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Phylogeography of *Oligosarcus acutirostris* (Characiformes: Characidae): testing biogeographic hypotheses in the Northeastern Mata Atlântica freshwater ecoregion

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The Northeastern Mata Atlântica freshwater ecoregion (NMAF) is recognized for the high degree of endemism of its ichthyofauna, whose evolutionary and biogeographic histories are still poorly understood. *Oligosarcus acutirostris* is a freshwater fish species endemic to the NMAF, which is distributed in coastal rivers and streams draining Bahia, Espírito Santo, and part of Minas Gerais states in eastern Brazil. Its widespread distribution in currently isolated river basins along the NMAF prompted this study, which aimed to understand what scenarios would be involved in determining its current distribution pattern, and to contribute to a better understanding of the biogeographic history of the NMAF. For this, mitochondrial and nuclear DNA sequences were analyzed based on samples from different localities along the species distribution, including its type locality. Overall, phylogeographic analyses indicate a strong genetic structure within the species evidenced mainly by the non-sharing of haplotypes between most of the basins analyzed. According to the AMOVA results, the current distribution of haplotypes is better explained by the Pleistocene coastal paleodrainages. The results are also used to test and complement a biogeographic hypothesis previously proposed for the drainages of the NMAF.

Keywords: Atlantic Forest, Biogeography, Coastal paleodrainages, Ichthyofauna, Neotropical Region.

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A ecorregião de água doce Mata Atlântica Nordeste (NMAF) é reconhecida pelo alto grau de endemismo da sua ictiofauna, cujas histórias evolutiva e biogeográfica ainda são pouco compreendidas. *Oligosarcus acutirostris* é uma espécie de peixe de água doce endêmica da NMAF, que está distribuída em rios e riachos costeiros que drenam os estados da Bahia, Espírito Santo e parte de Minas Gerais, no leste do Brasil. Sua ampla distribuição em bacias atualmente isoladas ao longo da NMAF motivou este estudo, que teve como objetivos entender quais cenários estariam envolvidos na determinação do seu padrão atual de distribuição e contribuir para uma melhor compreensão da história biogeográfica da NMAF. Para isto, foram analisadas sequências de DNA nuclear e mitocondrial, a partir de amostras de diferentes localidades ao longo da distribuição da espécie, incluindo sua localidade tipo. No geral, as análises filogeográficas indicam forte estruturação genética na espécie, evidenciada principalmente pelo não compartilhamento de haplótipos entre a maioria das bacias analisadas. De acordo com os resultados da AMOVA, a distribuição atual dos haplótipos é melhor explicada pelas paleodrenagens costeiras do Pleistoceno. Os resultados obtidos também são utilizados para testar e complementar hipótese biogeográfica previamente proposta para as drenagens da NMAF.

Palavras-chave: Biogeografia, Floresta Atlântica, Ictiofauna, Paleodrenagens costeiras, Região Neotropical.

INTRODUCTION

The Atlantic Forest (AF) hydrographic basins occupy the eastern part of the Brazilian crystalline shield, a region of high topographic complexity, shaped mainly by both Quaternary and Tertiary tectonic activities (Ribeiro, 2006; Thomaz, Knowles, 2018). According to Abilhoa *et al*. (2011), 269 native species of freshwater fishes occur across the AF drainages, distributed among 89 genera and 21 families. These values are certainly underestimated, mainly concerning species and genera due to the most recent description of new taxa from river basins draining this domain (*e.g*., Ferreira *et al*., 2011; Birindelli *et al*., 2013; Pereira *et al*., 2014; Zanata, Camelier, 2015; Zanata *et al*., 2017; Barreto *et al*., 2018; Silva-Junior *et al*., 2020; Oliveira-Silva *et al*., 2022). Furthermore, the ichthyofauna from the AF rivers and streams stands out for its high degree of endemism, and the pattern of species distribution is the result of millions of years of landscape evolution (Bizerril, 1994; Ribeiro, 2006; Menezes *et al*., 2007; Camelier, Zanata, 2014; Silva *et al*., 2020). However, the evolutionary history of AF ichthyofauna is still not fully understood, with limited information on its biogeographic history (Barreto *et al*., 2022), especially of the rivers from northeastern Brazilian coastal basins like those of the Northeastern Mata Atlântica freshwater ecoregion (NMAF; *sensu* Abell *et al*., 2008). The NMAF ecoregion includes all coastal drainages in eastern Brazil in the area from the rio Sergipe in the north to the rio Itabapoana in the south (Abell *et al*., 2008; Hales, Petry, 2013; Camelier, Zanata, 2014).

Camelier, Zanata (2014) carried out a taxonomic inventory of freshwater fishes from 25 drainages of the NMAF ecoregion plus a biogeographic analysis using the Parsimony Analysis of Endemicity (PAE) method to propose a general hypothesis about the relationships of the main river basins comprising this ecoregion. According to the PAE results, there are two main groups of drainages within the NMAF ecoregion, which were defined based on their shared species of freshwater fishes and denominated as North and Central-South Groups (Camelier, Zanata, 2014). The North Group comprises eight drainages from the rio Sergipe to the rio Paraguaçu. On the other hand, the Central-South Group is composed by 16 river basins (from north to south: rio de Contas, Cachoeira, Almada, Una, Pardo, Jequitinhonha, Buranhém, Frades, Jucuruçu, Itanhém, Peruípe, Mucuri, Itaúnas, São Mateus, Doce, and Itapemirim), and it is formed by a polytomy including two groups (*i.e.*, Central Group and South Group) plus the rio Itapemirim basin (see Camelier, Zanata, 2014, fig. 1). According to these authors, the Central-South Group is supported by the presence of *Oligosarcus acutirostris* Menezes, 1990 whose absence in a few drainages of this group is probably due to undersampling.

Several species of *Oligosarcus* Günther, 1864 habitat preferentially upland areas (*i.e.*, headwater streams draining both Brazilian crystalline shield and Argentinian and Bolivian Andean chain) while others primarily occur in the lowland including the Brazilian eastern coastal plain (Menezes, 1988; Ribeiro, Menezes, 2015). *Oligosarcus acutirostris*, originally described from the rio Itapemirim basin, is endemic to the Central-South Group of the NMAF ecoregion occurring from the rio Almada in the north to the rio Itabapoana in the south (Menezes *et al*., 2007; Camelier, 2010; Burger *et al*., 2011; Camelier, Zanata, 2014). It is considered a typical lowland species of *Oligosarcus*, which distribution is restricted to the Atlantic slope coastal plains (Ribeiro, Menezes, 2015; Wendt *et al*., 2019).

Lowland areas are more prone to promote expansions of distributional ranges as compared to upland ones, due to more common favorable dispersion processes, such as the high degree of lateral movements represented by the oscillation of meanders along the whole extension of the floodplain through time and megafan dynamics (Lima, Ribeiro, 2011; Ribeiro, Menezes, 2015). This hypothesis may explain the widespread distribution of *O. acutirostris* along the NMAF in the Central-South Group. Its relatively broad distribution along those rivers makes this species an interesting phylogeographic model to study genetic diversity patterns on isolated freshwater systems within the AF. Here, it is interesting to highlight that some of the main river basins draining the Brazilian AF and whose ichthyofauna exhibits a high degree of endemism and interesting biogeographical patterns are included in this group (*e.g*., Contas, Jequitinhonha, Doce, Mucuri; see Ribeiro, 2006; Camelier, Zanata, 2014). According to Pio, Carvalho (2021), the knowledge of these patterns along the Atlantic Rainforest is still limited, and conservation planning is hindered. In this paper, phylogeographic analyses of *O. acutirostris* were performed, based on molecular data (nuDNA and mtDNA), to understand both the evolutionary history of the species and the river basins of the Central-South Group. *Oligosarcus acutirostris* distributional pattern plus the geomorphological processes and events that are involved with its current distribution are discussed. Therefore, we discussed the putative effects of the current configuration of basins and paleodrainages rearrangements on the genetic structuring of this species and compared these results with the biogeographic hypothesis proposed by Camelier, Zanata (2014). We think that the obtained results will be useful for shed light on both the biogeographic history of the aquatic biota of the AF domain and its river basins, mainly those draining the NMAF ecoregion.

