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Temporal genetic structure of a stock of *Prochilodus lineatus* (Characiformes: Prochilodontidae) in the Mogi-Guaçu River ecosystem, São Paulo, Brazil

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Prochilodus lineatus is a species of migratory fish widely distributed in the Paraná River basin, found mainly in the Grande, Pardo and Mogi-Guaçu rivers located in a well-developed region of the state of São Paulo. This study analyzes the genetic diversity and population structure in shoals of *P. lineatus* based on temporal analysis of specimens sampled over the years 2003, 2005, 2006, 2009, 2010, and 2015 in the Mogi-Guaçu River, São Paulo, at the region of Cachoeira de Emas. Genetic analysis performed using the D-Loop and seven microsatellite marker revealed significant genetic variability in all sampled groups. Moderate levels of structuring between groups were identified with the microsatellite markers (Fst = 0.14), while the mitochondrial marker did not reveal patterns of genetic structuring (Fst = 0.01). The genetic variability fluctuated over time, characterizing patterns of structuring among the analyzed samples. The occurrence of environmental alterations resulting in increased mortality rates, as well as changes in the water level in the ecosystem, among other factors, could determine changes in the reproductive behavior of species. The lack of favorable environmental conditions for reproduction in the basin, as reflected by tests of population bottlenecks, could have resulted in the differentiation of populations of *P. lineatus* over time.

Keywords: Curimbata, D-loop, Genetic diversity, Genetic structure, Microsatellites.

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Prochilodus lineatus é uma espécie de peixe migratório amplamente distribuído na bacia do rio Paraná, principalmente nos rios Grande, Pardo e Mogi-Guaçu localizados em uma região bem desenvolvida do estado de São Paulo. Este estudo analisou a diversidade genética e a estrutura populacional em cardumes de *P. lineatus* com base na análise temporal de espécimes amostrados ao longo dos anos de 2003, 2005, 2006, 2009, 2010 e 2015 na Cachoeira de Emas no rio Mogi-Guaçu, São Paulo, Brasil. A análise genética realizada com o marcador D-Loop e sete microssatélites revelou variabilidade genética significativa em todos os grupos amostrados. Níveis moderados de estruturação entre os grupos foram identificados com os marcadores microssatélites (Fst = 0.14), enquanto o marcador mitocondrial não revelou padrões de estruturação genética (Fst = 0.01). A variabilidade genética identificada no estoque oscilou ao longo do tempo, caracterizando padrões de estruturação entre as amostras analisadas. A ocorrência de alterações ambientais resultando em aumento das taxas de mortalidade, bem como mudanças no nível de água no ecossistema, entre outros fatores, podem determinar mudanças no comportamento reprodutivo das espécies. A falta de condições ambientais favoráveis para a reprodução na bacia, pode ter resultado na diferenciação das populações de *P. lineatus* ao longo do tempo.

Palavras-chave: Curimbatá, Diversidade genética, D-loop, Estrutura genética, Microssatélites.

INTRODUCTION

Freshwater ecosystems are currently imperiled by anthropogenic activities worldwide (Jeremias *et al*., 2018; Strungaru *et al*., 2019; Iordache *et al*., 2020). In general, rivers undergo serious degradation due to uncontrolled wastewater disposals, affecting several species and causing the depletion of their natural populations by mortality (Martins *et al*., 2012; Warr, Greenfield 2012; Paschoal *et al*., 2020). Species that exhibit migratory behavior are more susceptible to the effect of anthropogenic and environmental changes since environmental fragmentation and mortality generally hinders their reproductive process (Izzo *et al*., 2016; Nieminen *et al*., 2017).

Despite the remarkable abundance and wide distribution, previous studies have reported that variation in water quality has also caused severe reductions in many migratory species including *Prochilodus lineatus* (Valenciennes, 1837) (Agostinho *et al*., 2007; Garcez *et al*., 2011; Machado, Foresti, 2012; Rueda *et al*., 2013; Perini *et al*., 2021). Among the main migratory species of Neotropical fish, the curimbata *P. lineatus* is an ordinary species that dwells in the Paraná River basin and is found mainly in the Grande, Pardo and Mogi-Guaçu rivers (Godoy, 1975). Although it is more frequently detected during its reproductive migration, individuals of this species can be captured during the whole year along these rivers and in the Cachoeira de Emas region in the city of Pirassununga, São Paulo (Godoy, 1975; Agostinho, Gomes, 2018). During the reproductive period, *P. lineatus* forms large fish aggregations, which are usually found downstream of waterfalls or dams (Godoy, 1975). Juveniles grow fast; males may attain

20 cm in the first year and may already be mature after the first year of life (Gomes, Agostinho, 1997). Moreover, *P. lineatus* is considered an important species, playing a very important role in preventing sediment accumulation and promoting the transfer of energy in the food chain. Nevertheless, *P. lineatus* could be more exposed to toxicants, since many pollutants concentrate in the sediments (Weber *et al*., 2013).

Anthropogenic activities can affect genetic variation over both long- and short-time scales (Blaber *et al*., 2000). Population structuring and the accumulation of genetic differences between different groups can lead to reproductive isolation, gene flow disruption, decrease in the effective population size, increased inbreeding, making these groups more susceptible to the process of extinction (Frankham *et al*., 2014; Krishan *et al*., 2015). Given that, it is increasingly important to examine factors that influence the loss of biodiversity of migratory species (Strayer, Dudgeon, 2010; Van Leeuwen *et al*., 2018; Morita *et al*., 2019).

Despite many environmental processes occurring too slowly to be appreciated in short to medium-term surveys, there is an increasing interest in temporal studies analyzing the effects of environmental changes on molecular genetics (Barcia *et al*., 2005; Fullerton *et al*., 2011; Prunier *et al*., 2018). In this sense, the use of molecular markers such as microsatellites (SSRs) and the sequencing of the mitochondrial DNA control region (D-loop) has proven to be an excellent tool for estimating recent and ancient genetic variation in populations (Avise, 1994; Guo *et al*., 2019). These molecular markers allow quantifying the genetic variability between/among shoals, providing the understanding of the mechanisms involved in the genetic structuring of populations more accurately, and such information made it possible to propose better conservation strategies for the species (Dowling *et al*., 2015; Ferreira *et al*., 2017; Castellanos-Galindo *et al*., 2019; Guzman *et al*., 2020).

Long-term temporal studies of genetic variability are needed to understand the dynamics of populations and the effects of natural and human-induced forces on populations. In this context, the present study aimed to identify possible structural changes in the stock of *Prochilodus lineatus* from the Mogi-Guaçu River based on the analysis of specimens collected at Cachoeira de Emas over the period between 2003 and 2015, using nuclear and mitochondrial molecular markers in a temporal genetic analysis. The information presented here could provide a first insight into the genetic structure of the species in this ecosystem, and the consequences of anthropic actions on wild stocks of the species. This information could be further used in the proposition of adequate management and conservation programs for the curimbata and other species in the ecosystem.

MATERIAL AND METHODS

Study site and sampling collection. The study area comprises the locality of Cachoeira de Emas in the Mogi-Guaçu River, Pirassununga, São Paulo State, Brazil (Fig. 1). *Prochilodus lineatus* is a plentiful fish species from the Paraná River basin, especially within the waters of the Grande, Pardo and Mogi-Guaçu rivers (Godoy, 1975). A total of 209 adult specimens were obtained at one study site (Cachoeira de Emas) in the Mogi-Guaçu River from seven temporal collections (Sep03, Jan05, Aug05, Jan06,

Jan09, Sep10 and Feb15) (Tab. **S1**). All individuals were adults, possibly of reproductive age (1–2 years), showing body lengths above 25 cm (Vazzoler, 1996). Tissue fragments of each individual were collected and preserved in 95% alcohol before being deposited in the collection of the Laboratório de Biologia e Genética de Peixes (LBP) at UNESP, in Botucatu, São Paulo, Brazil. The total genomic DNA was obtained from fin tissue fragments of the individuals using the protocol proposed by Ivanova *et al.* (2006). The genetic analysis was performed using microsatellites and mitochondrial DNA (D-loop) sequences as genomic markers.

