub/504901>
- **Jombart T.** Adegnet: a R package for the multivariate analysis of genetic markers. *Bioinformatics.* 2008; 24(11):1403–05. <https://doi.org/10.1093/bioinformatics/btn129>
- **Kassambara A, Mundt F.** Package ‘factoextra’. Extract and visualize the results of multivariate data analyses. 2017; 76(2):10–18637. <https://doi.org/10.32614/CRAN.package.factoextra>
- **Kimura MA.** A Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol.* 1980; 16:111–20. <https://doi.org/10.1007/BF01731581>

- **Kumar S, Stecher G, Li M, Knyaz C, Tamura K.** MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol. Evol.* 2018; 35(6):1547–49. <https://doi.org/10.1093/molbev/msy096>
- **Lê S, Josse J, Husson F.** FactoMineR: an R package for multivariate analysis. *J. Stat. Soft.* 2008; 25(1):1–18. <https://doi.org/10.18637/jss.v025.i01>
- **Leigh JW, Bryant D.** POPART: full-feature software for haplotype network construction. *Methods Ecol Evol.* 2015; 6(9):1110–16. <https://doi.org/10.1111/2041-210X.12410>
- **Librado P, Rozas J.** DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics.* 2009; 25(11):1451–52. <https://doi.org/10.1093/bioinformatics/btp187>
- **Lima SMQ, Berbel-Filho WM, Araújo TFP, Lazzarotto H, Tatarenkov A, Avise JC.** Headwater Capture Evidenced by Paleo-Rivers Reconstruction and Population Genetic Structure of the Armored Catfish (*Pareiorhaphis garbei*) in the Serra do Mar Mountains of Southeastern Brazil. *Front Gen.* 2017; 8:199. <https://doi.org/10.3389/fgene.2017.00199>
- **Lima SMQ, Berbel-Filho WM, Vilasboa A, Lazoski C, Volpi TA, Lazzarotto H et al.** Rio de Janeiro and other palaeodrainages evidenced by the genetic structure of an Atlantic Forest catfish. *J Biogeogr.* 2021; 48(6):1475–88. <https://doi.org/10.1111/jbi.14091>
- **Lujan NK, Armbruster JW, Lovejoy NR, López-Fernández H.** Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae. *Mol Phylogenet Evol.* 2015; 82:269–88. <https://doi.org/10.1016/j.ympev.2014.08.020>
- **Mejia E, Buckup PA.** Species boundaries of the whiptail catfish *Rineloricaria* (Siluriformes: Loricariidae) from the Paraíba do Sul River drainage, southeastern Brazil, with species redescrptions and description of a new species. *J Fish Biol.* 2024; 105(1):288–13. <https://doi.org/10.1111/jfb.15780>
- **Mejia E, Ferraro GA, Buckup PA.** A new species of *Rineloricaria* (Siluriformes: Loricariidae) from coastal drainages of Rio de Janeiro, southeastern Brazil. *Neotrop Ichthyol.* 2023; 21(1):e220083. <https://doi.org/10.1590/1982-0224-2022-0083>
- **Mejia E, Ferraro GA, Souto-Santos ICA.** A new species of whiptail catfish *Rineloricaria* (Siluriformes: Loricariidae) from Macaé and São João river basins, southeastern Brazil. *Neotrop Ichthyol.* 2025; 23(2):e240087. <https://doi.org/10.1590/1982-0224-2024-0087>
- **Miller SA, Dykes DD, Polesky HF.** A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic acids res.* 1988; 16(3):1215. <https://doi.org/10.1093/nar/16.3.1215>
- **Morris PJ, Yager HM, Sabaj Pérez MH.** ACSImagebase: a digital archive of catfish images compiled by participants in the all catfish species inventory. 2006. Available from <http://acsi.acnatsci.org/base>
- **Nei M.** Molecular evolutionary genetics. Columbia University Press; 1987.
- **Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RN et al.** Package 'vegan'. Community ecology package. 2013; 2(9):1–295. Available from: <https://CRAN.R-project.org/package=vegan>
- **Oliveira-Silva L, Batalha-Filho H, Camelier P, Zanata AM.** Past riverine connectivity effects in population structure and distribution of an endemic freshwater fish from northeastern Brazilian rivers: phylogeographic, taxonomic, and conservation implications. *Freshw Biol.* 2023; 68(10):1685–702. <https://doi.org/10.1111/fwb.14150>
- **Padial JM, Miralles A, De la Riva I, Vences M.** The integrative future of taxonomy. *Front Zool.* 2010; 7:1–16. <https://doi.org/10.1186/1742-9994-7-16>
- **Paradis E.** pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics.* 2010; 26(3):419–20. <https://doi.org/10.1093/bioinformatics/btp696>
- **Pereira LHG, Hanner R, Foresti F, Oliveira C.** Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? *BMC Genet.* 2013; 14:1–14. <https://doi.org/10.1186/1471-2156-14-20>