MATERIAL AND METHODS

Taxon sampling, DNA extraction, and sequencing. Tissue samples of 28 individuals of *Oligosarcus acutirostris* were obtained from the following fish collections: Laboratório de Biologia e Genética de Peixes (LBP), Universidade Estadual Paulista, Botucatu; Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS), and Museu Nacional, Rio de Janeiro (MNRJ). We used specimens from all distributional range of *O. acutirostris*, *i.e.*, several coastal drainages from the Almada in the north to the rio Itabapoana in the south, including specimens from the rio Itapemirim, the type locality of this species. Besides specimens of *O. acutirostris*, taxon sampling also included individuals of other two species of the *Oligosarcus* [*O. hepsetus* (Cuvier, 1829) and *O. macrolepis* (Steindachner, 1877), which are included in the same clade of *O. acutirostris* according to Wendt *et al*., 2019] plus [*Astyanax lacustris* (Lütken, 1875) and *Deuterodon intermedius* (Eigenmann, 1908), genera of Characidae closely related to *Oligosarcus* according to Oliveira *et al*., 2011; Mirande, 2018; and Wendt *et al*., 2019]. Sequences of these other species plus one sequence of *O. acutirostris* (from the rio Santo Antônio) were obtained from the GenBank database deposited by Wendt *et al.* (2019). All specimens' vouchers of *O. acutirostris*, including the individual from the rio Santo Antônio, were identified to the species level based on diagnostic morphological traits proposed in the literature (*e.g.*, Menezes, 1987). Species used in the molecular analyses, specimens' vouchers, identification codes of samples, localities, and GenBank accession numbers are given in Tab. 1. Other institutions: Departamento de Zoologia e Botânica, Universidade Estadual Paulista "Júlio de Mesquita Filho", São José do Rio Preto (DZSJRP), Museu de Zoologia João Moojen, Universidade Federal de Viçosa, Viçosa (MZUFV).

Total DNA was extracted from muscle tissues preserved in 96% ethanol using the Wizard Genomic DNA purification kit (Promega; http://www.promega.com), according to manufacturer's instructions. Partial sequences of one mitochondrial marker (cytochrome c oxidase subunit I, COI) and one nuclear (myosin heavy chain 6 cardiac muscle alpha, Myh6) were amplified by Polymerase Chain Reaction (PCR) with the primers already described (*i.e.*, Ward *et al*., 2005 and Melo *et al*., 2011: COI; Li *et al*., 2007: Myh6). Amplifications were performed in a total volume of 15 µl, with 1.5 µl of 10X buffer, 0.6 µl (COI) and 0.8 µl (Myh6) of MgCl2 (50 nM), 2.4 µl of dNTPs (200 nM of each), 0.6 µl each primer, 0.12 µl Taq Platinum® Polymerase (Invitrogen), 8.58 µl (COI) and 8.38 µl (Myh6) of double-distilled water, and 0.6 µl template DNA. The thermo-cycler profile consisted one cycle of an initial denaturation step at 94ºC (COI) and 95ºC (Myh6) for four min; followed by 40 cycles of chain denaturation (30 s at 92ºC) for COI and 35 cycles (40 s at 95ºC) for Myh6, annealing (COI: 30 s at 54ºC for primers FishF1 and FishR1 and 58ºC for COI L6252 and H7271-COXI; Myh6: one min at 60–62ºC), and nucleotide extension (COI: one min at 72ºC; Myh6: one min and 30 s at 72ºC); plus a final extension step at 72ºC for 10 min (COI) and seven min (Myh6). In all PCR reactions, negative controls without DNA were used to check contaminations. The PCR products were first visually identified on a 1% agarose gel and then purified using precipitation in 20% polyethylene glycol (PEG) (Sambrook *et al*., 1989) following the instructions of the manufacturer. Then, the purified PCR products were bidirectionally sequenced using the BigDye Terminator 3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems; www.appliedbiosystems.com). The sequences were read in an automatic sequencer ABI 3500 XL Genetic Analyser (Applied Biosystems) at the Centro de Pesquisas Gonçalo Moniz – Fiocruz, Bahia, Brazil. All sequences produced in this study are available in the GenBank database (accession numbers in Tab. 1).

TABLE 1 | Species, lot number, vouchers, locality, and GenBank accession numbers for the samples used in this study. All samples from Brazil: BA = Bahia, ES = Espírito Santo, MG = Minas Gerais, and RJ = Rio de Janeiro states. The (–) represents absence of sequence for that marker. CT, MNTI and TEC = fish tissue collection from the respective institutions.

Species	Lot number	Voucher	Locality	GenBank accession numbers	
				COI	**Myh6**
Oligosarcus acutirostris	LBP 8331	LBP 40093	Rio do Braço, Almada River basin, Ilhéus, BA	OQ877236	OQ928424
Oligosarcus acutirostris		LBP 40094		OQ877237	OQ928425
Oligosarcus acutirostris		LBP 40095		OQ877225	OQ928426
Oligosarcus acutirostris	UFRGS 22533	TEC 7226A	Santo Antônio River basin, Santa Cruz de Cabrália, BA	MN011638.1	MK991872.1
Oligosarcus acutirostris		TEC 7226B		OQ877216	OQ928415
Oligosarcus acutirostris		TEC 7226C		OQ877215	OQ928416
Oligosarcus acutirostris	UFRGS 22532	TEC 7225A	João de Tiba River basin, Santa Cruz de Cabrália, BA	OQ877224	OQ928411
Oligosarcus acutirostris		TEC 7225B		OQ877232	OQ928412
Oligosarcus acutirostris		TEC 7225C		OQ877233	OQ928413
Oligosarcus acutirostris		TEC 7225D		OQ877234	OQ928414
Oligosarcus acutirostris		TEC 7225E		OQ877235	OQ928436
Oligosarcus acutirostris	LBP 8340	LBP 40116	Mucuri River basin, Carlos Chagas, MG	–	OQ928417
Oligosarcus acutirostris		LBP 40117		–	OQ928418
Oligosarcus acutirostris	LBP 10185	LBP 47682	Mucuri River basin, Carlos Chagas, MG	OQ877218	OQ928419
Oligosarcus acutirostris		LBP 47683		OQ877221	OQ928420
Oligosarcus acutirostris		LBP 47684		OQ877219	OQ928421
Oligosarcus acutirostris		LBP 47685		OQ877217	OQ928422
Oligosarcus acutirostris		LBP 47686		OQ877220	OQ928423
Oligosarcus acutirostris	MNRJ 40098	MNTI 6464	Córrego Cupido, Barra Seca River basin, Sooretama, ES	–	OQ928428
Oligosarcus acutirostris	MNRJ 37750	MNTI 3907	Córrego Miracema, Doce River basin, Colatina, ES	OQ877227	OQ928430
Oligosarcus acutirostris		MNTI 3908		OQ877228	OQ928431
Oligosarcus acutirostris	MNRJ 41831	MNTI 8998	Rio Santa Maria do rio Doce, Doce River basin, Santa Teresa, ES	OQ877229	OQ928433
Oligosarcus acutirostris		MNTI 9000		OQ877230	OQ928434
Oligosarcus acutirostris	MNRJ 41877	MNTI 9039	Rio Santa Maria do rio Doce, Doce River basin, Santa Teresa, ES	OQ877231	OQ928435
Oligosarcus acutirostris	MNRJ 36661	MNTI 3235	Rio Castelo, Itapemirim River basin, Castelo, ES	–	OQ928429
Oligosarcus acutirostris	MNRJ 41713	MNTI 8739	Itapemirim River basin, Itapemirim, ES	OQ877223	–
Oligosarcus acutirostris		MNTI 8740		OQ877222	OQ928432
Oligosarcus acutirostris	LBP 3461	LBP 20230	Córrego Juvêncio, Itabapoana River basin, Campo dos Goycatazes, RJ	OQ877226	OQ928427
Oligosarcus macrolepis	DZSJRP 19136-1		Jequitinhonha River basin, MG	MN119429.1	–
Oligosarcus macrolepis	DZSJRP 19136-2			MN119430.1	–
Oligosarcus hepsetus	UFRGS 18821	TEC 4456	Rio Preto, Paraíba do Sul River basin, Paraíba do Sul, RJ	MN119407.1	MK991874.1
Astyanax lacustris	UFRGS 19055	TEC 4030	Northeastern Mata Atlântica freshwater ecoregion, RJ	KY327441.1	MK991888.1
Deuterodon intermedius	MZUFV 4458	CT 2801	Doce River basin	KY327432.1	MK991898.1