Microsatellites amplification. The 209 individuals sampled from 2003 to 2015 were analyzed using seven microsatellite loci, of which six were previously described for *P. lineatus* (PL3, PL9, PL14, PL119, PL139 and PL216; Rueda *et al.*, 2013) and one (AG72) was obtained through heterologous amplification from *Megaleporinus macrocephalus* (Garavello & Britski, 1988) (Morelli *et al.*, 2007). Both primers were labeled with FAM and HEX fluorescence. PCR reactions were performed in five separate runs for each multiplex in a thermocycler (ABI 3130, Applied Biosystems), using specific primers

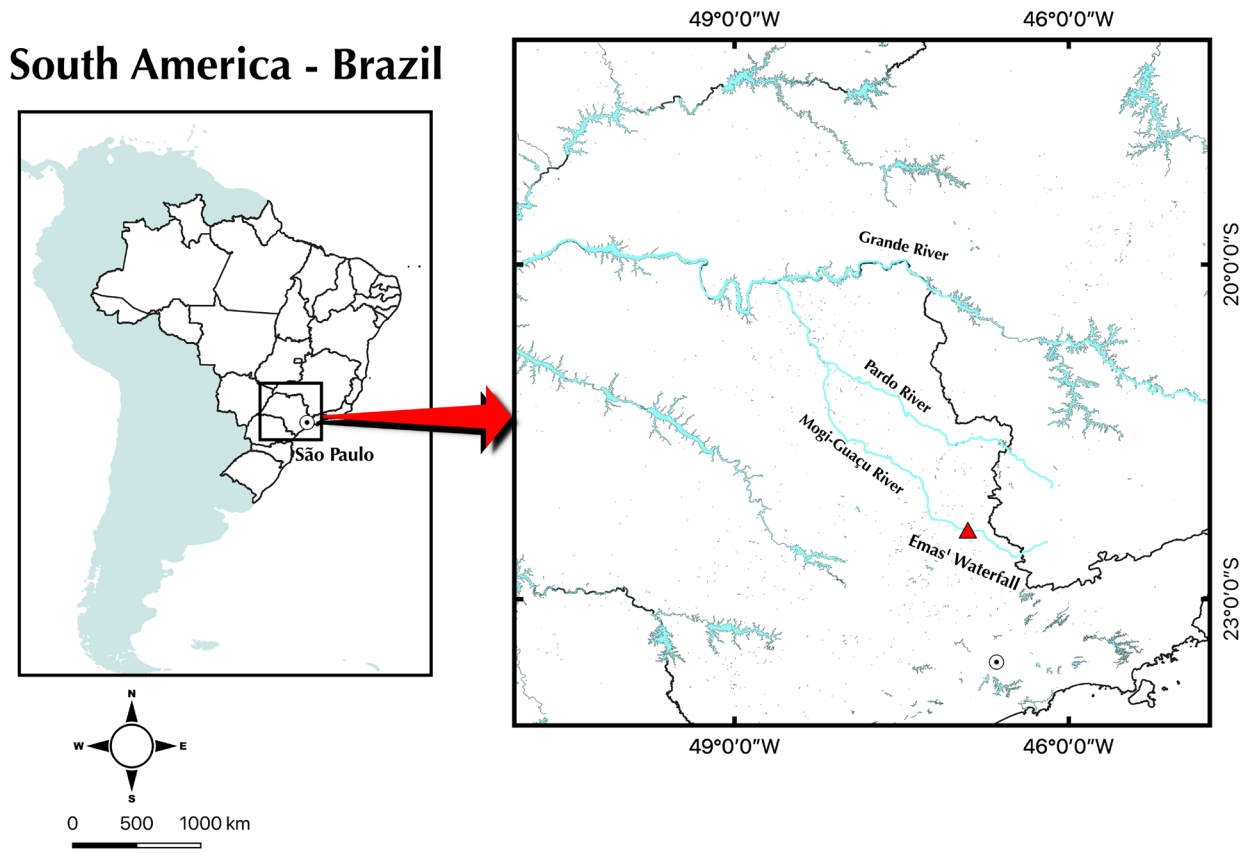

FIGURE 1 | Map of the State of Sao Paulo showing the main components of the hydrographic system in the Southeast of Brazil. In detail square, the collection site of samples located in Cachoeira de Emas, Pirassununga-SP. The Mogi-Guaçu River is a component of the Upper Paraná River basin.

in each multiplex. The following reagents were used for each sample: 3.25 µl H_2O; 2.4 µl dntps; 1.5 µl buffer; 0.2 µl primers f; 0.2 µl primers r; 0.75 µl $MgCl_2$; 0.3 µl taq; 1 µl DNA; Formamide; and size standard Rox. The products were genotyped in ABI 3130 sequencer (Applied Biosystems) according to the methodology proposed by Schuelke (2000). Microsatellite profiles were manually analyzed using the Gene mapper 4.3 program (Applied Biosystems).

Mitochondrial DNA amplification. Polymerase chain reaction (PCR) was performed using a set of forward primers (F-TTF: GCCTAAGAGCATCGGTCTTGTAA) and reverse primers (F-12R: GTCAGGACCATGCCTTTGTG) described by Sivasundar *et al*. (2001) following the protocol suggested by Platinum TaqDna Polymerase (Invitrogen). PCR reaction (total volume of 25 µL) contained 0.5 µM of each primer, 0.2 mM of dNTPs, 1.5 mM of MgCl2, 0.02 µl of Platinum TaqDna Polymerase (Invitrogen), 1 × amplification buffer and 2 ng/µL of DNA template. Thermal conditions were as follows: initial denaturation at 95 °C for 5 min, 35 cycles of denaturation (94 °C 40 s), annealing (50 °C 40 s) and elongation 72 °C 40 s, with final elongation at 72 °C for 10 min. Samples from 209 individuals were sequenced over seven temporal collections (Sep03, Jan05, Aug05, Jan06, Jan09, Sep10, and Feb10). Fragments of approximately 600bp were purified and sequenced in the ABI 3130 Genetic Analyzer (Applied Biosystems) with the BigDyeTM Thermator v 3.1 Cycle Sequencing Ready Reaction (Applied Biosystems) kit. Reverse and forward fragments were sequenced, and the consensus sequences were aligned using the Geneious 4.8.5 software (Kearse *et al*., 2012).

Population structure analysis. The temporal genetic structure of *P. lineatus* was investigated using the Bayesian clustering STRUCTURE v.2.3.3 software (Pritchard *et al*., 2000; Falush *et al*., 2003). The Markov chain Monte Carlo (MCMC) was run for 1 million generations, with an initial burn-in of 10% steps discarded, and 20 iterations of each K and the admixture model. The true number of populations is expected to be the value of K that maximizes the estimated model log-likelihood, log (P(X|K)) (Falush *et al*., 2003), and the K values were selected using the delta K (Evanno *et al*., 2005) method described by (Earl, VonHoldt, 2012) in Structure Harvester (https://taylor0.biolo gy.ucla.edu/structureHarvester). The AMOVA (Excoffier *et al*., 1992) examined the temporal genetic heterogeneity between groups using the Arlequin v.3.5.1.3 program (Excoffier, Lischer, 2010). Genetic population structure was inferred using F_{ST} values estimated with the Arlequin v.3.5.1.3 program (Excoffier, Lischer, 2010) and the genetic differentiation index Djost proposed by Jost (2008).

