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# Evidence on the paleodrainage connectivity during Pleistocene: Phylogeography of a hypoptopomatine endemic to southeastern Brazilian coastal drainages

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The coastal basins of southeastern Brazil are influenced by climatic changes that caused sea-level oscillations during the Pleistocene. These marine transgressions and regressions can generate isolation and connection among coastal rivers. In this region, freshwater fishes are excellent models for phylogeographic studies because their distributions may have been affected by geographical and ecological changes resulting from these processes. Therefore, the main objective of this study was to evaluate the effects of Pleistocene sea-level changes on the genetic structure of the loricariid *Hisonotus leucofrenatus* throughout its area of occurrence. Two genes were sequenced: Cytochrome Oxidase subunit 1 (mitochondrial gene) and rpS7 ribosomal protein gene intron 1 (nuclear gene) from specimens representing 14 river drainages. The genetic data corroborate a divide for freshwater fish by the Serra do Tabuleiro mountain in Santa Catarina State. This divide determines two main genetic groups in *H. leucofrenatus*: one group to the south and one to the north of this mountain range. The genetic structure observed coincides with the limits of estimated paleodrainage systems for the region, supporting that marine transgressions and regressions during the Pleistocene influenced the biogeographical history of *H. leucofrenatus*.

**Keywords:** Atlantic Rainforest, Biogeography, *Hisonotus leucofrenatus*, Loricariidae, Pleistocene sea-level changes.

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As bacias costeiras do sul do Brasil são influenciadas pelas mudanças climáticas que causaram oscilações no nível do mar durante o Pleistoceno. Essas transgressões e regressões marinhas geraram isolamento e conexão entre os rios. Nessa região, as espécies de peixe são excelentes modelos para estudos filogeográficos, pois suas distribuições podem ter sido afetadas por mudanças históricas e ecológicas decorrentes desses processos. Portanto, o objetivo principal deste estudo foi testar os efeitos das alterações do nível do mar durante o Pleistoceno na estrutura genética das populações do loricarídeo *Hisonotus leucofrenatus* ao longo de sua área de ocorrência. Dois genes foram sequenciados: Citocromo Oxidase subunidade 1 (gene mitocondrial) e o intron 1 da proteína ribossomal rpS7 (gene nuclear) de espécimes representando 14 bacias de drenagens. A estrutura genética observada corrobora uma divisão para peixes de água doce separada pela Serra do Tabuleiro, em Santa Catarina. Essa divisória determina dois grupos principais genéticos em *H. leucofrenatus*: um grupo ao sul e outro ao norte desse divisor. A estrutura genética também coincide com os limites dos sistemas de paleodrenagens estimados para a região, sustentando que as transgressões e regressões marinhas durante o Pleistoceno influenciaram a história biogeográfica de *H. leucofrenatus*.

**Palavras-chave:** Biogeografia, *Hisonotus leucofrenatus*, Loricariidae, Mata Atlântica, Mudanças no nível do mar no Pleistoceno.

## INTRODUCTION

The Neotropical region's freshwaters are highly diverse, representing approximately one-fifth of the more than 35,000 known extant fish species (Nelson *et al.*, 2016; Birindelli, Sidlauskas, 2018; Fricke *et al.*, 2021). The coastal rivers of eastern Brazil stand out by their high proportion of endemic species (Bizerril, 1994; Schaefer, 1997; Mittermeier *et al.*, 2005; Albert *et al.*, 2011). This high proportion of endemics is thought to be related to the isolation of these drainages from larger continental basins (Ribeiro, 2006; Thomaz, Knowles, 2018; Wendt *et al.*, 2019).

Among the geographic processes associated with geographic distribution and genetic structuring of populations within the coastal rivers of southeastern Brazil, the two more often cited are headwater stream capture and isolation/connection due to marine transgressions and regressions (Ribeiro, 2006; Roxo *et al.*, 2014; Thomaz, Knowles, 2018; Thomaz *et al.*, 2019). Stream capture, when part of a river drainage basin is diverted into an adjacent basin, is an important geographic event affecting the distribution of freshwater organisms (Albert *et al.*, 2011, 2018; Souza *et al.*, 2020). From a biogeographic perspective, stream capture involves first geodispersal (*e.g.*, erosion of barriers) and latter vicariance (*e.g.*, emergence of new geographic barriers to dispersal and gene flow) (Albert, Crampton, 2010). Another contrasting geographic event is the connection and disruption of basins caused by marine transgressions and regressions that may profoundly affect the population dynamics on the coastal drainages (Thomaz *et al.*, 2015; Shelley *et al.*, 2020). During the Pleistocene glacial periods, the Brazilian

coast was impacted by lower sea levels, and the exposed paleodrainages connected currently isolated riverine basins and may have served as dispersal corridors (Dias *et al.*, 2014; Thomaz *et al.*, 2015, 2017).