Sequence edition, alignment, and datasets. Electropherograms were inspected and assembled in contigs using *Geneious Prime* 2021.1.1 (https://www.geneious.com). Consensus sequences were aligned using MUSCLE (Edgar, 2004) in MEGA v. 5.2.2 (Tamura *et al*., 2011) under default parameters. After alignments, the matrix was checked visually for any obvious misalignments and to detect potential cases of sequencing error due to contamination, paralogy, or pseudogenes using *Geneious*. Nucleotide variation and substitution patterns were examined using MEGA. To evaluate the occurrence of substitution saturation in the sequences, the index of substitution saturation (Iss) described by Xia *et al.* (2003) and Xia, Lemey (2009) in DAMBE 5.3.48 (Xia, 2013) was estimated. For the nuclear gene, heterozygous sites were assigned using International Union of Pure and Applied Chemistry (IUPAC) ambiguity codes. The gametic phase was determined using PHASE algorithm (Stephens *et al*., 2001) in DnaSP 5 (Librado, Rozas, 2009) under default settings, assuming 0.7 as a minimum posterior probability. Haplotypes with probabilities below this threshold were excluded from further analyses. Three datasets were produced; one for each of the gene individually (COI and Myh6) plus one for both markers, which were concatenated into a single matrix using MEGA X (Kumar *et al*., 2018) (https://www.megasoftware.net/). Only specimens with sequences of both markers were included in this concatenated matrix. The dataset used for each analysis is indicated when necessary.

Phylogenetic analyses. Phylogenetic relationships among samples of *O. acutirostris* from 11 localities and between this species and the others were inferred by Bayesian inference (BI) method. Sequences from *Astyanax lacustris* and *Deuterodon intermedius* were used to root the phylogenetic analyses. The best-fit nucleotide evolution model was estimated independently for each partition using MrModeltest v. 2.2 (Nylander, 2004) based on the Akaike Information Criterion (AIC).

Bayesian inference analysis was conducted in MrBayes v. 3.2.6 (Ronquist *et al*., 2012) at the CIPRES Science Gateway (Miller *et al*., 2010). Two independent Bayesian runs of 10 million generations with four chains of Markov chain Monte Carlo (MCMC) each were performed, saving trees each 500 generations. Chain convergence (Effective Sample Size – ESS values > 200) was checked using the likelihood plots for each run using Tracer v. 1.7.1 (Rambaut, Drummond, 2009). The Potential Scale Reduction Factor (PSRF) was also used to check chain convergence and burn-in; values close to one indicate good convergence between runs (Gelman, Rubin, 1992). After a graphical analysis of the evolution of the likelihood scores, and checking for the stationarity of all model parameters, the first twenty thousand generations (10%) were discarded as burn-in. The trees were visualized and edited in Figtree 1.3.1 (Rambaut, 2009).

Phylogeographic analyses. Population structure tests and summary statistics were based on COI and Myh6 matrices separately. We generated median-joining networks (Bandelt *et al*., 1999) using the program PopART (Leigh, Bryant, 2015, http://popart.otago.ac.nz/index.shtml) to study the relationships between haplotypes and their geographic distribution.

To test different scenarios of population structure within *O. acutirostris* along its distribution, we carried out an Analysis of Molecular Variance (AMOVA; Excoffier *et al*., 1992) in the program ARLEQUIN v. 3.5.2.2 (Excoffier, Lischer, 2010, http://cmpg.

unibe.ch/software/arlequin35/Arlequin35.html) based on mtDNA. We implemented a three hierarchical level analysis, in which we tested different groups configuration: (i) a null model in which individuals of *O. acutirostris* sampled in the same basin were merged into a single population to quantify the amount of genetic structure amongst them testing the "basin stability", considering each basin as a single group isolated from the others (current conformation); (ii) connections between tributaries from the same coastal paleodrainage; and (iii) close relationship between the basins based on the biogeographic hypothesis proposed by Camelier, Zanata (2014). Therefore, for scenario i, seven groups were considered, which are the sampled river basins currently isolated from each other: Almada, Santo Antônio, João de Tiba, Mucuri, Doce, Itapemirim, and Itabapoana. For scenario ii, six groups were considered based on coastal paleodrainages proposed by Thomaz, Knowles (2018); in this case, coincidentally, each basin represents a distinct paleodrainage, except for the Santo Antônio and João de Tiba rivers, tributaries of the same coastal paleodrainage. For scenario iii, two tests were carried out for the Central-South Group of the NMAF ecoregion proposed by Camelier, Zanata (2014): (a) with three groups – Almada (Central Group), Santo Antônio + João de Tiba + Mucuri + Doce (South Group), and Itapemirim + Itabapoana; (b) with two groups – Almada (Central Group) and all other basins belonging to the South Group. For scenario "b", the geographic proximity of the drainages of the South Group and Itapemirim and Itabapoana river basins was considered as an alternative hypothesis to the polytomy found by these authors within the Central-South Group. Summary statistics, such as nucleotide diversity per site (π), number of haplotypes (h), and haplotype diversity (Hd) were calculated in software DnaSP for each scenario analyzed in this study.

RESULTS

We obtained a molecular dataset with 1,191 bp: 604 bp of COI (N = 29, 120 variable sites, 49 informative) and 587 bp of Myh6 (N = 29, 29 variable sites, 11 informative). The sequences did not show indels, stop-codons or sequencing errors due to contamination. The Phi test result did not indicate statistically significant evidence for recombination in the nuclear locus (Myh6, p = 1,0). The best-fit models of evolution estimated by MrModeltest were GTR+G and GTR for COI and Myh6 matrices, respectively.

In all phylogenetic analyses, the specimens of *O. acutirostris* are recovered as closely related to each other (Fig. 1). According to mtDNA, the individuals from Almada river basin are more related to each other, and the same is true for the specimens from rio Mucuri (Fig. 1A). Furthermore, the topology based on COI showed a close relationship between individuals from Itabapoana and Itapemirim basins and between those from Santo Antônio and João de Tiba river basins, although with low statistical support (pp = 67%). The topology based on Myh6 recovered two clades into *O. acutirostris*, one is represented by specimens from rio Almada plus rio Mucuri that is the sister group of the clade formed by specimens from the remaining river basins analyzed (Fig. 1B). The internal relationships in both clades, however, were not clearly resolved. According to the phylogenetic hypothesis obtained from the concatenated matrix, the relationships among the samples of *O. acutirostris* were not clearly resolved (Fig. 1C). Despite the polytomy, the topology indicated that individuals from rio Almada basin are more related to each other. Furthermore, according to this topology, there is a close relationship between some individuals from the Itapemirim and Itabapoana river basins, although with low statistical support (pp = 78%).