Genetic diversity. Genetic diversity for the mitochondrial (D-loop) marker estimating the number of haplotypes, haplotype diversity rather (h), nucleotide diversity (π), and polymorphic sites were evaluated using the DnaSP v.5.10.01 program (Librado, Rozas, 2009). The *F*stat v.2.9.3 program (Goudet, 2002) was used in the analysis of genetic diversity with the SSR markers to estimate the total number of alleles per locus (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and the inbreeding rate (F_{IS}). The Microcheker 2.2.1 program (van Oosterhout *et al*., 2004) was used for the detection of possible genotyping errors such as the presence

of stutter, dropout and null alleles. Possible deviations of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were calculated using the Genepop v4.2 program (Raymond, Rousset, 1995; Rousset, 2008). P values for HWE were corrected for multiple tests (P = 0.05/number of combinations) by applying a sequential Bonferroni correction (Rice, 1989).

Demographic analysis and effective population size. The neutrality test based on the model of Tajima D (Tajima, 1989) and Fu FS (Fu, 1997) were used for demographic analyzes. The analysis of a mismatch distribution was performed with the programs Arlequin 3.5.1.3 (Excoffier, Lischer, 2010) and Bottleneck v.1.2.02 (Piry *et al*., 1999). These tests are used to verify the occurrence of recent population expansion or reduction (bottleneck) (Fu, Li, 1993). Past changes in effective population size were also explored with the coalescent-based Bayesian skyline plot (BSP) created in BEAST v1.6.1 software (Drummond, Rambaut, 2007). The Markov chain Monte Carlo (MCMC) sampling for intervals along the genealogy was determined from coalescent events (Drummond *et al*., 2005) and the mutation rate was estimated considering1.935x10-2 mutations per site per million years (Mondin *et al*., 2018). All BEAST input.xml files were created in Beauti v1.6.1 software available in the package. The runs used a strict molecular clock set to one and therefore time was in mutational units (substitution/site). The results from multiple runs were combined using the LogCombiner v1.6.1 program available in the BEAST package and examined in Tracer v1.6 (Drummond, Rambaut, 2007) to check adequate mixing and convergence of the MCMC. The effective sample size for all parameters was > 200.

RESULTS

Population structure. The structure analysis conducted under the admixture model and K = 1–7 populations indicated the highest likelihood (ln(P)D) in a population structure of K = 3 (4790.34 ± 0.079), a result corroborated by the estimation of ΔK (Fig. 2). Likewise, the results from AMOVA for microsatellites revealed moderate genetic structure among all the groups (*F*st = 0.14; p ≤ 0.007; Tab. 1). In addition to the three temporal clusters identified with STRUCTURE, pairwise *F*st, *R*st and D 'Jost analyses also suggested moderate significant differentiation in genetic structure over the years (Tab. **S2**). However, the genetic differentiation for mitochondrial DNA was found either among populations within groups, with low genetic structure (*F*st = 0.01; p ≤ 0.08; Tab. **S3**). Nevertheless, a significant genetic differentiation between pairwise groups was found for mitochondrial DNA between Jan05xJan09 (*F*st = 0.11), Sep10xJan05 (*F*st = 0.09), Jan09xJan06 (*F*st = 0.05), as well as, between Jan05xSep10 (*F*st = 0.03) (p ≤ 0.07; Tab. 2; Tab. **S4**).

Genetic diversity. A total of 209 consensus D-loop sequences of 600 base pairs in length were obtained for *P. lineatus.* The analysis of mitochondrial data of *P. lineatus* collected in a sampling period of six years revealed an extensive genetic variability expressed by 51 polymorphic sites and 71 haplotypes. The haplotype diversity (h) ranged from 0.94 (Sep10) to 0.98 (Sep03/Aug05), whereas the nucleotide diversity (π) ranged from 0.08 (Jan09/Sep10) to 0.12 (Feb15) (Tab. 3). The greater genetic diversity

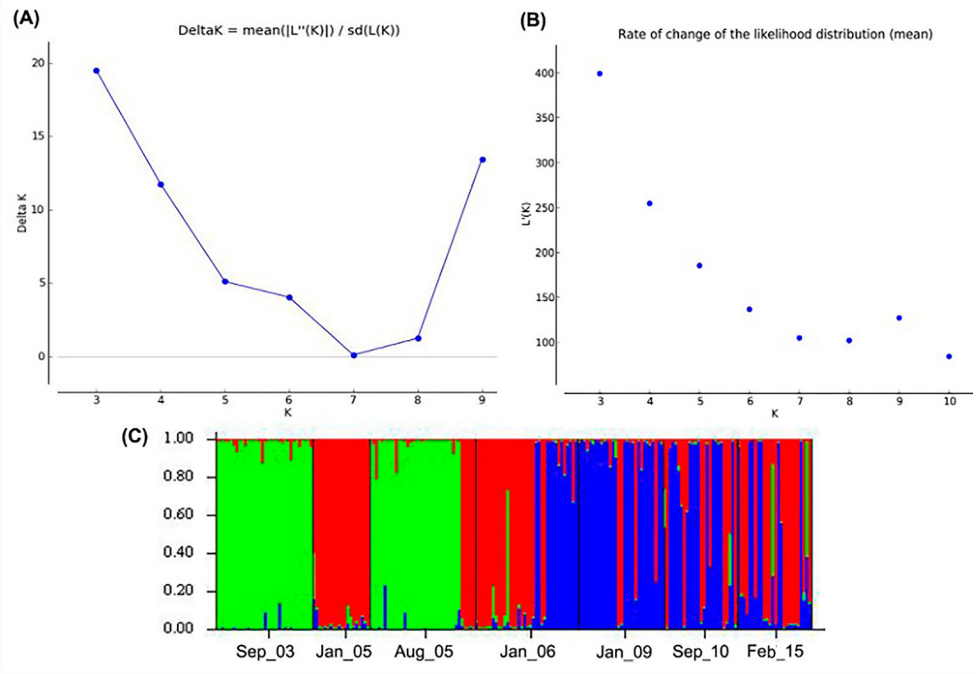

FIGURE 2 | Graph of the Bayesian analysis of population structure of microsatellites for *Prochilodus lineatus*. **A.** Delta(k) showing the highest value in a population structure of $K = 3$; **B.** The estimated mean log-likelihoods [ln(PrK)]; **C.** Structure bar plot. Black lines separate the different sampled populations based on temporal collection.

TABLE 1 | Analysis of Molecular Variance (AMOVA) of different groups of *Prochilodus lineatus* using microsatellite markers. *Statistically significant values $p \leq 0.007$.

Source of variation	Sum of squares	Components of variance	Percentage of variation	F' Statistics
Among the populations	129	0.41	14	F_{ST}: 0.14*
Among individuals within populations	510	0.64	22	F_{IS}: 0.26*
Within populations	311	1.82	63	F_{IT}: 0.36*
Total	951	2.88		

TABLE 2 | Analysis of F_{ST} coupled with *Prochilodus lineatus* collected along the period of 2003 to 2015 obtained with the D-loop marker. *Statistically significant values at the level of 5%.