The south and southeastern coastal drainages of Brazil encompass three Freshwater Ecoregions of the World (FEOW): Tramandaí-Mampituba, Southeastern Mata Atlântica, and Ribeira de Iguape (Abell *et al.*, 2008; Thomaz, Knowles, 2018). Ecoregions are widely recognized and applied geospatial units for conservation planning, developed to represent patterns of environmental and ecological variables known to influence the distribution of biodiversity at broad scales (Abell *et al.*, 2008). Rivers of southeastern Brazil flow within the Atlantic Rain forest, a major biodiversity hotspot. This biome has been reduced to less than 8% of its original range. Currently, its fragments harbor one of the largest percentages of endemic species in the world (Morellato, Haddad, 2000; Rodrigues, 2005; Carnaval *et al.*, 2009). Freshwater ecosystems and their diverse communities of species may be the most endangered (Abell *et al.*, 2008). Species of freshwater fish are often unable to cross barriers and represent an evolutionarily conserved system most susceptible to climate change (Dias *et al.*, 2014). Knowledge of genetic diversity patterns on isolated freshwater systems within the Atlantic Rainforest is still limited, and conservation planning is hindered.

To evaluate phylogeographic patterns in this region, we accessed the genetic diversity of *Hisonotus leucofrenatus* (Miranda Ribeiro, 1908), which belongs to the Hypoptopomatinae, a group of small-sized loricariids including 252 species (Fricke *et al.*, 2021). *Hisonotus* Eigenmann & Eigenmann, 1889, is the most species-rich genus of Hypoptopomatinae and recent contentious taxonomic circumscription suggests that this group encompasses about 50 species (Roxo *et al.*, 2019; Reis *et al.*, 2019). *Hisonotus leucofrenatus* occurs in coastal drainages of southern and southeastern Brazil, between Tramandaí (southern boundary) and Ribeira de Iguape (northern boundary) (Fig. 1; Carvalho, Reis, 2011), in which inhabits lowland portions of rivers within slow to medium flowing waters (Malabarba *et al.*, 2013), characteristics that are associated to species of large distribution (Carvajal-Quintero *et al.*, 2019). Habitat and other ecological specificities are often associated with phylogeographic structure (Papadopoulou, Knowles, 2016). In this sense, the lowlander *H. leucofrenatus* contrasts with other fish species in this region that were studied in a phylogeographic framework (*e.g.*, *Hollandichthys* Eigenmann, 1910, a forest specialist in Thomaz *et al.*, 2015; *Diapoma itaimbe* (Malabarba & Weitzman, 2003) and *Bryconamericus lethostigmus* (Gomes, 1947) rapid dwellers in Hirschmann *et al.*, 2015, 2017; and Thomaz, Knowles, 2020) that often show a per basin genetic structure.

Thus, this study main objective is to evaluate how Pleistocene sea-level changes may have affected the genetic diversification of *Hisonotus leucofrenatus*. If paleodrainage connections during marine regressions served as an opportunity for dispersal among currently isolated rivers, they might have left a signature in the genetic diversity of this species, otherwise, if proposed paleodrainages fail to describe past connections correctly, it can be suggested that processes such as river capture may have a more significant role (*e.g.*, Lima *et al.*, 2017). We also evaluate how physical characteristics of these paleodrainages (*e.g.*, area, number of tributaries) may correlate with populations' genetic diversity in this region (Thomaz *et al.*, 2017).

## MATERIAL AND METHODS

**Samples.** We included 80 specimens of *H. leucofrenatus* in the analyses distributed throughout its area of occurrence, in a total of 14 currently isolated drainages, from Tramandaí River system in Rio Grande do Sul State to Ribeira de Iguape River basin in São Paulo State (Fig. 1) corresponding to the known species distribution (Carvalho, Reis, 2011). The number of samples per each drainage vary between 3 and 12 specimens and are often from distant collecting localities, trying to maximize the genetic diversity observed at each drainage (Tab. 1; S1). Samples came from museum historical collections, but three recent collection expeditions were conducted to obtain genetic data in regions that there were no tissue samples. Fish expeditions were done with permits (SISBIO/ICMBio #9318–1, 9220–1, 8796–1). All fishes were euthanized using clove oil - eugenol (following Lucena *et al.*, 2013), and tissues were collected from fin clips or muscle. All samples are deposited in the fish collection of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS) (S1).