- **Pio NL, Carvalho TP.** Evidence on the paleodrainage connectivity during Pleistocene: Phylogeography of a hypoptopomatine endemic to southeastern Brazilian coastal drainages. *Neotrop Ichthyol.* 2021; 19(2):e200128. <https://doi.org/10.1590/1982-0224-2020-0128>
- **Piol MVA, Reis JAT, Caiado MAC, Mendonça ASF.** Performance evaluation of flow duration curves regionalization methods. *RBRH.* 2019; 24:e9. <https://doi.org/10.1590/2318-0331.241920170202>
- **QGIS Development Team.** QGIS geographic information system. Chicago, IL: Open Source Geospatial Foundation Project; 2017. Available from: <https://qgis.org/en/site/>
- **R Development Core Team.** R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2016. Available from: <https://www.r-project.org/>
- **Regan CT.** III. A Monograph of the fishes of the family Loricariidae. *Trans Zool Soc Lond.* 1904; 17(3):191–350.
- **Reis RE, Cardoso AR.** Two new species of *Rineloricaria* from southern Santa Catarina and northeastern rio Grande do Sul, Brazil (Teleostei: Loricariidae). *Ichthyol Explor Freshw.* 2001; 12(4):319–32.
- **Ribeiro AC.** Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil: an example of faunal evolution associated with a divergent continental margin. *Neotrop Ichthyol.* 2006; 4(2):225–46. <https://doi.org/10.1590/S1679-62252006000200009>
- **Ribolli J, Scaranto BM, Shibatta OA, Bombardelli RA.** DNA barcoding confirms the occurrence of *Rhamdia branneri* and *Rhamdia voulezi* (Siluriformes: Heptapteridae) in the Iguaçu River basin. *Neotrop Ichthyol.* 2017; 15(1):e160147. <https://doi.org/10.1590/1982-0224-20160147>
- **Sabaj MH.** Codes for Natural History Collections in Ichthyology and Herpetology. *Copeia.* 2020; 108(3):593–669. <https://doi.org/10.1643/ASIHCONDONS2020>
- **Schaefer SA.** The Neotropical cascudinhos: systematics and biogeography of the *Otocinclus* catfishes (Siluriformes: Loricariidae). *Proc Acad Nat Sci Philadelphia.* 1997; 148:1–120.
- **Shelley JJ, Swearer SE, Dempster T, Adams M, Feuvre MC, Hammer MP et al.** Plio-Pleistocene sea-level changes drive speciation of freshwater fishes in north-western Australia. *J Biogeogr.* 2020; 47(8):1727–38. <https://doi.org/10.1111/jbi.13856>
- **Souza V, Oliveira-Silva L, Dutra RT, Camelier P.** Phylogeography of *Oligosarcus acutirostris* (Characiformes: Characidae): testing biogeographic hypotheses in the Northeastern Mata Atlântica freshwater ecoregion. *Neotrop Ichthyol.* 2023; 21(2):e220084. <https://doi.org/10.1590/1982-0224-2022-0084>
- **Stamatakis A.** RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics.* 2014; 30(9):1312–13. <https://doi.org/10.1093/bioinformatics/btu033>
- **Steindachner F.** Beiträge zur Kenntniss der Flussfische Südamerikas (III) und Ichthyologische Beiträge (XI). *Anz Akad Wiss Wien.* 1881; 18(11):97–100.
- **Swofford DL.** PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Mass; 2002.
- **Thomaz AT, Carvalho TP, Malabarba LR, Knowles LL.** Geographic distributions, phenotypes, and phylogenetic relationships of *Phalloceros* (Cyprinodontiformes: Poeciliidae): insights about diversification among sympatric species pools. *Mol Phylogenet Evol.* 2019; 132:265–74. <https://doi.org/10.1016/j.ympev.2018.12.008>
- **Thomaz AT, Knowles LL.** Flowing into the unknown: inferred paleodrainages for studying the ichthyofauna of Brazilian coastal rivers. *Neotrop Ichthyol.* 2018; 16(3):e180019. <https://doi.org/10.1590/1982-0224-20180019>
- **Thomaz AT, Malabarba LR, Bonatto SL, Knowles BLL.** Testing the effect of palaeodrainages versus habitat stability on genetic divergence in riverine systems: study of a Neotropical fish of the Brazilian coastal Atlantic Forest. *J Biogeogr.* 2015; 12(2):389–401. <https://doi.org/10.1111/jbi.12597>
- **Thomaz AT, Malabarba LR, Knowles LL.** Genomic signatures of paleodrainages in a freshwater fish along the southeastern coast of Brazil: Genetic structure reflects past riverine properties. *Heredity.* 2017; 119(4):287–94. <https://doi.org/10.1038/hdy.2017.46>