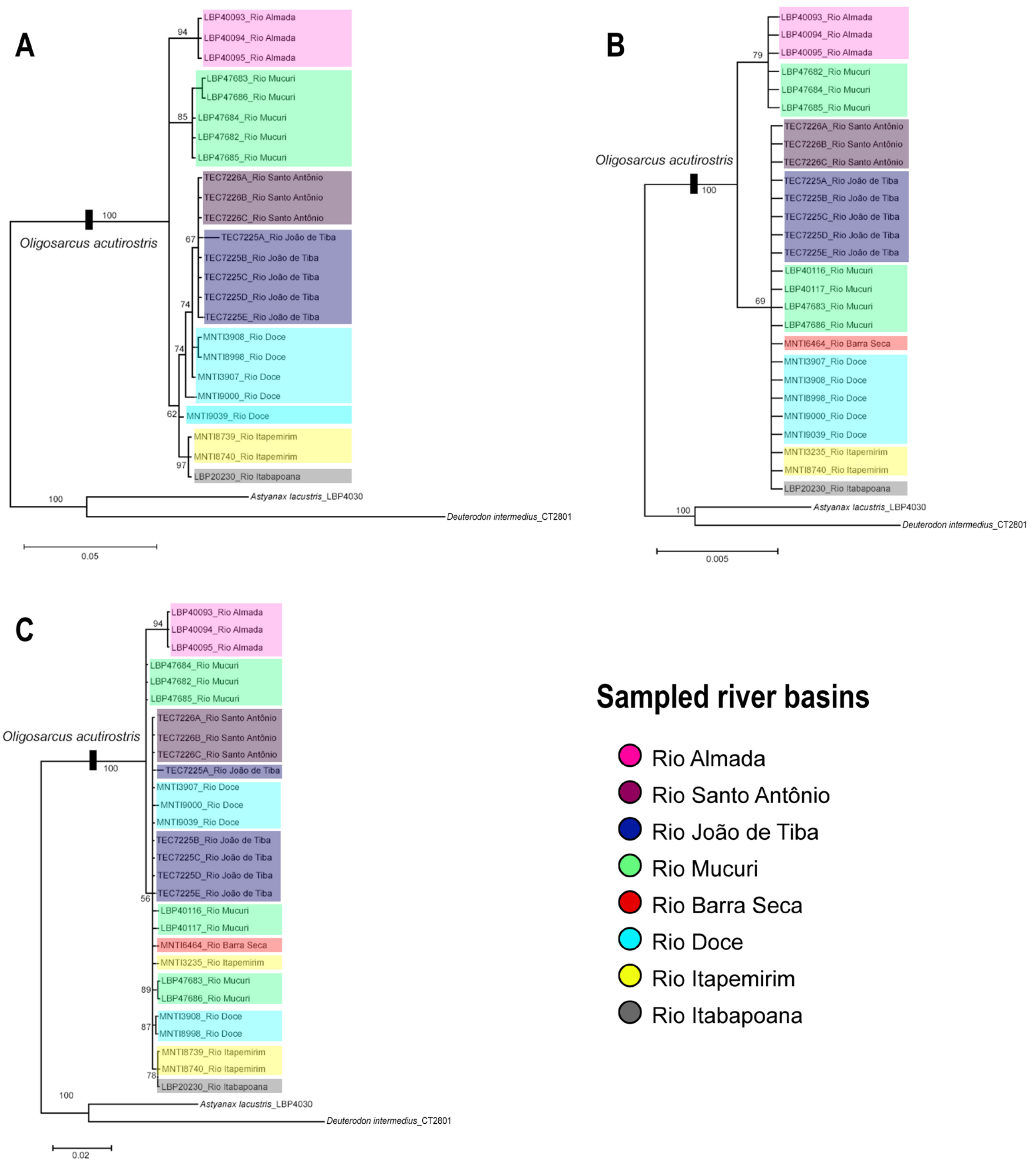

FIGURE 1 | Bayesian phylogenetic trees of *Oligosarcus acutirostris* obtained in this study indicating the relationships among analyzes specimens. Numbers at branches are posterior probabilities. **A.** Topology based on mitochondrial gene cytochrome oxidase I (COI, 604 bp); **B.** Topology based on nuclear gene myosin heavy chain 6 cardiac muscle alpha (Myh6, 587 pb); and **C.** Topology based on concatenated dataset (COI+Myh6, 1,191 bp). The highlighted colors in the topologies represent each sampled river basin as indicated in the legend (below/right) and showed in Fig. 2A.

The haplotype networks were congruent with the phylogenetic inferences (Fig. 2). The haplotype network based on COI revealed a strong genetic structuring within *O. acutirostris* (Fig. 2B). In fact, this structuration was even more evident because there was no shared haplotype among several analyzed populations, indicating a clear association between genetic structure and river basins. We found 11 haplotypes in the COI sequences, nine of them exclusive to following river basins: Almada (one haplotype), João de Tiba (three), Mucuri (two), and Doce (four). On the other hand, there is one haplotype shared between Santo Antônio and João de Tiba river basins and other shared between Itabapoana and Itapemirim drainages. The exclusive haplotype of the rio Almada is separated from all others by at least six mutation steps, while the two haplotypes exclusive to the rio Mucuri are separated by at least seven mutation steps from the others.

In agreement with the phylogenetic inference, the haplotype network results showed a closer relationship among specimens of *O. acutirostris* from Santo Antônio, João de Tiba, Doce, Itapemirim, and Itabapoana to each other than between these and those from Almada and Mucuri drainages. Also, the haplotype network based on COI is presented with the inclusion of samples of *O. macrolepis* and *O. hepsetus* to show both intra and interspecific genetic structuring (as shown in the Fig. **S1**: samples of *O. acutirostris* are separated from these species by at least 11 and 22 mutation steps, respectively). On the other hand, the haplotype network based on Myh6 (Fig. 2C) showed absence of genetic structuration, with a central haplotype shared among all basins, except for rio Almada. In this drainage, only one haplotype was found (separated by three mutation steps from the central one), which is shared with the rio Mucuri. Also, exclusive haplotypes were found in João de Tiba, Mucuri, and Itapemirim river basins, separated by only one mutation step from the central haplotype.

The AMOVA analyzes, based on mtDNA, indicated a high percentage of variation among all groups analyzed in the different scenarios, recovering the genetic structuration, and corroborating the hypotheses raised (Tab. 2). According to AMOVA results, two of three scenarios analyzed, *i.e.*, (i) past connections between tributaries of the same coastal paleodrainage and (i) relationship between basins based on biogeographic hypothesis of Camelier, Zanata (2014) (respectively AMOVA 2 and 3 + 4, Tab. 2), contributed to explain the distributional pattern currently presented by *O. acutirostris*. For scenario ii, it is worth noting that two tests were performed, one in which the Central and South Groups and the Itapemirim + Itabapoana river basins were considered separately (as proposed in the polytomy presented by Camelier, Zanata, 2014) and another in which these latter drainages were considered as belonging to the South Group. For this scenario, AMOVA results showed that only the second test helps to explain the current distribution pattern of *O. acutirostris* (see AMOVA 3 and 4, Tab. 2). Although these two scenarios contributed to explain the currently distributional pattern of *O. acutirostris*, the AMOVA results showed that the scenario that best explains it is the past connections created by coastal paleodrainages occurring in the NMAF ecoregion (AMOVA 2, 79.6% of variation, Tab. 2).

Summary statistics values based on COI matrix (604 bp) for each analyzed scenario are presented in Tab. 3. Considering each independent river basin, Doce and Mucuri drainages presented the highest and lowest values of haplotype diversity, respectively (*i.e.*, Hd = 0.9 and Hd = 0.6). The highest value of nucleotide diversity was found in rio João de Tiba (π = 0.6%). Paleodrainages 110– Mucuri and 114– Doce showed highest