**Molecular data.** DNA extractions were made using a CTAB protocol (Doyle, Doyle, 1987). PCR was used to amplify the mitochondrial gene Cytochrome Oxidase I (cox1) (primers LCO1490 and HCO 21908 primers in Folmer *et al.*, 1994) and nuclear gene rpS7 ribosomal protein gene intron 1 (rpS7; S7RPEX1F and S7RPEX2R primers in Chow, Hazama, 1998). Heterozygous sites in the rpS7 nuclear gene were coded with IUPAC ambiguity codes. For the nuclear gene rpS7 intron 1 only a subset of 27 specimens was sequenced due to difficulties in amplification, this datasets represents most drainages and all paleodrainages. PCRs were carried out in 20 µL reactions containing 10–50 ng DNA, 0.2 µM of each primer, 0.2 mM of each dNTP, 1X Buffer, 1.5 µM MgCl<sub>2</sub> and 1U Platinum Taq DNA polymerase (Invitrogen, São Paulo, Brazil). PCR conditions followed recommendations reported for the respective primers. PCR products were checked by electrophoresis in agarose gel, purified using ExoSap (Exonuclease I and Shrimp Alkaline Phosphatase, GE Healthcare, Piscataway, NJ, USA) and sequenced cox1 and rpS7 genes in both directions by MACROGEN Inc., Seoul, Korea, and ACTGENE Ltda., Porto Alegre, Brazil. Sequences were edited and aligned in Geneious 7.1.3.0, that uses the Muscle algorithm (Edgar, 2004). All sequences were uploaded in the GenBank (S1).

**Phylogeographic and phylogenetic reconstruction methods.** The haplotype diversity, nucleotide diversity, neutrality tests Fu's FS (Fu, 1997) and Tajima's D (Tajima, 1989) were calculated on DNASP v.5 (Librado, Rozas, 2009) for the cox 1 gene. The haplotype network was constructed with the cox1 gene using the Median-Joining method in PopArt (Bandelt *et al.*, 1999). PartitionFinder 2.1.1 (Lanfear *et al.*, 2016) was used to find the best nucleotide substitution models. The cox1 gene was partitioned by codon positions and rpS7 considered as a single partition and the Bayesian Information Criterion (BIC) was used to model selection in PartitionFinder. Phylogenetic relationships between populations were inferred using Bayesian Inference in BEAST 2.3 (Bouckaert *et al.*, 2019) and both genes (cox1 and rpS7) separately. A strict molecular clock was used to estimate the divergence time between lineages, appropriate for analysis within species or among closely related species (Li, Drummond,

**TABLE 1** | Number of samples sequenced for Cytochrome Oxidase subunit 1 (cox1) and rpS7 ribosomal protein gene intron 1(rpS7) genes by drainage (populations) and its respective paleodrainage.

Drainage	Paleodrainage	cox1	rpS7
Ribeira de Iguape	ribeira de iguape	7	2
Paranaguá	paranaguá	7	2
Guaratuba	itajaí	4	1
Cubatão Norte	itajaí	4	2
Itapocu	itajaí	4	2
Itajaí	itajaí	13	5
Tijucas	florianópolis	5	2
Biguaçu	florianópolis	5	2
Cubatão Sul	florianópolis	4	2
Tubarão	tramandaí	7	1
Urussanga	tramandaí	4	-
Araranguá	tramandaí	5	1
Mampituba	tramandaí	3	1
Tramandaí	tramandaí	8	3

2011). We use cox1 evolutionary rates proposed by Bermingham *et al.* (1997) of 0.01/site/million years, followed by other phylogeographic studies in the region (Thomaz *et al.*, 2015; Hirschmann *et al.*, 2015, 2017). The haplotype trees were based on 40 million MCMC steps. The trees were sampled every 1000 steps; chain efficiency was observed in TRACER 1.5 (Rambaut *et al.*, 2018), and after that, trees were summarized using Maximum Clade Credibility tree criterion in treeAnnotator with 10% burn-in.