- **Vera-Alcaraz HS, Pavanelli CS, Zawadzki CH.** Taxonomic revision of the *Rineloricaria* species (Siluriformes: Loricariidae) from the Paraguay river basin. *Neotrop Ichthyol.* 2012; 10(2):285–311. <https://doi.org/10.1590/S1679-62252012000200006>
- **Vieira F, Gasparini JL, Macieira RM.** Guia ilustrado dos peixes da bacia do rio Benevente-ES. Vitória. ACQUA Consultoria e Recuperação de Ambientes Aquáticos Ltda. 2014.
- **Vieira-Guimarães F, Sarmento-Soares LM, Nobre DM, Silva JP, Martins-Pinheiro RF.** Biogeographic patterns of the freshwater fishes from the state of Espírito Santo, eastern Brazil. *Stud. Neotrop Fauna Environ.* 2024; 59(3):727–46. <https://doi.org/10.1080/01650521.2023.2258798>
- **Ward RD, Hanner R, Hebert PD.** The campaign to DNA barcode all fishes, FISH-BOL. *J Fish Biol.* 2009; 74(2):1329–56. <https://doi.org/10.1111/j.1095-8649.2008.02080.x>
- **Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN.** DNA barcoding Australia's fish species. *Philos Trans R Soc Lond B Biol Sci.* 2005; 360(1462):1847–57. <https://doi.org/10.1098/rstb.2005.1716>
- **Weitzman SH, Menezes NA, Weitzman MJ.** Phylogenetic biogeography of the Glandulocaudini (Teleostei Characiformes, Characidae) with comments on the distribution of other freshwater fishes in eastern and Southeastern Brazil. In: Vanzolini PE, Heyer WR, editors. *Proceedings of a Workshop on Neotropical Distribution Patterns* Academia Brasileira de Ciências, Rio de Janeiro; 1988. p.379–427.
- **Wickham H.** ggplot2. *WIREs Comp Stat.* 2011; 3(2):180–85. <https://doi.org/10.1002/wics.147>

AUTHORS' CONTRIBUTION

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

Paulo A. Backup: Conceptualization, Funding acquisition, Supervision, Validation, Writing—review and editing.

FUNDING INFORMATION

This research was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (to EM: CAPES/PROEX 88887.613913/2021–00; to PAB: CNPq/CAPES 88882.156885/2016–01), Conselho Nacional de Pesquisa de Desenvolvimento Científico (to PAB: CNPq 308493/2026–5), and Fundação de Amparo à Pesquisa no Estado do Rio de Janeiro – FAPERJ grants (to Rosana Mazzoni as coordinator, E–26/210.053/2023).

ETHICAL STATEMENT

This study was based on museum specimens and tissue samples already deposited in institutional collections. No live animals were handled by the authors.

DATA AVAILABILITY STATEMENT

Sequence data are available in GenBank and BOLD Systems (accession numbers and sample IDs in Tab. S1). Additional data and code will be deposited in a public repository upon acceptance.

AI STATEMENT

The authors used a generative AI tool only to improve English language clarity and readability. All scientific content, analyses, and interpretations were produced and verified by the authors.

COMPETING INTERESTS

The authors declare no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary Material File

PEER REVIEW FILE

Peer Review File

PEER REVIEW REPORT

Reviewers:

Anonymous reviewer #1

Anonymous reviewer #2

The peer review report is available at: Neotropical Ichthyology

HOW TO CITE THIS ARTICLE

- **Mejia E, Buckup PA.** Past connection and isolation of *Rineloricaria* (Siluriformes: Loricariidae) across southeastern Brazilian coastal drainages: evidence for palaeodrainage connectivity and description of a new species. *Neotrop Ichthyol.* 2026; 24(3):e260024. <https://doi.org/10.1590/1982-0224-2026-0024>

Neotropical Ichthyology

OPEN ACCESS



This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Distributed under Creative Commons CC-BY 4.0

© 2026 The Authors.
Diversity and Distributions Published by SBI



Official Journal of the
Sociedade Brasileira de Ictiologia