	Sep_03	Jan_05	Aug_05	Jan_06	Jan_09	Sep_10
Sep_03	*					
Jan_05	0.01	*				
Aug_05	0.00	0.05	*			
Jan_06	0.00	0.01	0.01	*		
Jan_09	0.01	0.11*	0.00	0.03*	*	
Sep_10	0.01	0.09*	0.00	0.04	0.00	
Feb_15	0.00	0.00	0.02	0.00	0.05	0.05*

was also characterized by the number of haplotypes in each group, and the groups with a large index of variability were those characterized by the high number of haplotypes. The haplotype H5 was the most frequent in the network and appears internally, while the other haplotypes present peripheral distribution and lower frequencies (Fig. 3). The genetic variability of the 7 microsatellite loci for each collection sampled is described in Tab. 3. The highest mean number of alleles (Na = 12.00) and mean number of effective alleles (Ne = 8.05) were found in Sep03, while Jan05 had the lowest average for alleles (Na = 6.71), showing the smallest mean number of effective alleles (Ne = 3.40). Interestingly, the lowest number of average alleles and effective alleles was observed over the same sample period (Jan05). The average observed and expected heterozygosities (Ho and He), ranged from 0.41 (Sep10) to 0.58 (Jan09) and from 0.59 (Jan05) to 0.77 (Jan09), respectively. The inbreeding coefficient values (FIS) were significant in 35 of 49 comparisons in the seven groups analyzed, suggesting the existence of heterozygote deficiency. Likewise, the departure of HWE was detected in 27 out of 49 loci, which significantly deviated from expectations after Bonferroni correction (Tab. **S2**; $p \leq 0.007$). Analysis in Micro-Checker did not show any presence of null alleles, stuttering, or large allele dropout. The greatest source of variability for microsatellites was found within the individuals among collections (63%, F_{IT} 0.36), rather than within collections (22%, F_{IS} = 0.26) or among collections (14%, F_{ST} = 0.14) (Tab. 1).

Demographic analysis and population expansion. The Tajima D (Tajima, 1989) and Fu F_S (Fu, 1997) tests showed more extensive values of differentiation in the populations of Sep03 and Jan09 (Tab. 3), but all indices presented negative values. The Bayesian skyline plots to track historical fluctuation of effective population size in *P. lineatus* samples set by D-loop sequences showed a demographic balance in 6 out of the 7 groups analyzed (Fig. 4; Fig. **S5**), except for the group of Feb15 that presented a sharp curve of population expansion.

TABLE 3 | Genetic variability data and results of Tajima's D and Fu's Fs neutrality tests in different groups of *Prochilodus lineatus* through microsatellite and D-loop markers. N: number of samples; Na: number of average alleles; Ne: number of effective alleles; Ho: observed heterozygosity; He: expected heterozygosity; F_{IS}: intrapopulation index. h: haplotype diversity, π: nucleotide diversity. *Statistically significant values at the level of 5%.

	N	Na	Ne	Ho	He	F_{IS}	Genetic diversity	h	ϖ	Polymorphic sites	Tajima's	Fu's
Sep_03	29.14	12.00	8.05	0.53	0.74	0.24	0.76	0.98	0.10	30	-1.06*	-14.96*
Jan_05	14.57	6.71	3.40	0.45	0.59	0.21	0.56	0.97	0.11	18	0.16	-3.78*
Aug_05	31.71	10.29	5.56	0.56	0.70	0.20	0.52	0.98	0.09	22	-1.09	-9.36*
Jan_06	29.00	8.29	4.64	0.42	0.65	0.33	0.67	0.97	0.09	21	-0.71	-6.72*
Jan_09	23.57	11.57	7.07	0.58	0.77	0.17	0.56	0.96	0.08	28	-1.39	-10.46*
Sep_10	21.71	9.57	5.18	0.41	0.66	0.33	0.41	0.94	0.08	27	-1.54	-8.5*
Feb_15	16.71	7.57	4.20	0.48	0.64	0.23	0.72	0.97	0.12	27	-0.68	-6.23*

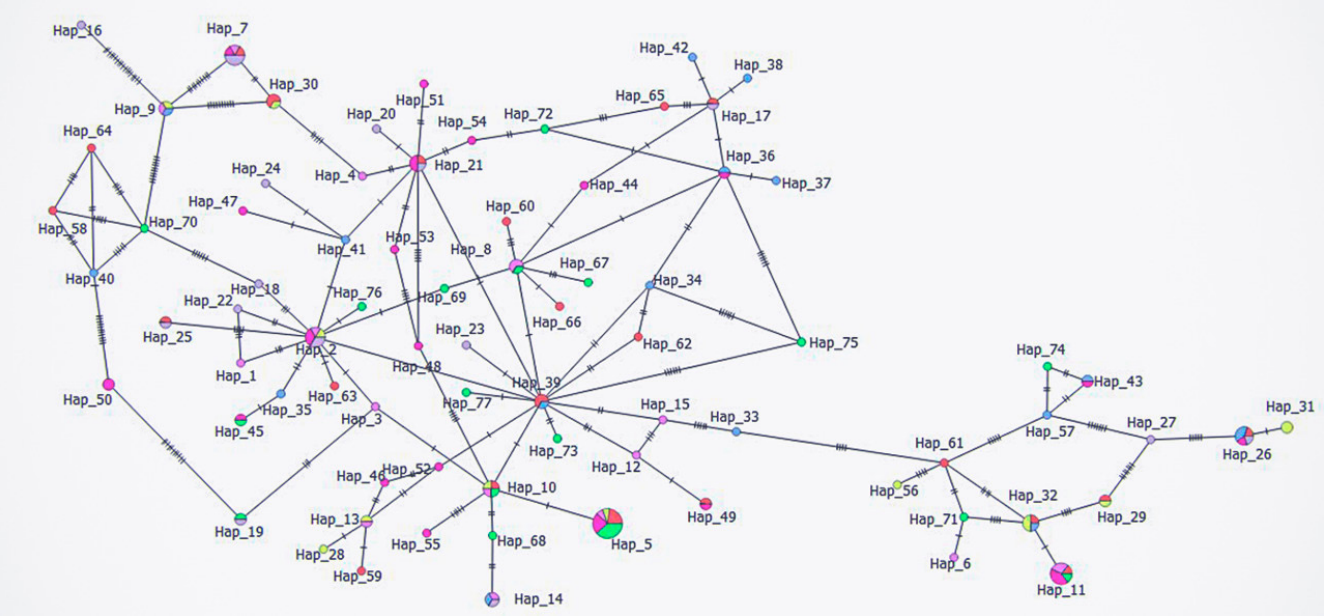

FIGURE 3 | Median-joining network of *Prochilodus lineatus*, based on haplotypes of D-loop marker. Sizes of the circled are proportional to the frequencies of the haplotypes at issue. The colors indicate the groups according to the collections: red circles: Sep_03; yellow circles: Jan_05; purple circles: Aug_05; blue circles: Jan_06; pink circles: Jan_09; green circles: Sep_10; pastel pink circles: Feb_15. Hatch marks represent the number of mutations by which haplotypes differ.