Calculation of Fstatistic ( $\Phi_{st}$ ) and analysis of molecular variance (AMOVA) were done in ARLEQUIN 3.5 (Excoffier *et al.*, 2005) using the cox1 gene. Groups on AMOVA, were made considering paleodrainages following (Thomaz, Knowles, 2018), and each population was equal to the currently isolated drainages by the sea (Tab. 1; S1). We also follow the same nomenclature of paleodrainages as in Thomaz, Knowles (2018, tab. 1). Since some paleodrainages, have the same names as current drainages, here we used the first word capitalized for drainages and lower case for paleodrainages. Samples on rivers draining to the Paranaguá bay area were grouped into two populations in order to have more than one specimen per population in the AMOVA analysis: one population with samples from nearest rivers (draining the west portion) and another population corresponding to the northern tributaries (Fig. 1), similar to phylogeographic structure proposed for other groups in the region (Tschá *et al.*, 2017). These two populations compose the paleodrainage of paranaguá for the AMOVA configuration. Samples from Ribeira de Iguape river drainage were excluded from AMOVA analysis because there is a single population (drainage) to a single paleodrainage. To evaluate whether there was a correspondence between genetic variation and paleodrainages features, we examined the correlation between genetic diversity (mean number of pairwise differences -  $\pi$ ) and paleodrainage physical characteristics such as number of tributaries, contemporary area and exposed area (Papadopoulou *et al.*, 2011; Thomaz *et al.*, 2017; Thomaz, Knowles, 2018; S2) using the linear regression function in Microsoft Excel.

## RESULTS

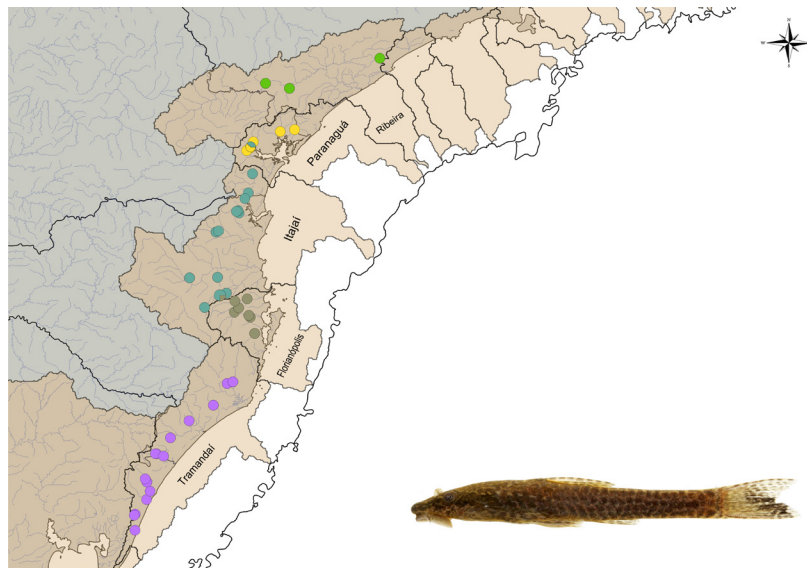
A total 80 specimens from the entire distribution of *H. leucofrenatus* (Fig. 1) were sequenced (678 base pairs) for the mitochondrial *cox1* gene. For the nuclear gene *rpS7* intron 1 a subset of 27 specimens (608 base pairs) was sequenced (Tab. 1; **S1**). Best fit nucleotide substitution models were TrNef (TrN equal base frequencies, Tamura, Nei, 1993) for first codon position and HKY (Hasegawa *et al.*, 1985) for second and third codon positions of the mitochondrial *cox1* and HKY+G for the nuclear *rpS7*. Details on the number of sequenced specimens, number of observed mitochondrial haplotypes, haplotype diversity (*h*), nucleotide diversity ( $\pi$ ), Tajima's *D*, Fu's *FS* and theta values are in Tab. 2. Only *cox1* values are listed, as the sampling of nuclear *rpS7* is relatively low – this later gene was used only for phylogenetic reconstructions.