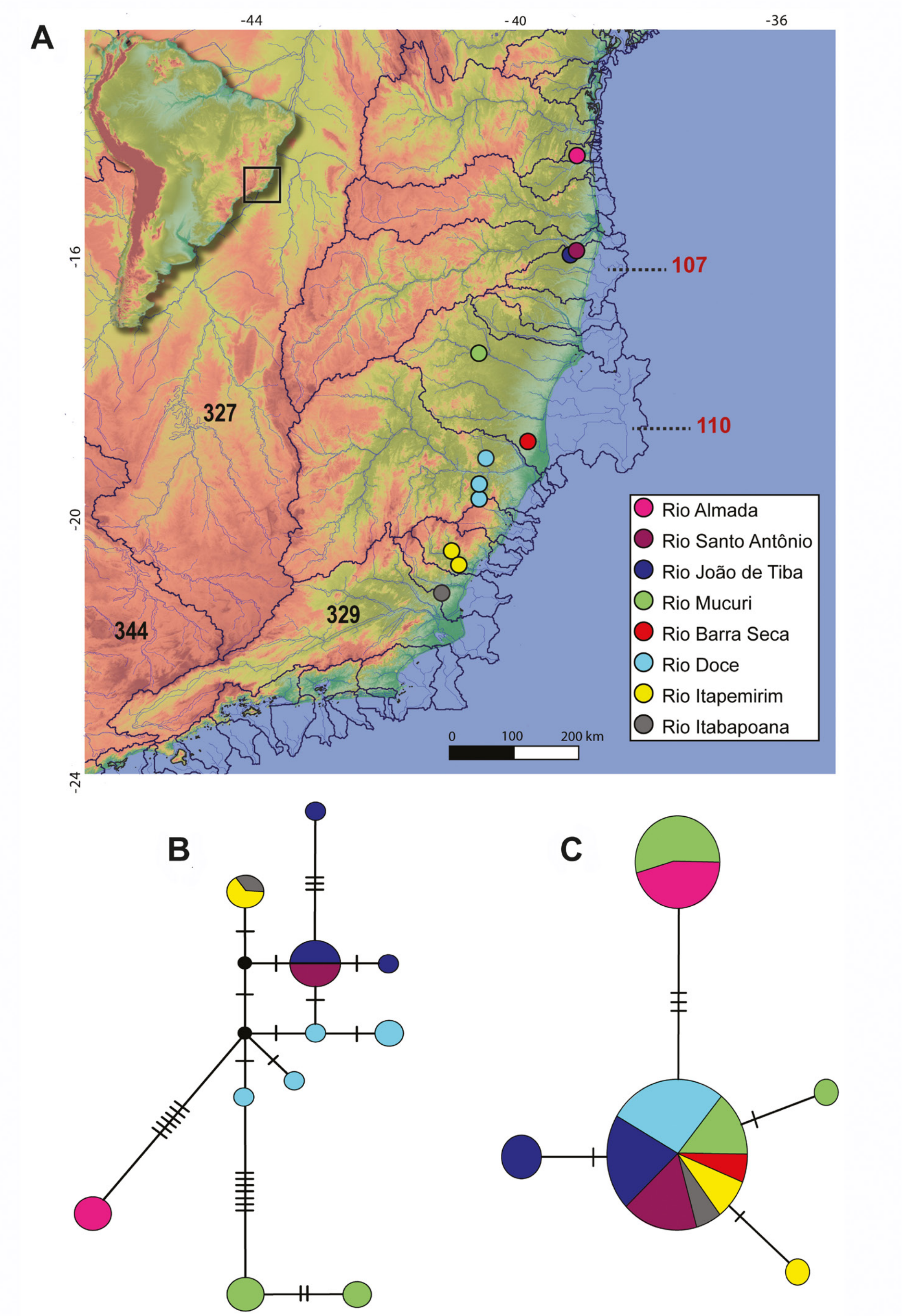

FIGURE 2 | **A.** Map showing part of the Northeastern Mata Atlântica freshwater ecoregion (NMAF) and the distribution of *Oligosarcus acutirostris* samples analyzed in this study. Adjacent freshwater ecoregions *sensu* Abell *et al*. (2008): (327) São Francisco, (329) Paraíba do Sul, and (344) Upper Paraná. Costal paleodrainages based on Thomaz, Knowles (2018): (107) Santo Antônio + João de Tiba and (110) Mucuri + Barra Seca; **B** and **C.** Haplotype networks showing the genetic connectivity among the haplotypes of *O. acutirostris*, which are represented by circles, the size proportional to their frequencies. Each trace corresponds to a single mutation. Colors correspond to the sampled river basins as in Fig. 2A; **B.** Haplotype network based on COI dataset (604 bp, 24 specimens, 11 haplotypes). Black circles represent median vectors; **C.** Haplotype network based on Myh6 dataset (587 bp, 27 specimens, five haplotypes).

values of haplotypic diversity, with the highest value to paleodrainage 114– Doce (Hd = 0.9). This paleodrainage also presented the highest value of nucleotide diversity (π = 0.5%). Finally, for the third scenario analyzed, as mentioned above, two tests were carried out for the Central-South Group of the NMAF ecoregion proposed by Camelier, Zanata (2014), test (a) with three groups – Almada (Central Group), Santo Antônio + João de Tiba + Mucuri + Doce (South Group) and Itapemirim + Itabapoana; test (b) with two groups – Almada (Central Group) and Santo Antônio + João de Tiba + Mucuri + Doce + Itapemirim + Itabapoana (South Group). The results of the summary statistics for each of the tests (a) and (b) are also presented in Tab 3. In both analyzes, the South Group presented values of haplotypic diversity greater than 0.5, with Hd = 0.86 in (a) and Hd = 0.89 in (b). Also, in both cases, the nucleotide diversity is greater than 1%.

TABLE 2 | Analysis of Molecular Variance (AMOVA) based on mitochondrial gene cytochrome oxidase I (COI, 604 bp) of samples of *Oligosarcus acutirostris*. For AMOVA 1, seven populations were considered (*i.e.*, currently isolated river basins): Almada, Santo Antônio, João de Tiba, Mucuri, Doce, Itapemirim, and Itabapoana; for AMOVA 2, six populations were considered (*i.e.*, river basins representing the coastal paleodrainages proposed by Thomaz, Knowles, 2018): Almada, Mucuri, Doce, Itapemirim, Itabapoana, and Santo Antônio + João de Tiba; for AMOVA 3, three populations were considered based on biogeographic relationships proposed by Camelier, Zanata (2014) with some changes: Almada (*i.e.*, Central Group), Santo Antônio + João de Tiba + Mucuri + Doce (*i.e.*, South Group) and Itapemirim + Itabapoana; for AMOVA 4, two populations were considered: Almada (Central Group) and Santo Antônio + João de Tiba + Mucuri + Doce + Itapemirim + Itabapoana (South Group including the last two river basins in this group). * = $p < 0.01$.

AMOVA	Source of variation	%variation	Fixation index
1: Currently isolated river basins	Among groups	63.29	FCT = 0.63291
	Among populations within groups	23.62	FSC = 0.64356
	Within populations	13.08	FST = 0.86916*
2: Coastal paleodrainages	Among groups	79.56	FCT = 0.79561*
	Among populations within groups	8.05	FSC = 0.39409
	Within populations	12.38	FST = 0.87616*
3: Central Group; South Group; Itapemirim + Itabapoana	Among groups	22.78	FCT = 0.22783*
	Among populations within groups	65.51	FSC = 0.84837*
	Within populations	11.71	FST = 0.88292*
4: Central Group; South Group	Among groups	33.00	FCT = 0.33005
	Among populations within groups	56.96	FSC = 0.85026*
	Within populations	10.03	FST = 0.89968*

TABLE 3 | Summary statistics for cytochrome oxidase I (COI, 604 bp) of *Oligosarcus acutirostris* by scenarios analyzed in this study. Coastal paleodrainages were proposed according to Thomaz, Knowles (2018) and biogeographical relationships were based on Camelier, Zanata (2014). Null values are represented by (–). N: sample size; h: number of haplotypes; Hd: haplotype diversity; sd: standard deviation; π: nucleotide diversity per site.

River basins	N	h	Hd/sd	ϖ/sd
Almada	3	1	–	–
Santo Antônio	3	1	–	–
João de Tiba	5	3	0.70000/0.218	0.00602/0.00307
Mucuri	5	2	0.60000/0.175	0.00199/0.00058
Doce	5	4	0.90000/0.161	0.00490/0.00128
Itapemirim + Itabapoana	3	1	–	–
Coastal paleodrainages	**N**	**h**	**Hd/sd**	**ϖ/sd**
101 – Almada	3	1	–	–
107 – Santo Antônio + João de Tiba	8	3	0.46400/0.200	0.00230/0.00126
110 – Mucuri	5	2	0.60000/0.175	0.00199/0.00058
114 – Doce	5	4	0.90000/0.02592	0.00490/0.00128
119 + 120 – Itapemirim + Itabapoana	3	1	–	–
Biogeographical relationships A	**N**	**h**	**Hd/sd**	**ϖ/sd**
Almada (Central Group)	3	1	–	–
Santo Antônio + João de Tiba + Mucuri + Doce (South Group)	18	9	0.86900/0.061	0.01303/0.00210
Itapemirim + Itabapoana	3	1	–	–
Biogeographical relationships B	**N**	**h**	**Hd/sd**	**ϖ/sd**
Almada (Central Group)	3	1	–	–
Santo Antônio + João de Tiba + Mucuri + Doce + Itapemirim + Itabapoana (South Group)	21	10	0.89000/0.046	0.01249/0.00204

DISCUSSION

Mitonuclear discordances in *Oligosarcus acutirostris* and concatenated analysis. Topologies based on mitochondrial and nuclear markers were relatively different, especially concerning the relationships between individuals from the Almada and Mucuri river basins. According to nuDNA topology, samples from the rio Almada and some specimens from the rio Mucuri are more closely related (although with low statistical support; Fig. 1B), which was not recovered by mtDNA. The topology based on COI marker also showed a closer relationship between specimens from the Santo Antônio and João de Tiba river basins and those from the Itapemirim and Itabapoana drainages, which was not detected in the nuDNA-based analysis. Several studies show that it is relatively common for species to exhibit high population differentiation when the analysis is based on a mitochondrial marker, but to show less structuring in nuDNA, due to higher population size and mutational speed (*e.g.*, Godinho *et al*., 2006; Martins *et* *al*., 2009; Rato *et al*., 2015). The rapid evolution of mtDNA can lead to high nucleotide sequence variation and result in the coexistence of many different haplotypes within a species (Provan *et al*., 2001; Avise, 2009). Therefore, the peculiarities of mtDNA lead it to have more information in general when we are looking within species and achieve reciprocal monophyly in a shorter time than nuDNA, especially in samples with low or no gene flow (Moore, 1995), such as those analyzed here. Some studies have highlighted the importance of the combined use of mtDNA and nuDNA in phylogenetic analyzes to improve their analytical performance (*e.g*., Slowinski, Page, 1999; Toews, Brelsford, 2012), because the evolution of a population or species leaves traces in both genomes, allowing a more comprehensive explanation of its evolutionary history (Avise, 2009). In this study, the topology based on concatenated data also recovered the close relationship among individuals from the rio Almada each other, as well as the close relationship between some specimens from the rio Itapemirim and individuals from Itabapoana. This result is also interesting because it proposes an unprecedented relationship for the Itabapoana river basin within the NMAF. Here, it is important to highlight that Camelier, Zanata (2014) did not include this basin in their biogeographical analysis. Although these studies were based on distinct data sources (*i.e*., genetics within species and species distribution), they are likewise important for a more complete understanding of the biogeographical history of the river basins draining the AF and its ichthyofauna.