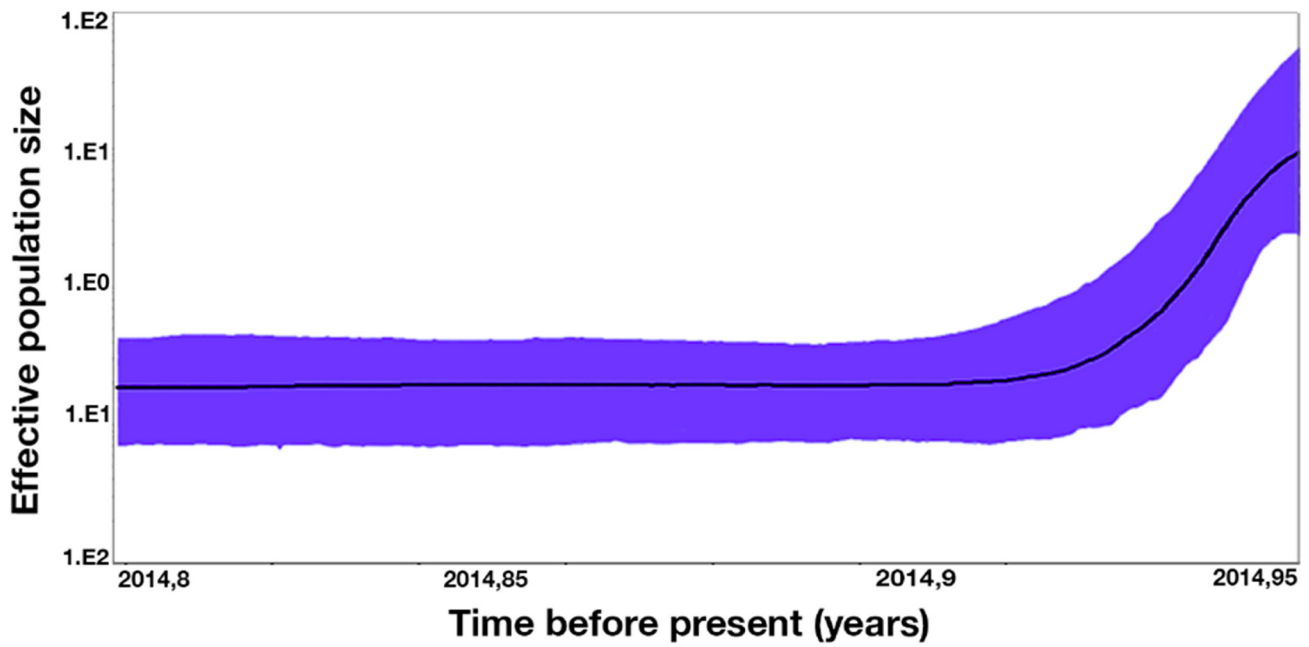

FIGURE 4 | Bayesian skyline plot (BSP) showing change in effective population size of *Prochilodus lineatus* in Feb_15 group from Cachoeira de Emas in the Mogi-Guaçu River based on Dloop marker. The y-axis, population size × generation time*; x-axis, time (indicated in thousands of years ago). *Generation time measured in million years. Solid lines represent median estimates, and shaded areas represent the 95% HPD limits.

DISCUSSION

Genetic diversity plays an important role in the origin, survival, and adaptability of species (Dachapak *et al*., 2017). The results of the present study provide interesting insights concerning the temporal genetic diversity of *P. lineatus* occurring in individuals captured in the Mogi-Guaçu River at the region of Cachoeira de Emas. The analysis of the mitochondrial DNA (D-loop) region revealed a high diversity of haplotypes ($h > 0.5$) and lower nucleotide diversity ($\pi < 0.005$), which is similar to the results of other studies with *P. lineatus* in the Paraná River basin (Yazbeck, Kalapothakis, 2007; Ferreira *et al*., 2017; Perini *et al*., 2021). Our finding was not in contrast to patterns observed in many other Chraraciform fish species, such as *Brycon opalinus* of the Paraíba do Sul basin (Hilsdorf *et al*., 2002), in which nucleotide diversity (π) ranged from 0.00 to 1.35%, *Leporinus elongatus* (Martins *et al*., 2003) from 1.78 to 7.70% including another study with *Prochilodus lineatus* (Sivasundar *et al*., 2001) which varied from 0.3 to 3.6%.

The results of lower nucleotide diversity observed in the Feb15 group might be the result of the accumulation of mutations since in a short time, haplotype diversity is easier to accumulate than nucleotide polymorphisms (Zhang *et al*., 2020). Although the recovery of the variability in the populations over the years is probably due to the large effective population size, as expected for populations with high migration rates (Santos *et al*., 2007). The migratory movements of these species enable large populations to be maintained (high effective population sizes), minimizing the loss of genetic diversity through genetic drift (Santos *et al*., 2007). Therefore, *P. lineatus* possesses one of the largest fish populations in the Grande River basin, with no signal of bottlenecks (Revaldaves *et al*., 1997; Sivasundar *et al*., 2001; Aguirre-Pabón *et al*., 2013; Perini *et al*., 2021). In addition, large effective population sizes also increase the rate of allelic recombination (Agostinho *et al*., 2003; Ferreira *et al*., 2015). Thus, over time, the frequency of common alleles may increase and thus promote their reestablishment in the population (Charlesworth, Willis, 2009). Another possibility for recovering the previous condition of variability in fish stocks would be the contribution of complementary stocks found in the Pardo River, the secondary component of the river basin, which could lead to the re-establishment of the populations over the years. These results could agree with the analysis of Bayesian skyline plots. Even if not observed in the other groups, Feb15 presented a pronounced population expansion between 2014,9 and 2014,95 TBP, providing independent evidence for range expansion of *Prochilodus lineatus.* In addition, patterns containing high h (> 0.5) values combined with low π ($< 0.5\%$) values observed by this group often demonstrate the occurrence of accumulation of mutations (Grant, Bowen, 1998).

On the contrary, and despite their high genetic diversity, the *P. lineatus* population has undergone significant heterozygosity deficiency. Our findings detected a large decline in the observed heterozygosity values (Ho) relative to expected heterozygosity (He) and the groups of Aug05 and Jan09 showed the larger differences. Similar levels were obtained by Yazbeck, Kalapothakis (2007), with values of Ho = 0.00–1.00 in the intermediate section of the Paraná River, as well in components of the Paraná River basin (Ho = 0.634–0.82; He = 0.803–0.874) (Ferreira *et al*., 2017).

The Intrapopulation Index F_{IS} used to estimate the rate of inbreeding within the groups (Chistiakov *et al*., 2006), and divergences between He and Ho generate a positive value

for the F_{IS}. The values found were higher than those reported by Ferreira *et al.* (2017) for the same species in a spatial study developed in the Paraná River basin (F_{IS} = –0.04 to 0.09). Therefore, the high homozygous indices are common in impacted populations, and even large populations are subject to the restriction of the genetic variability due to anthropic actions (Mastrochirico *et al*., 2018). In this sense, events of population change caused by environmental damage are usually followed by a consequent reduction of genetic variability and an increase in the rate of inbreeding in the remaining biological stocks (Faulks *et al*., 2017).

In addition, another fact that may have influenced the decline of heterozygosity values of these populations may involve environmental disturbances that occurred in the Mogi-Guaçu, Pardo, and Grande hydrographic complex (IBAMA, 2003). Several freshwater fish including *P. lineatus* experienced severely mass mortality due to exposure to pesticides in the tributaries (IBAMA, 2003; Campagna *et al*., 2006), which should lead to a direct loss of genetic diversity and a further indirect reduction of genetic diversity via population bottlenecks and associated elevated genetic drift effects. Second, a general modification of the rainfall regime occurred after 2009 for some time in the region, interfering with a low water level of rivers in the ecosystem, and causing disturbances affecting the reproductive behavior of species with possible negative impacts on the ecosystem in the following years. In addition, the evidence of a heterozygosity deficiency with different allelic frequencies observed here could be also explained by subpopulation structure, suggesting a possible Wahlund effect, selection of specific alleles and inbreeding (Hartl, Clark, 2007; Ribolli *et al*., 2017).

It should be pointed out, that the ecosystem of the Upper Parana basin has a few tributaries with a free course sufficiently large for the trophic and reproductive migrations of fish species, with stretches favorable to the life of the rheophilic fish species. Thus, in this hydrographic complex, the Mogi-Guaçu and Pardo Rivers present themselves as fundamental for the maintenance of the variability of the stock of *P. lineatus* and, consequently, for other species with the same behavior. Furthermore, although this process can recover a level of variability that allows the stability of the species in the ecosystem, it does not guarantee the recovery of the previously existing genetic variability, since the lost rare alleles could hardly be recovered.

Our results also identified a temporal genetic structuring of *P. lineatus* sampled in the same site over 12 years for the first time. The application of the AMOVA test revealed moderate genetic structure (*F*st = 0.14) for microsatellites, while mitochondrial DNA shows low genetic structure levels (*F*st = 0.01). Although, the pairwise *F*st values indicate significant mitochondrial DNA differentiation between some groups (*F*st = 0.11). These results suggest that even tenuous, there is a temporal structuring in the stock over the years, being more evident in the periods of 2003/2005 and 2009/2010 in which a reduction in the number of alleles was observed.