**Phylogenetic history and divergence time estimates.** Our phylogenetic estimates support two highly diverged clades in both molecular markers (Fig. 2A – *cox1*; Fig. 2B – *rpS7*), herein named north and south. According to molecular calibration only using the mitochondrial marker, this divergence occurred at about 2.6 Ma (95% confidence intervals of 1.9 – 3.5 Ma). In the north group, a clade representing individuals from Ribeira de Iguape River drainage in the *cox1* tree form a monophyletic unit sister to a large group containing several populations from other southern basins (Fig. 2A; **S3**), this relationship is not supported by the *rpS7* gene (Fig. 2B; **S4**). The group formed by Ribeira de Iguape River specimens diverged at about 1.3 Ma (95% confidence intervals of 0.8 – 2.8 Ma) from other southward populations in the north group (**S3**). Most paleodrainages form well supported monophyletic units with some degree of lineage sharing between paranaguá and itajaí and also between itajaí and florianópolis paleodrainages in both *rpS7* and *cox1* genes. Divergence age estimates of these less

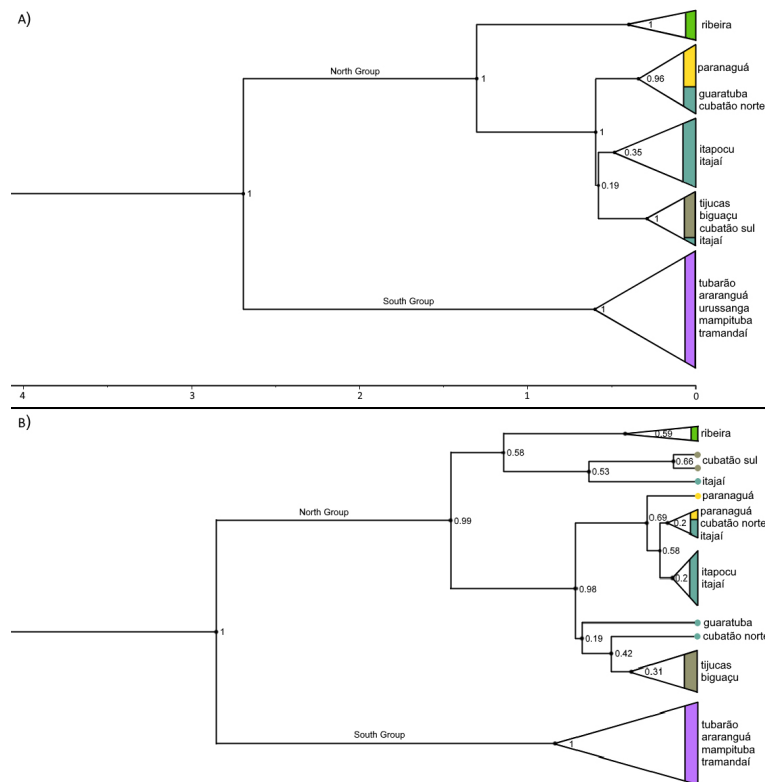
**TABLE 2 |** Genetic diversity of *cox1*: sample size (N), haplotype number (H), haplotype diversity (Hd), mean number of pairwise differences ( $\pi$ ), D of Tajima statistic (D) and FS test (FS). \*Value of  $P < 0.001$ ; \*\*Population not considered for the AMOVA analyses.

	Paleodrainage	N	H	Hd	$\pi$	D	P	FS	P
	tramandaí/ mampituba	27	11	0.8775 ± 0.0386	2.427350 ± 1.357928	-0.4924	0.337	-3.64016	0.024
	florianópolis	12	3	0.6667 ± 0.0910	0.787879 ± 0.613118	0.55418	0.767	0.21735	0.456
<i>cox1</i>	itajaí	25	10	0.8933 ± 0.0354	2.166667 ± 1.242691	-0.6008	0.319	-3.35801	0.031
	paranaguá	6	4	0.8000 ± 0.1721	1.333333 ± 0.954521	-129.503	0.079	-1.25217	0.07
	ribeira**	7	4	0.7143 ± 0.1809	1.61904 ± 1.082592	-102.379	0.186	-0.53807	0.211
<b>Source of variation</b>					<b>d.f.</b>	<b>% of variation</b>			
Among paleodrainage					3	90.6			
Among populations					10	4.31			
Within population					56	5.1			
FCT					0.90596*				
FSC					0.45801*				
FST					0.94903*				