Biogeographic inferences based on distributional pattern and evolutionary history of *Oligosarcus acutirostris*. The close relationship between Itapemirim and Itabapoana mentioned above is also corroborated by the sharing of haplotypes of *O. acutirostris* among them. The distribution of lineages of freshwater fishes across isolated basins may be explained by geomorphological events such as river captures (see Bishop, 1995; Wilkinson *et al*., 2006) and/or coastal paleoconnections due to sea level retreat (Ribeiro, 2006; Albert *et al*., 2011; Thomaz *et al*., 2015; 2017; Thomaz, Knowles, 2018). In the case of *O. acutirostris*, however, it does not appear to have been the latter explanation, since the Itabapoana and Itaperimim river basins belonged to different coastal paleodrainages during the maximum glacial period of the Pleistocene (see Thomaz, Knowles, 2018: 119- Itapemirim and 120- Itabapoana). Therefore, the presence of the same haplotype in these river basins is probably due to more recent contact or headwater capture events between their tributaries, as suggested by Reis *et al.* (2021) as an explanation for the occurrence of the loricariid catfish *Loricariichthys melanurus* Reis, Vieira & Pereira, 2021 in these drainages. According to several authors (*e.g*., Ribeiro, 2006; Buckup, 2011; Dagosta *et al*., 2014; Camelier *et al*., 2018), this phenomenon is very common in Brazilian coastal basins and represents one of the main reasons for the sharing of ichthyofauna between adjacent drainages. Although no hypothesis of river capture has been proposed between the Itapemirim and Itabapoana rivers, they constitute a hydrologically homogeneous region with tributaries that are geographically very close (Silva-Junior, 2014; Piol *et al*., 2019). Abrupt sharp changes in the flow direction of some tributaries at current boundaries of these basins (see Piol *et al*., 2019: Fig. 1) may be due to river piracy. These sharp changes, known as the “elbow of capture”, are the most cited evidence for the occurrence of river captures (Bishop, 1995) and ichthyofauna exchange, as suggested by several studies using freshwater fishes (*e.g*., Ribeiro *et al*., 2006; Camelier *et al*., 2018; Abreu *et al*., 2020; Souza *et al*., 2020; Silva *et al*., 2020; Mendes *et al*., 2022; Oliveira-Silva *et al*., 2022).

The comparison between the current conformation of river basins sampled in the present study with the coastal paleodrainages proposed by Thomaz, Knowles (2018) suggests that most of them was associated with an independent paleodrainage of the NMAF ecoregion during the Pleistocene, except for the Santo Antônio + João de Tiba and Mucuri + Barra Seca, which correspond to paleodrainage 107 and 110, respectively. Contemporary river basins draining the northeastern Brazil, including those along the NMAF ecoregion, may represent interesting scenarios for predicting patterns of genetic divergence, given the limited connectivity promoted among rivers during sea level retreat in this area (Thomaz, Knowles, 2018). According to these authors, the current disconnection among the basins, rather than coastal paleodrainages, may best predict patterns of genetic divergence in this area. This hypothesis is not supported here because the results show that the current distribution of *O. acutirostris* in the NMAF ecoregion is best explained by the past history through paleodrainages distribution (AMOVA 2, Tab. 2). In this scenario, the currently isolated basins of the Santo Antônio and João de Tiba rivers, as mentioned above, were part of a single paleodrainage #107 which would have allowed the spread of *O. acutirostris* and could explain the current sharing of haplotypes between these basins. On the other hand, it is important to highlight that the current scenario of disconnection among NMAF basins is similar to that of the Pleistocene, with these basins being related to different coastal paleodrainages that are also isolated from each other. Perhaps this is why the current and past drainage arrangements results are so similar, although AMOVA 1 was not significant. A similar result was indicated by Medeiros *et al.* (2021) for species of *Microcambeva* Costa & Bockmann, 1994 in the NMAF.

As mentioned, other geological/hydrological phenomena, in addition to coastal paleodrainages, may justify the sharing of ichthyofauna (both at specific and population levels) between adjacent basins currently isolated in the eastern part of Brazil, as is the case with river captures. The sharing of haplotypes between the Itapemirim and Itabapoana river basins, for example, may be a result of these events, since they formed independent coastal paleodrainages during the Pleistocene (see Thomaz, Knowles, 2018), representing an interesting alternative hypothesis that should be tested to justify the sharing of haplotypes between these basins that are so geographically close. Here, however, it is important to point out that the small sample size of each basin may result in sampling the most common haplotypes, thus the addition of other taxa, more samples and markers is essential to check these patterns. Even so, it is important to highlight that the current distribution pattern of *O. acutirostris* and its haplotypes could be the result of the combined effect of these hydrological phenomena at different times. The joint action of these phenomena has helped to explain the distributional pattern of several species of freshwater fishes in the Neotropical Region (*e.g.*, Ribeiro, 2006; Buckup, 2011; Camelier *et al.*, 2018; Barreto *et al.*, 2022).

According to the results of AMOVA 4 (Tab. 2), the biogeographic relationship among the river basins proposed by Camelier, Zanata (2014) also helps to explain the current distribution of *O. acutirostris*, although at a lower level. Of the two tests performed, the scenario that best explained the results found was the one that included both the Itaperimim and Itabapoana drainages within the South Group. Therefore, we presented here an alternative proposal to that of Camelier, Zanata (2014) for the position of the Itapemirim river basin, which would be part of this group and would resolve the polytomy [*i.e.*, (Central Group, South Group, Itapemirim river basin)] presented by these authors. On the other hand, it is important to highlight that although the relationships between these basins proposed by Camelier, Zanata (2014) have an important influence on the distribution of *O. acutirostris*, the scenario that best explained the results found were those related to the coastal paleodrainages, followed by the current isolation among the basins (Tab. 2). As mentioned earlier, the fact that these basins were also isolated from each other during the Pleistocene and formed distinct paleodrainages in most cases makes these two scenarios very similar, suggesting continued separation between these drainages. These results are consistent with literature suggesting that the presence of a conspicuous barrier to gene flow separating populations over long periods of time (Avise, 2000; Martins, Domingues, 2011) reflects the presence of high genetic divergence and allopatric lineages (Avise *et al*., 1987; Avise, 2000; Thomaz, Knowles, 2020). According to several authors (*e.g*., Avise, 2000; Thomaz, Knowles, 2020), due to the current disconnection between watersheds, it is common for fish species from different and independent water bodies to be genetically structured, as is the case of *O. acutirostris*. Therefore, this result is consistent with what was expected for freshwater fish species that occur in isolated coastal basins for which the landscape and ocean serve as geographic barriers, confining these species to the basins in which they occur after their formation (Vari, 1988; Camelier, Zanata, 2014; Hirschmann *et al*., 2015).