Some divergences involving the markers used were observed. In summary, the results on microsatellite markers suggest that there is a genetic structure in *P. lineatus* populations and undergone significant heterozygosity deficiency, but it is not uniformly supported by the D-loop. Although, the pairwise *F*st values indicate significant mitochondrial DNA differentiation between some groups. The effective population size of mitochondrial DNA is one-fourth that of a nuclear-autosomal gene. Whereas mitochondrial markers reflect evolutionary processes operating through the maternal germline, microsatellites

reflect evolutionary processes of both sexes that have occurred more recently, *e.g.*, over a few thousand years (Schlötterer, 2000). Therefore, evolutionary relationships may be oversimplified and historical events within and between populations may not be correctly detected with mitochondrial DNA data (Zhang, Hewitt, 2003). In this way, microsatellite markers may better reflect the genetic changes in this study and could be considered more accurate to test temporal structure.

The Bayesian analysis using Ln(P)D and delta de Evanno for the microsatellites corroborated the pattern observed, in which three populations were identified and the observed reduction of heterozygosity. As previously mentioned, the departure from HWE and the positive FIS values obtained would be explained by the existence of a population structure in our study area. Furthermore, the low deviation of Hardy-Weinberg equilibrium indicates that *P. lineatus* present a genetic structure probably a consequence of changing genetic flow followed by the genetic drift of their subpopulations.

Studies have shown that modification of ecological environments by human activities can lead to the genetic structure of freshwater fish (Meldgaard *et al*., 2007; Perkin *et al*., 2012; Nanninga *et al*., 2014; Pereira *et al*., 2016). The occurrence of genetic population structuring in *P. lineatus* was related before by Rueda *et al.* (2013) using spatial analysis and attributed to the presence of different shoals occurring during autumn-winter (*F*st = 0.14) and autumn-spring (*F*st = 0.12) in the lower section of the Uruguay River. Additionality, Perini *et al*. (2021) also showed a significant spatial genetic structure (*F*st = 0.009 to 0.022) and three genetic clusters inhabiting the Grande-Pardo-Mogi Guaçu River system. However, temporal analysis with *P. lineatus* has not developed in the Paraná Basin until the present study. Furthermore, temporal structure values reported in this study are similar to those reported for *Salminus brasiliensis* in the Paraná River (Ribolli *et al*., 2017). The temporal structuring of a population could be associated with a possible decrease in the competition for resources such as food, space, sexual partners, and due to a different use of the stream corridor through time and across life stages (Braga-Silva, Galetti, 2016; Ribolli *et al*., 2017). Furthermore, such genetic structure detected at the Emas sample site could be due to distinct seasonal stocks (Rueda *et al*., 2013) or spawning waves consisting of different genetic populations (Braga-Silva, Galetti, 2016; Perini *et al*., 2021), obtained during the rainy season and/or to population dynamics of sink/source due to fish shoal migrations to feed during the dry season. Moreover, it is possible that environmental actions could contribute to the genetic differentiation of these species. Many cases of massive fish mortality in the Mogi-Guaçu River basin, São Paulo state, have been attributed to the presence of toxicants, high loads of organic matter, and toxins from algal bloom (IBAMA, 2003; Campagna *et al*., 2006; Meschiatti, Arcifa, 2009). The massive mortality of 30 tons of freshwater fish including *P. lineatus* (October of 2002), was related to exposure to pesticides in Paraná tributaries. Thus, a possible explanation for the population structure reported here could be associated with drastically reduced populations occurring in the Mogi-Guaçu River, Upper Paraná River basin (IBAMA, 2003). Added to the fact that the main fishing area of the region is organized around its breeding ground, there are many pollution sources along the basin in which the fish performs its migration (Esteves, Pinto Lôbo, 2001).

Furthermore, it must be considered that populations are dynamic entities in the ecosystem, governed by biological and environmental factors and subordinated to

natural selection laws. As has been postulated, the rainfall regime is one of the main environmental factors underlying the reproductive process (Melo *et al*., 2013) of rheophilic fish species and for the synchronization of the biological mechanisms of gonadal development. The perturbation of these factors can result in the alteration of the biological cycle of the species and, ultimately, affect the population structure and dynamics of the species in the ecosystem (Lopes *et al*., 2018). Therefore, the genetic structuring described here might have to be considered in future conservation actions, considering that the biota of the Mogi-Guaçu River is highly impacted by human activities.

Overall, our findings show that populations of *P. lineatus* are capable of undergoing temporal changes in population genetic structure and diversity over short time periods. The evidence of temporal population structuring in the main channel of the Mogi-Guaçu River emphasizes the importance of unaltered tributaries in this area to conserve migratory fish populations. Finally, our findings could provide a basis for future management and conservation initiatives to protect migratory fish and their breeding environments.

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REFERENCES

- **Agostinho AA, Gomes LC, Suzuki HI, Júlio HF, Jr.** Migratory fishes of the upper Paraná River basin, Brazil. In: Gomes LC, Fernandes DR, Suzuki HI, Júlio Junior HF, Carolsfeld J, Harvey B, Ross C, Baer A, editors. Migratory fishes of South America: biology, fisheries and conservation status. Ottawa:World Fisheries Trust: The World Bank: International Development Research Centre; 2003. p.19–98.
- **Agostinho AA, Gomes LC, Pelicice FM.** Ecologia e manejo de recursos pesqueiros em reservatórios do Brasil. Maringá: EDUEM; 2007.
- **Agostinho AA, Gomes LC.** Biodiversity and fisheries management in the Paraná River basin: Successes and failures. In: Would fisheries trust (org.). The blue millenium project: Managing fisheries for biodiversity. Victoria: Would Fisheries Trust - CRDI, UNEP; 2018. Available from: http://repositorio.uem.br:8080/jspui/handle/1/5331
- **Aguirre-Pabón J, Barandica JN, García LC.** Mitochondrial DNA variation of the bocachico *Prochilodus magdalenae* (Characiformes, Prochilodontidae) in the Magdalena River Basin, Colombia. Aquat Conserv. 2013; 23(4):594–605. https://doi.org/10.1002/aqc.2339
- **Avise JC.** Molecular Markers, Natural History and Evolution. New York: Chapman & Hall; 1994.
- **Barcia AR, López GE, Hernández D, García-Machado E.** Temporal variation of the population structure and genetic diversity of *Farfantepenaeus notialis* assessed by allozyme loci. Mol Ecol. 2005; 14(10):2933–42. https://doi.org/10.1111/j.1365-294X.2005.02613.x
- **Blaber SJM, Cyrus DP, Albaret JJ.** Effects of fishing on the structure and functioning of estuarine and nearshore ecosystems. ICES J Mar Sci. 2000; 57(3):590–602. https://doi.org/10.1006/jmsc.2000.0723