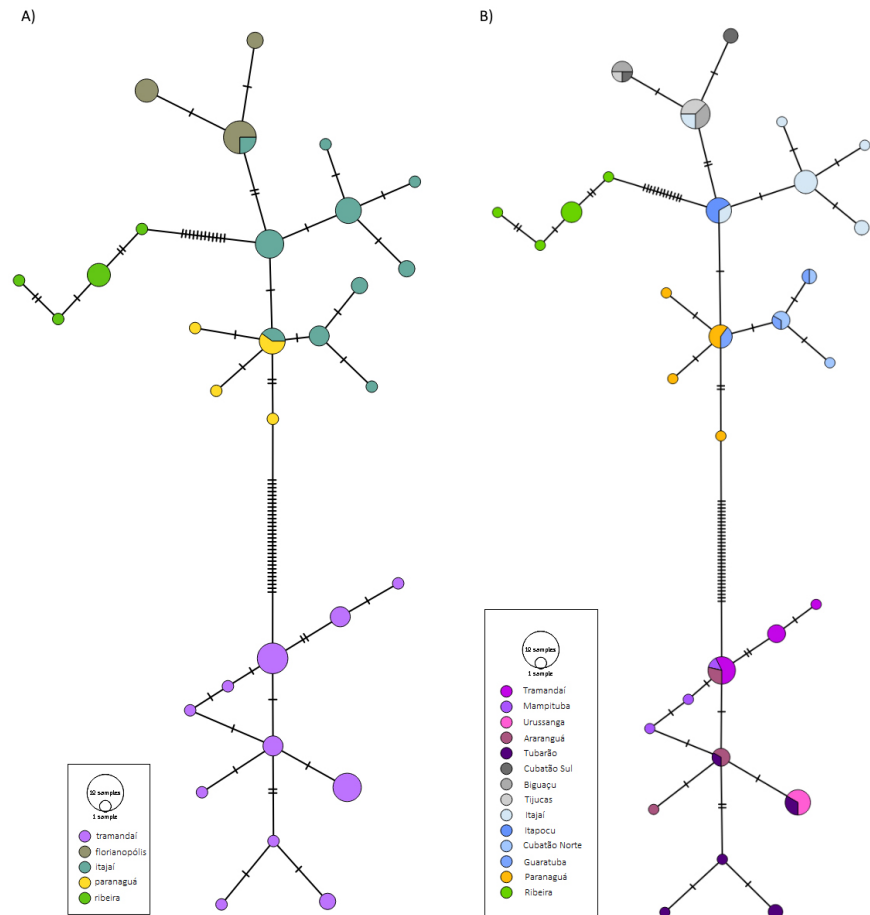
**FIGURE 1** | Map of geographic distribution and included samples of *Hisonotus leucofrenatus* from coastal drainages of southeastern Brazil. Colors of the circles represent groups sampled according limits of paleodrainage systems for the region and sample photo of *Hisonotus leucofrenatus* from Tramandaí River system, UFRGS 16528 (photo by L. R. Malabarba).



**FIGURE 2** | Phylogeny of *Hisonotus leucofrenatus* samples. **A.** Cytochrome Oxidase subunit 1; and **B.** rps7 ribosomal protein gene intron 1 sequences. Numbers represent posterior probabilities of the respective node. Terminals collapsed into large and well supported group and colors represent paleodrainages according to Fig. 1. In (A) X axis represents time (Ma).

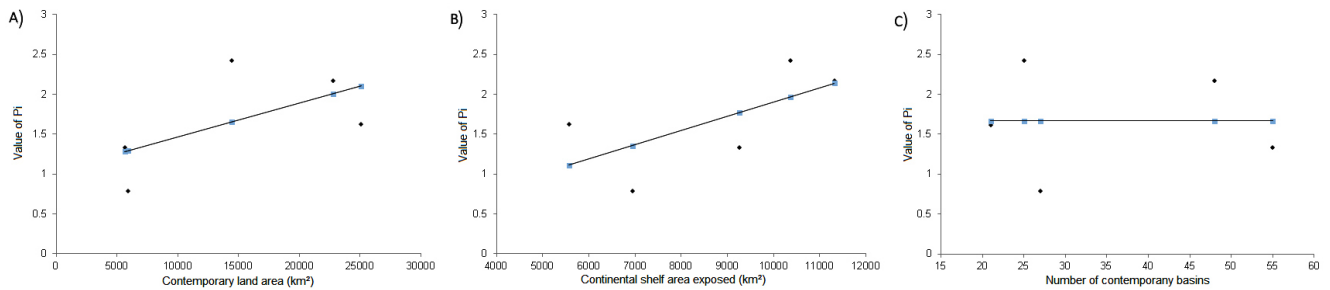
inclusive monophyletic units vary between 0.6 Ma (tramandaí) and 0.3 Ma (florianópolis). Similar patterns of geographic structure by paleodrainage are observed in the haplotype network for mtDNA data that shows two consistent and genetically distinct groups: north (including paleodrainages: ribeira de iguape, paranaguá, itajaí and florianópolis) and south (limited to a single paleodrainage, tramandaí – see Fig. 3A). Only two among 32 haplotypes (Tab. 1) are shared between paleodrainages. Extensive haplotype sharing between drainages (populations) is observed throughout *H. leucofrenatus* distribution, except the Ribeira de Iguape River drainage with its haplotypes (Fig. 3B).