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REFERENCES

- **Abell R, Thieme ML, Revenga C, Bryer M, Kottelat M, Bogutskaya N, Coan B, Mandrak N, Balderas SC, Bussing W, Stiassny MLJ, Skelton P, Allen GR, Unmack P, Naseka A, Ng R, Sindorf N, Robertson J, Armijo E, Higgins JV, Heibel TJ, Wikramanayake E, Olson D, López HL, Reis RE, Lundberg JG, Pérez MHS, Petry P.** Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. BioScience. 2008; 58(5):403–14. https://doi.org/10.1641/B580507
- **Abilhoa V, Braga RR, Bornatowski H, Vitule JR.** Fishes of the Atlantic Rain Forest streams: ecological patterns and conservation. Changing diversity in changing environment. IntechOpen. 2011; 259–82. https://doi.org/10.5772/24540
- **Abreu JMS, Saraiva ACS, Albert JS, Piorski NM.** Paleogeographic influences on freshwater fish distributions in northeastern Brazil. J S Am Earth Sci. 2020; 102:102692. https://doi.org/10.1016/j.jsames.2020.102692

- **Albert J, Petry P, Reis RE.** Major biogeographic and phylogenetic patterns. In: Albert JS, Reis RE, editors. Historical biogeography of Neotropical freshwater fishes. California: University of California Press; 2011. p.21–57
- **Avise JC.** Phylogeography: retrospect and prospect. J Biogeogr. 2009; 36(1):3–15. https://doi.org/10.1111/j.1365-2699.2008.02032.x
- **Avise JC.** Phylogeography: the history and formation of species. Cambridge: Harvard University Press; 2000.
- **Avise JC, Arnold J, Ball RM, Jr Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC**. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu Rev Ecol Syst. 1987; 18:489–522. http://www.jstor.org/stable/2097141
- **Bandelt HJ, Forster P, Röhl A.** Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol. 1999; 16(1):37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036
- **Barreto SB, Knowles LL, Mascarenhas R, Affonso PRADM, Batalha-Filho H.** Drainage rearrangements and in situ diversification of an endemic freshwater fish genus from north-eastern Brazilian rivers. Freshwater Biol. 2022; 67(5):759–73. https://doi.org/10.1111/fwb.13879
- **Barreto SB, Silva AT, Batalha-Filho H, Affonso PRAM, Zanata AM.** Integrative approach reveals a new species of *Nematocharax* (Teleostei: Characidae). J Fish Biol. 2018; 93(6):1151–62. https://doi.org/10.1111/jfb.13834
- **Birindelli JL, Britski HA, Garavello JC.** Two new species of *Leporinus* Agassiz (Characiformes: Anostomidae) from eastern basins of Brazil, and redescription of *L. melanopleura* Günther. Neotrop Ichthyol. 2013; 11(1):9–23. https://doi.org/10.1590/S1679-62252013000100002
- **Bishop P.** Drainage rearrangement by river capture, beheading and diversion. Prog Phys Geogr. 1995; 19(4):449–73. https://doi.org/10.1177/030913339501900402
- **Bizerril CRSF.** Análise taxonômica e biogeográfica da ictiofauna de água doce do leste brasileiro. Acta Biol Leopold. 1994; 16(1):51–80.
- **Buckup PA.** The Eastern Brazilian Shield. In: Albert JS, Reis RE, editors. Historical Biogeography of Neotropical Freshwater Fishes. California: University of California Press; 2011. p.203–10
- **Burger R, Zanata AM, Camelier P.** Estudo taxonômico da ictiofauna de água doce da bacia do Recôncavo Sul, Bahia, Brasil. Biota Neotrop. 2011; 11(4):273–90. https://doi.org/10.1590/S1676-06032011000400024
- **Camelier P.** Estudo taxonômico e aspectos biogeográficos da ictiofauna de água doce de drenagens costeiras do Estado da Bahia, Brasil, com a descrição de seis espécies novas. [Master Dissertation]. Salvador: Universidade Federal da Bahia; 2010.
- **Camelier P, Zanata AM.** Biogeography of freshwater fishes from the Northeastern Mata Atlântica freshwater ecoregion: distribution, endemism, and area relationships. Neotrop Ichthyol. 2014; 12(4):683–98. https://doi.org/10.1590/1982-0224-20130228
- **Dagosta FC, Marinho MM, Camelier P.** A new species of *Hyphessobrycon* Durbin (Characiformes: Characidae) from the middle rio São Francisco and upper and middle rio Tocantins basins, Brazil, with comments on its biogeographic history. Neotrop Ichthyol. 2014; 12(2):365–75. https://doi.org/10.1590/1982-0224-20130179
- **Edgar RC.** MUSCLE: multiple sequence alignment with high accuracy and high throughput. NAR. 2004; 32(5):1792–97. https://doi.org/10.1093/nar/gkh340
- **Excoffier L, Lischer HE.** Arlequin suite ver 3.5: a new series of programs toper form population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010; 10(3):564–67. https://doi.org/10.1111/j.1755-0998.2010.02847.x
- **Excoffier L, Smouse PE, Quattro JM.** Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 1992; 131(2):479–91. https://doi.org/10.1093/genetics/131.2.479
- **Ferreira KM, Menezes NA, Quagio-Grassioto I.** A new genus and two new species of Stevardiinae (Characiformes: Characidae) with a hypothesis on their relationships based on morphological and histological data. Neotrop Ichthyol. 2011; 9(2):281–98. https://doi.org/10.1590/S1679-62252011000200005

- **Gelman A, Rubin DB.** Inference from iterative simulation using multiple sequences. Statist Sci. 1992; 7(4):457–72. https://doi.org/10.1214/ss/1177011136
- **Godinho R, Mendonça B, Crespo EG, Ferrand N.** Genealogy of the nuclearβ-fibrinogen locus in a highly structured lizard species: comparison with mtDNA and evidence for intragenic recombination in the hybrid zone. Heredity. 2006; 96(6):454–63. https://doi.org/10.1038/sj.hdy.6800823
- **Hales J, Petry P.** Northeastern Mata Atlântica ecoregion. 2013. Avalaible from: http://www.feow.org/ecoregions/details/northeastern_mata_atlantica
- **Hirschmann A, Malabarba LR, Thomaz AT, Fagundes NJR**. Riverine habitat specificity constrains dispersion in a Neotropical fish (Characidae) along Southern Brazilian drainages. Zool Scr. 2015; 44(4):374–82. https://doi.org/10.1111/zsc.12106
- **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
- **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
- **Li C, Ortí G, Zhang G, Lu G.** A practical approach to phylogenomics: the phylogeny of ray-finned fish (Actinopterygii) as a case study. BMC Evol Biol. 2007; 7(1):1–11. https://doi.org/10.1186/1471-2148-7-44
- **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 FCT, Ribeiro AC.** Continental-scale tectonic controls of biogeography and ecology. In: Albert JS, Reis RE, editors. Historical Biogeography of Neotropical Freshwater Fishes. California: University of California Press; 2011. p.145–84.
- **Martins FM, Domingues MV.** Filogeografia. In: Carvalho CJB, Almeida EAB, editors. Biogeografia da América do Sul: Padrões e Processos. São Paulo: Roca; 2011. p.137–50
- **Martins FM, Templeton AR, Pavan AC, Kohlbach BC, Morgante JS.** Phylogeography of the common vampire bat (*Desmodus rotundus*): marked population structure, Neotropical Pleistocene vicariance and incongruence between nuclear and mtDNA markers. BMC Evol Biol. 2009; 9(1):1–13. https://doi.org/10.1186/1471-2148-9-294
- **Medeiros LS, Sarmento-Soares LM, Lima SMQ.** A new psammophilous catfish of the genus *Microcambeva* (Teleostei: Trichomycteridae) from the Rio Doce basin, southeastern Brazil. Ichthyol Explor Freshw. 2021; 1147:1–13.
- **Melo BF, Benine RC, Mariquela TC, Oliveira C.** A new species of *Tetragonopterus* Cuvier, 1816 (Characiformes: Characidae: Tetragonopterinae) from the rio Jari, Amapá, northern Brazil. Neotrop Ichthyol. 2011; 9(1):49–56. https://doi.org/10.1590/S1679-62252011000100002
- **Mendes IS, Melo BF, Damasceno JS, Teixeira DF, Carvalho DC.** Phylogeography of *Hypomasticus copelandii* (Teleostei, Anostomidae) Reveals Distinct Genetic Lineages along Atlantic Coastal Drainages of Eastern Brazil. Diversity. 2022; 14(1):29. https://doi.org/10.3390/d14010029
- **Menezes NA.** Três espécies novas de *Oligosarcus* Gunther, 1864 e redefinição taxonômica das demais espécies do gênero (Osteichthyes, Teleostei, Characidae). Bol Zool. 1987; 11(11):1–39. https://doi.org/10.11606/issn.2526-3358.bolzoo.1987.122368
- **Menezes NA.** Implications of the distribution patterns of the species of *Oligosarcus* (Teleostei; Characidae) from central and southern South America. In: Heyer WR, Vanzolini PE, editors. Proceedings of a Workshop on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências; 1988. p.295–304
- **Menezes NA, Weitzman SH, Oyakawa OT, Lima FCT, Castro RMC, Weitzman MJ.** Peixes de água doce da Mata Atlântica: lista preliminar de espécies e comentários sobre conservação de peixes de água doce neotropicais. São Paulo: Museu de Zoologia da Universidade de São Paulo; 2007.
- **Miller MA, Pfeiffer W, Schwartz T.** Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans; 2010. https://doi.org/10.1109/GCE.2010.5676129