- **Braga-Silva A, Galetti PM, Jr.** Evidence of isolation by time in freshwater migratory fish *Prochilodus costatus* (Characiformes, Prochilodontidae). Hydrobiologia. 2016; 765:159–67. https://doi.org/10.1007/s10750-015-2409-8
- **Campagna AF, Eler MN, Espíndola ELG, Senhorini JA, Rêgo RF, Silva LOL.** Dimethoate 40% organosphosphorous pesticide toxicity in *Prochilodus lineatus* (Prochilodontidae, Characiformes) eggs and larvae. Braz J Biol. 2006; 66(2B):633–40. https://doi.org/10.1590/S1519-69842006000400007
- **Castellanos-Galindo GA, Casella E, Mejía-Rentería JC, Rovere A.** Habitat mapping of remote coasts: Evaluating the usefulness of lightweight unmanned aerial vehicles for conservation and monitoring. Biol Conserv. 2019; 239:108282. https://doi.org/10.1016/j.biocon.2019.108282
- **Charlesworth D, Willis JH.** The genetics of inbreeding depression. Nat Rev Genet. 2009; 10:783–96. https://doi.org/10.1038/nrg2664
- **Chistiakov DA, Hellemans B, Volckaert FAM.** Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. Aquaculture. 2006; 255(1–4):1–29. https://doi.org/10.1016/j.aquaculture.2005.11.031
- **Dachapak S, Somta P, Poonchaivilaisak S, Yimram T, Srinives P.** Genetic diversity and structure of the combi pea (*Vigna vexillata* (L.) A. Rich) gene pool based on SSR marker analysis. Genetica. 2017; 145(2):189–200. https://doi.org/10.1007/s10709-017-9957-y
- **Dowling NA, Dichmont CM, Haddon M, Smith DC, Smith ADM, Sainsbury K.** Guidelines for developing formal harvest strategies for data-poor species and fisheries. Fish Res. 2015; 171:130–40. https://doi.org/10.1016/j.fishres.2014.09.013
- **Drummond AJ, Rambaut A.** BEAST: Bayesian evolutionary analysis by sampling trees. BMC Ecol Evol. 2007; 7(214):1–08. https://doi.org/10.1186/1471-2148-7-214
- **Drummond AJ, Rambaut A, Shapiro B, Pybus OG.** Bayesian coalescent inference of past population dynamics from molecular sequences. Mol Biol Evol. 2005; 22(5):1185–92. https://doi.org/10.1093/molbev/msi103
- **Earl DA, von Holdt BM.** STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour. 2012; 4:359–61. https://doi.org/10.1007/s12686-011-9548-7
- **Esteves KE, Pinto Lôbo AV.** Feeding pattern of *Salminus maxillosus* (Pisces, Characidae) at Cachoeira das Emas, Mogi-Guaçu River (São Paulo State, Southeast Brazil). Rev Bras Biol. 2001; 61(2):267–76. https://doi.org/10.1590/S0034-71082001000200009
- **Evanno G, Regnaut S, Goudet J.** Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol. 2005; 14(8):2611–20. https://doi.org/10.1111/j.1365-294X.2005.02553.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
- **Excoffier L, Lischer HEL.** Arlequin suite ver 3.5: A new series of programs to perform 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
- **Falush D, Stephens M, Pritchard JK.** Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 2003; 164(4):1567–87. https://doi.org/10.1093/genetics/164.4.1567
- **Faulks LK, Kerezsy A, Unmack PJ, Johnson JB, Hughes JM.** Going, going, gone? Loss of genetic diversity in two critically endangered Australian freshwater fishes, *Scaturiginichthys vermeilipinnis* and *Chlamydogobius squamigenus*, from Great Artesian Basin springs at Edgbaston, Queensland, Australia. Aquat Conserv Mar Freshw Ecosyst. 2017; 27(1):39–50. https://doi.org/10.1002/aqc.2684
- **Ferreira DG, Galindo BA, Frantine-Silva W, Almeida FS, Sofia SH.** Genetic structure of a Neotropical sedentary fish revealed by AFLP, microsatellite and mtDNA markers: a case study. Conserv Genet. 2015; 16:151–66. https://doi.org/10.1007/s10592-014-0648-2

- **Ferreira DG, Souza-Shibatta L, Shibatta OA, Sofia SH, Carlsson J, Dias JHP *et al*.** Genetic structure and diversity of migratory freshwater fish in a fragmented Neotropical river system. Rev Fish Biol Fish. 2017; 27:209–31. https://doi.org/10.1007/s11160-016-9441-2
- **Frankham R, Bradshaw CJA, Brook BW.** Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biol Conserv. 2014; 170:56–63. https://doi.org/10.1016/j.biocon.2013.12.036
- **Fu YX.** Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 1997; 147(2):915–25. https://doi.org/10.1093/genetics/147.2.915
- **Fu YX, Li WH.** Statistical tests of neutrality of mutations. Genetics 1993; 133(3):693–709. https://doi.org/10.1093/genetics/133.3.693
- **Fullerton AH, Lindley ST, Pess GR, Feist BE, Steel EA, McElhany P.** Human influence on the spatial structure of threatened Pacific Salmon metapopulations. Conserv Biol. 2011; 25(5):932–44. https://doi.org/10.1111/j.1523-1739.2011.01718.x
- **Garcez R, Calcagnotto D, Almeida-Toledo LF.** Population structure of the migratory fish *Prochilodus lineatus* (Characiformes) from rio Grande basin (Brazil), an area fragmented by dams. Aquat Conserv Mar Freshw Ecosyst. 2011; 21(3):268–75. https://doi.org/10.1002/aqc.1176
- **Godoy MP.** Peixes do Brasil: subordem Characoidei: bacia do rio Mogi-Guassu. Piracicaba: Franciscana; 1975.
- **Gomes LC, Agostinho AA.** Influence of the flooding regime on the nutritional state and juvenile recruitment of the curimba, *Prochilodus scrofa*, Steindachner, in upper Paraná River, Brazil. Fish Manag Ecol. 1997; 4(4):263–74. https://doi.org/10.1046/j.1365-2400.1997.00119.x
- **Goudet J.** FSTAT (version 2.9. 3.2): a program to estimate and test gene diversities and fixation indices; 2002.
- **Grant WAS, Bowen BW.** Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J Hered. 1998; 89(5):415–26. https://doi.org/10.1093/jhered/89.5.415
- **Guo W, Guo C, Wang Y, Hu W, Mei J.** Population structure and genetic diversity in yellow catfish (*Pelteobagrus fulvidraco*) assessed with microsatellites. J Genet. 2019; 98(26):1–04. https://doi.org/10.1007/s12041-019-1070-9
- **Guzman P, Fatorić S, Ishizawa M.** Monitoring climate change in World Heritage properties: A review for the potential application of landscape approaches in the State of Conservation System. Climate. 2020; 8(3):39. https://doi.org/10.3390/cli8030039
- **Hartl DL, Clark AG.** Principles of population genetics. Sunderland: Sinauer Associates; 2007.
- **Hilsdorf AWS, Azeredo-Espin AML, Krieger MH, Krieger JE.** Mitochondrial DNA diversity in wild and cultured populations of *Brycon opalinus* (Cuvier, 1819) (Characiformes, Characidae, Bryconinae) from the Paraíba do Sul Basin, Brazil. Aquaculture. 2002; 214(1–4):81–91. https://doi.org/10.1016/S0044-8486(02)00132-1
- **Iordache AM, Nechita C, Pluhacek T, Iordache M, Zgavarogea R, Ionete RE.** Past and present anthropic environmental stress reflect high susceptibility of natural freshwater ecosystems in Romania. Environ Pollut. 2020; 267(115505):1–10. https://doi.org/10.1016/j.envpol.2020.115505
- **Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis (IBAMA).** Portaria IBAMA N° 49 [Internet]. Brasil; 2003. Available from: https://www.icmbio.gov.br/cepsul/images/stories/legislacao/Portaria/2007/p_ibama_49_2007_revogada_normasperiodoreproducaopeixes_pr_revogada_p_ibama_27_2008.pdf
- **Ivanova NV, Dewaard JR, Hebert PDN.** An inexpensive, automation-friendly protocol for recovering high-quality DNA. Mol Ecol Notes. 2006; 6(4):998–1002. https://doi.org/10.1111/j.1471-8286.2006.01428.x
- **Izzo C, Doubleday ZA, Grammer GL, Gilmore KL, Alleway HK, Barnes TC *et al*.** Fish as proxies of ecological and environmental change. Rev Fish Biol Fish. 2016; 26:265–86. https://doi.org/10.1007/s11160-016-9424-3
- **Jeremias G, Barbosa J, Marques SM, Asselman J, Gonçalves FJM, Pereira JL.** Synthesizing the role of epigenetics in the response and adaptation of species to climate change in freshwater ecosystems. Mol Ecol. 2018; 27(13):2790–806. https://doi.org/10.1111/mec.14727