**Paleodrainage genetic structure and diversity.** Paleodrainage structure is strongly evidenced by both network and phylogenetic analyses (Figs. 2–3A). AMOVAs suggests a greater partitioning of genetic variation among paleodrainage (90.6%) than among populations (current drainages) within paleodrainages (4.3%) and within populations (5%) (Tab. 2). The linear regressions pointed to a positive correlation and high r-square values between paleodrainage contemporary land area and continental shelf area exposed when compared with index of genetic diversity (mean number of pairwise differences -  $\pi$ ) but not a strong correlation with the number of isolated drainages in each paleodrainage (Figs. 4A–C). Other descriptive values of genetic diversity in paleodrainages are found in Tab. 1.



**FIGURE 3** | Haplotype network using Median-Joining for only *cox1* sequences of *Hisonotus leucofrenatus* samples. **A.** Colors represent groups by paleodrainage; and **B.** Colors represent populations by current drainages. We use upper case in the first word of the noun for drainages (e.g., Tramandaí River system) to differentiate from the homonymous paleodrainages (e.g., tramandaí).





**FIGURE 4 |** Linear regression plots of the relationship between genetic diversity (mean number of pairwise differences  $\pi$ ) and physical characteristics of paleodrainage (contemporary land area, continental shelf area exposed and number of contemporary basins) as described by Thomaz, Knowles, 2018 (S2). **A.** Contemporary land area ( $R^2 = 0.90$ ); **B.** Continental shelf area exposed ( $R^2 = 0.77$ ); **C.** Number of contemporary basin ( $R^2 = 0.21$ ).

## DISCUSSION

The freshwater fishes in the coastal region of Brazil were strongly influenced by marine regressions and transgressions (Weitzman *et al.*, 1988; Thomaz *et al.*, 2015), which alternately connected and disconnected populations through Pleistocene glacial cycles. The phylogeographic structure of *H. leucofrenatus* corroborates proposed paleodrainage configurations on the southeastern coast of Brazil during periods of marine regression (Dias *et al.*, 2014; Thomaz *et al.*, 2015, 2017; Thomaz, Knowles, 2018). This shows that freshwater fishes in this region used these paleoconnections during sea regression periods, and interchanges among adjacent drainages were rare. Our data evidenced that paleodrainages, promoted by marine regressions, shaped the genetic profile of these populations (Thomaz *et al.*, 2015, 2017; Tschá *et al.*, 2017). The influence on patterns of genetic diversity seems to be associated with paleodrainage physical features as well (Figs. 4A–B; Thomaz *et al.*, 2017). The species–area relationship is a robust biogeographic predictor for richness and endemism (Albert *et al.*, 2011), and as seems to be the case for genetic diversity (Fan *et al.*, 2019). In our estimates, we found that both the current area and past geographic area (area submerged in the continental platform) strongly correlates with genetic diversity, but not the number of drainages (Fig. 4C), suggesting that the current isolation of coastal basins has less impact on the genetic structure of the populations (Thomaz *et al.*, 2017).

Factors intrinsic to species can result in specific distribution patterns (Whiteley *et al.*, 2004; Burrige *et al.*, 2008). For example, fish faunas from high latitudinal regions are dominated by large-bodied species, while the fish assemblages from river basin of the neotropical realms harbor smaller mean body sizes (Blanchet *et al.*, 2010). This biological trait is associated with the dispersal capacity of freshwater fishes, where small-bodied species have less dispersal capacity (Tedesco *et al.*, 2012; Radinger, Wolter, 2014). *Hisonotus leucofrenatus* as a small-bodied species seems to contrast this general rule, at least when we consider dispersal among drainages in the same paleodrainage. Studies in this region have shown that the corridors among drainages facilitate dispersal depending on species specific ecological restrictions (Hirschmann *et al.*, 2015, 2017; Thomaz *et al.*, 2015, Thomaz, Knowles, 2020). In terms of habitat