- **Mirande JM.** Morphology, molecules and the phylogeny of Characidae (Teleostei, Characiformes). Cladistics. 2018; 35(3):282–300. https://doi.org/10.1111/cla.12345
- **Moore WS.** Inferring phylogenies from mtDNA variation: Mitochondrial-Gene Trees versus nuclear-gene trees. Evolution. 1995; 49(4):718–26. https://doi.org/10.2307/2410325
- **Nylander JAA.** MrModeltestv2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, 2004.
- **Oliveira-Silva L, Santos SAD, Lopes MM, Zanata AM.** A new species of *Characidium* (Characiformes: Crenuchidae) from the rio Doce basin, Brazil. Neotrop Ichthyol. 2022; 20(1):e210125. https://doi.org/10.1590/1982-0224-2021-0125
- **Pereira EHL, Zanata A, Cetra M, Reis RE.** A remarkable sexually dimorphic new genus and species of Neoplecostomine catfish (Siluriformes, Loricariidae) from a coastal drainage of Eastern Brazil. Copeia. 2014; 2014(4):673–81. https://doi.org/10.1643/CI-14-075
- **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 JATD, Caiado MAC, Mendonça ASF.** Performance evaluation of flow duration curves regionalization methods. RBRH. 2019; 24:e9. https://doi.org/10.1590/2318-0331.241920170202
- **Provan J, Powell W, Hollingsworth PM.** Chloroplast microsatellites: new tools for studies in plant ecology and evolution. Trends Ecol Evol. 2001; 16(3):142–47. https://doi.org/10.1016/S0169-5347(00)02097-8
- **Rambaut A.** FigTree, version 1.3.1.2009. Available from: http://tree.bio.ed.ac.uk/software/figtree/
- **Rambaut A, Drummond AJ.** Tracer, version 1.5. 2009. Available from: http://beast.bio.ed.ac.uk/Main_Page
- **Rato C, Harris DJ, Carranza S, Machado L, Perera A.** The taxonomy of the *Tarentola mauritanica* species complex (Gekkota: Phyllodactylidae): Bayesian species delimitation supports six candidate species. Mol Phylogenet Evol. 2015; 94:271–78. https://doi.org/10.1016/j.ympev.2015.09.008
- **Reis RE, Vieira F, Pereira EHL.** A new species of the loricariid catfish genus *Loricariichthys* (Teleostei: Siluriformes) from Eastern Brazil. Ichthyol Herpetol. 2021; 109(2):557–66. https://doi.org/10.1643/i2020013
- **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
- **Ribeiro AC, Lima FCT, Ricomini C, Menezes NA.** Fishes of the Atlantic Rainforest of Boraceia: testimony of the Quaternary fault reactivation within a Neoproterozoic tectonic province in Southeastern Brazil. Ichthyol Explor Freshw. 2006; 17:625–30.
- **Ribeiro AC, Menezes NA.** Phylogenetic relationships of the species and biogeography of the characid genus *Oligosarcus* Günther, 1864 (Ostariophysi, Characiformes, Characidae). Zootaxa. 2015; 3949(1):41–81. https://doi.org/10.11646/zootaxa.3949.1.2
- **Ronquist F, Teslenko M, Mark PV, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Hueslsenbeck JP.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012; 61(3):539–42. https://doi.org/10.1093/sysbio/sys029
- **Sambrook J, Fritsch EF, Maniatis T.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press: Cold Spring Harbor. 1989.
- **Silva AT, Chagas RJ, Santos ACDA, Zanata AM, Rodrigues BK, Polaz CNM, Alves CBM, Vieira CS, Souza FB, Vieira F, Sampaio FAC, Ferreira H, Alvez HS, Sarmento-Soares LM, Pinho M, Martins-Pinheiro RF, Lima SM, Campiolo S, Camelier P.** Freshwater fishes of the Bahia State, Northeastern Brazil. Biota Neotrop. 2020; 20(4):e20200969. https://doi.org/10.1590/1676-0611-BN-2020-0969
- **Silva-Junior CA.** Análise regional de funções hidrológicas aplicáveis à avaliação de vazões mínimas nas bacias hidrográficas dos rios Itapemirim e Itabapoana (ES). [Master Dissertation]. Vitória: Universidade Federal do Espírito Santo; 2014.

- **Silva-Junior DE, Ramos TPA, Zanata AM.** A new species of *Parotocinclus* with reduced adipose fin (Loricariidae: Hypoptopomatinae), from the rio Jacuípe basin, Bahia State, Brazil. Neotrop Ichthyol. 2020; 18(2):e190137. https://doi.org/10.1590/1982-0224-2019-0137
- **Slowinski JB, Page RDM.** How should species phylogenies be inferred from sequence data? Syst Biol. 1999; 48(4):814–25. https://doi.org/10.1080/106351599260030
- **Souza MS, Thomaz AT, Fagundes NJR.** River capture or ancestral polymorphism: an empirical genetic test in a freshwater fish using approximate Bayesian computation. *Biol J Linn*. 2020; 131(3):575–84. https://doi.org/10.1093/biolinnean/blaa140
- **Stephens M, Smith NJ, Donnelly P.** A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. 2001; 68(4):978–89. https://doi.org/10.1086/319501
- **Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S.** MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011; 28(10):2731–39. https://doi.org/10.1093/molbev/msr121
- **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, Knowles LL.** Common barriers, but temporal dissonance: Genomic tests suggest ecological and paleo-landscape sieves structure a coastal riverine fish community. Mol Ecol. 2020; 29(4):783–96. https://doi.org/10.1111/mec.15357
- **Thomaz AT, Malabarba LR, Knowles LL.** Genomic signatures of paleodrainages in a freshwater fish along the southeastern coast of Brazil: genetic structurer effects past riverine properties. Heredity. 2017; 119(4):287–94. https://doi.org/10.1038/hdy.2017.46
- **Thomaz AT, Malabarba LR, Bonatto SL, Knowles LL.** 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; 42(12):2389–401. https://doi.org/10.1111/jbi.12597
- **Toews DP, Brelsford A.** The biogeography of mitochondrial and nuclear discordance in animals. Mol Eco. 2012; 21(16):3907–30. https://doi.org/10.1111/j.1365-294X.2012.05664.x
- **Vari RP.** The Curimatidae, a Lowland Neotropical fish family (Pisces: Characiformes): distribution, endemism, and phylogenetic biogeography. In: Vanzolini PE, Heyer WR, editors. Proceedings of a workshop on Neotropical distribution patterns. Rio de Janeiro: Academia Brasileira de Ciências.1988. pp.343–77.
- **Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN.** DNA barcoding Australia’s fish species. Philos T R Soc B. 2005; 360(1462):1847–57. https://doi.org/10.1098/rstb.2005.1716
- **Wendt EW, Silva PC, Malabarba LR, Carvalho TP.** Phylogenetic relationships and historical biogeography of *Oligosarcus* (Teleostei: Characidae): Examining riverine landscape evolution in southeastern South America. Mol Phylogenet Evol. 2019; 140:e106604. https://doi.org/10.1016/j.ympev.2019.106604
- **Wilkinson MJ, Marshall LG, Lundberg JG.** River behavior on mega fans and potential influences on diversification and distribution of aquatic organisms. J S Am Earth Sci. 2006; 21(1–2):151–72. https://doi.org/10.1016/j.jsames.2005.08.002
- **Xia X.** DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. Mol Biol Evol. 2013; 30(7):1720–28. https://doi.org/10.1093/molbev/mst064
- **Zanata AM, Camelier P.** Two new species of *Characidium* Reinhardt (Characiformes: Crenuchidae) from northeastern Brazilian coastal drainages. Neotrop Ichthyol. 2015; 13(3):487–98. https://doi.org/10.1590/1982-0224-20140106
- **Zanata AM, Lima FCT, Di Dario F, Gerhard P.** A new remarkable and Critically Endangered species of *Astyanax* Baird & Girard (Characiformes: Characidae) from Chapada Diamantina, Bahia, Brazil, with a discussion on durophagy in the Characiformes. Zootaxa. 2017; 4232(4):491–510.https://doi.org/10.11646/zootaxa.4232.4.2

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