specificity, *H. leucofrenatus* populations are mostly found in the lowlands (Malabarba *et al.*, 2013), an area that has higher connectivity during lower sea levels (Carvajal-Quintero *et al.*, 2019). These characteristics contrasts with some other investigated taxa in a phylogeographic context such as *Hollandichthys*, a forest specialist (Thomaz *et al.*, 2015; Thomaz, Knowles, 2020), and *Diapoma itaimbe*, *Bryconamericus lethostigmus*, and *Epactionotus* Reis & Schaefer, 1998, found almost exclusively in fast-flowing waters towards headwaters of river drainages (Hirschmann *et al.*, 2015, 2017; Delapieve *et al.*, 2020; Thomaz, Knowles, 2020). Within coastal drainages, some species show patterns of genetic structuring also by current drainage and not just preterit drainage (Lima *et al.*, 2017; Delapieve *et al.*, 2020), a feature not observed in *Hisonotus* (*e.g.*, AMOVA, Fig. 3B). In terms of macroevolution, hypoptopomatines inhabiting fast-flowing, rocky riverine habitats exhibited increased body size and higher lineage diversification rates (Roxo *et al.*, 2017), which can be associated with the geographical discontinuity and diminished connectivity of these types of habitats. Higher species richness and limited distributions are seen in the region for other loricariids as for *Epactionotus* (Delapieve *et al.*, 2020) and *Pareiorhaphis* Miranda Ribeiro, 1918 (Pereira, Reis, 2002; Cramer *et al.*, 2007), which contrasts with *H. leucofrenatus*, a single widespread species in these coastal basins.

*Hisonotus leucofrenatus* has a genetic diversity pattern that strongly correlates with paleodrainage configuration, evidencing its dispersal during the last glacial maximum. Dispersal between neighboring paleodrainages seem to be rare and its limits may compose strong barriers to gene flow within populations of fishes in this region, which is a potential factor for lineage diversification. One of the strong and oldest barriers affecting *H. leucofrenatus* populations is the one located between the paleodrainage tramandaí and florianópolis that is supported by strong genetic divergence between north and south groups (Figs. 2–3). In this area lies the Serra do Tabuleiro, a high promontory of the Serra do Mar that may act as a barrier for dispersal of fishes within the coastal basins of South Brazil. The role of this geographic feature as a barrier is also supported by species distributional limits (Carvalho, 2007) and genetic based evidence of other fish species (south barrier of Thomaz, Knowles, 2020). Furthermore, the genetic structure between populations of *H. leucofrenatus* support an additional previously proposed populational break (central break in Thomaz, Knowles, 2020 similar but southward in Tschá *et al.*, 2017) observed in the genetic structure of other species between Paranaguá Bay and Ribeira de Iguape River basin (Fig. 1). As discussed by these authors, no obvious geological structures would support this break, other than its paleodrainage discontinuity. This spatial break are not temporarily concomitant, which may also be supported by our age estimates (Fig. 2A), in general older within the Pleistocene than previously proposed to other species (Thomaz, Knowles, 2020).

The southeastern coastal drainages of Brazil encompass three Freshwater Ecoregions of the World: (Tramandaí-Mampituba, Southeastern Mata Atlântica and Ribeira de Iguape) that are widely applied geospatial units for conservation planning and biogeographical studies and represent environmental and ecological variables known to influence the distribution of biodiversity at broad scales (Abell *et al.*, 2008; Albert, Carvalho, 2011). In terms of genetic variation within these Freshwater Ecoregion patterns are, to some extension, discordant with limits proposed for these areas (*e.g.*, limits between Tramandaí-Mampituba and the Southeastern Mata Atlântica

ecoregions), which is true for other freshwater groups in this region (Thomaz, Knowles, 2020). For freshwater fish taxa in this region, it is more interesting, from a conservation standpoint, to look at the genetic structuring of paleodrainages, than the current proposed ecoregion configuration. Further studies on the coastal basin of southeastern Brazil should contrast these observed genetic patterns with proposed areas of species endemism and bioregionalization (e.g., Menezes, 1988; Bizerril, 1994; Abell *et al.*, 2008).

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#### AUTHOR'S CONTRIBUTION

**Nathália Luiz Pio:** Investigation, Methodology, Resources, Writing–original draft, Writing–review and editing.

**Tiago P. Carvalho:** Investigation, Methodology, Project administration, Resources, Writing–original draft, Writing–review and editing.

## Neotropical Ichthyology



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#### ETHICAL STATEMENT

Fish expeditions were done with permits (SISBIO/ICMBio #9318–1, 9220–1, 8796–1). All fishes were euthanized using clove oil – eugenol (following Lucena *et al.*, 2013).

#### COMPETING INTERESTS

The authors declare no competing interests.

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