- **Jost L.** GST and its relatives do not measure differentiation. Mol Ecol. 2008; 17(18):4015–26. https://doi.org/10.1111/j.1365-294X.2008.03887.x
- **Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S *et al*.** Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28(12):1647–49. https://doi.org/10.1093/bioinformatics/bts199
- **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
- **Lopes JM, Alves CBM, Peressin A, Pompeu PS.** Influence of rainfall, hydrological fluctuations, and lunar phase on spawning migration timing of the Neotropical fish *Prochilodus costatus*. Hydrobiology. 2018; 818:145–61. https://doi.org/10.1007/s10750-018-3601-4
- **Machado MRF, Foresti F.** Morphometric characteristics of *Prochilodus lineatus* (Valenciennes 1847), of the migratory and resident stock of the river Mogí-Guaçu, São Paulo State, Brazil. Acta Sci. 2012; 34(4):341–46. https://doi.org/10.4025/actascianimsci.v34i4.14445
- **Martins C, Wasko AP, Oliveira C, Foresti F.** Mitochondrial DNA variation in wild populations of *Leporinus elongatus* from the Paraná River basin. Genet Mol Biol. 2003; 26(1):33–38. https://doi.org/10.1590/S1415-47572003000100006
- **Martins N, Pereira R, Abrantes N, Pereira J, Gonçalves F, Marques CR.** Ecotoxicological effects of ciprofloxacin on freshwater species: Data integration and derivation of toxicity thresholds for risk assessment. Ecotoxicology. 2012; 21:1167–76. https://doi.org/10.1007/s10646-012-0871-x
- **Mastrochirico-Filho VA, Freitas MV, Ariede RB, Lira LVG, Mendes NJ, Hashimoto DT.** Genetic applications in the conservation of neotropical freshwater fish. In: Ray S, editor. Biological resources of water. London: Intechopen. 2018. p.249–84.
- **Meldgaard T, Crivelli AJ, Jesensek D, Poizat G, Rubin JF, Berrebi P.** Hybridization mechanisms between the endangered marble trout (*Salmo marmoratus*) and the brown trout (*Salmo trutta*) as revealed by in-stream experiments. Biol Conserv. 2007; 136(4):602–11. https://doi.org/10.1016/j.biocon.2007.01.004
- **Melo BF, Sato Y, Foresti F, Oliveira C.** The roles of marginal lagoons in the maintenance of genetic diversity in the Brazilian migratory fishes *Prochilodus argenteus* and *P. costatus*. Neotrop Ichthyol. 2013; 11(3):625–36. https://doi.org/10.1590/S1679-62252013000300016
- **Meschiatti AJ, Arcifa MS.** A review on the fishfauna of Mogi-Guaçu River basin: a century of studies. Acta Limnol Bras. 2009; 21(1):135–59.
- **Mondin LAC, Machado CB, Resende EK, Marques DKS, Galetti PM, Jr.** Genetic pattern and demographic history of *Salminus brasiliensis*: Population expansion in the pantanal region during the Pleistocene. Front Genet 2018; 9(1):1–08. https://doi.org/10.3389/fgene.2018.00001
- **Morelli KA, Revaldaves E, Oliveira C, Foresti F.** Isolation and characterization of eight microsatellite loci in *Leporinus macrocephalus* (Characiformes: Anostomidae) and cross-species amplification. Mol Ecol Notes. 2007; 7(1):32–34. https://doi.org/10.1111/j.1471-8286.2006.01484.x
- **Morita K, Sahashi G, Miya M, Kamada S, Kanbe T, Araki H.** Ongoing localized extinctions of stream-dwelling white-spotted charr populations in small dammed-off habitats of Hokkaido Island, Japan. Hydrobiologia. 2019; 840(1):207–13. https://doi.org/10.1007/s10750-019-3891-1
- **Nanninga GB, Saenz-Agudelo P, Manica A, Berumen ML.** Environmental gradients predict the genetic population structure of a coral reef fish in the Red Sea. Mol Ecol. 2014; 23(3):591–602. https://doi.org/10.1111/mec.12623
- **Nieminen E, Hyytiäinen K, Lindroos M.** Economic and policy considerations regarding hydropower and migratory fish. Fish Fish. 2017; 18(1):54–78. https://doi.org/10.1111/faf.12167
- **Paschoal LRP, Andrade DP, Pimpão DM, Torres S, Darrigran G.** Massive mortality of the giant freshwater mussel *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia: Mycetopodidae) during a severe drought in a Neotropical reservoir. An Acad Bras Ciênc. 2020; 92(Suppl. 2):1–13. https://doi.org/10.1590/0001-3765202020180811
- **Pereira LS, Ribas JLC, Vicari T, Silva SB, Stival J, Baldan AP *et al*.** Effects of ecologically relevant concentrations of cadmium in a freshwater fish. Ecotoxicol Environ Saf. 2016; 130:29–36. https://doi.org/10.1016/j.ecoenv.2016.03.046

- **Perini VR, Paschoalini AL, Bazzoli N, Rizzo E, Carvalho DC.** Metapopulation dynamics of the migratory fish *Prochilodus lineatus* (Characiformes: Prochilodontidae) in a lotic remnant of the Grande River, Southeastern Brazil. Neotrop Ichthyol. 2021; 19(4):e200046. https://doi.org/10.1590/1982-0224-2020-0046
- **Perkin JS, Gido KB.** Fragmentation alters stream fish community structure in dendritic ecological networks. Ecol Appl. 2012; 22(8):2176–87. https://doi.org/10.1890/12-0318.1
- **Piry S, Luikart G, Cornuet JM.** Computer note: BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered. 1999; 90(4):502–03. https://doi.org/10.1093/jhered/90.4.502
- **Pritchard JK, Stephens M, Donnelly P.** Inference of population structure using multilocus genotype data. Genetics. 2000; 155(2):945–59. https://doi.org/10.1093/genetics/155.2.945
- **Prunier JG, Dubut V, Loot G, Tudesque L, Blanchet S.** The relative contribution of river network structure and anthropogenic stressors to spatial patterns of genetic diversity in two freshwater fishes: A multiple-stressors approach. Freshw Biol. 2018; 63(1):6–21. https://doi.org/10.1111/fwb.13034
- **Raymond M, Rousset F.** An exact test for population differentiation. Evolution. 1995; 49(6):1280–83. https://doi.org/10.2307/2410454
- **Revaldaves E, Renesto E, Machado MFPS.** Genetic variability of *Prochilodus lineatus* (Characiformes, Prochilodontidae) in the upper Paraná River. Genet Mol Biol. 1997; 20(3):381–88. https://doi.org/10.1590/S0100-84551997000300005
- **Ribolli J, Hoeinghaus DJ, Johnson JA, Zaniboni-Filho E, Freitas PD, Galetti PM, Jr.** Isolation-by-time population structure in potamodromous Dourado *Salminus brasiliensis* in southern Brazil. Conserv Genet. 2017; 18:67–76. https://doi.org/10.1007/s10592-016-0882-x
- **Rice WR.** The sequential Bonferroni test. Evolution. 1989; 43(1):223–25.
- **Rousset F.** GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour. 2008; 8(1):103–06. https://doi.org/10.1111/j.1471-8286.2007.01931.x
- **Rueda EC, Carriquiriborde P, Monzón AM, Somoza GM, Ortí G.** Seasonal variation in genetic population structure of sábalo (*Prochilodus lineatus*) in the Lower Uruguay River. Rev Bras Genet. 2013; 141:401–07. https://doi.org/10.1007/s10709-013-9739-0
- **Santos MCF, Ruffino ML, Farias IP.** High levels of genetic variability and panmixia of the tambaqui *Colossoma macropomum* (Cuvier, 1816) in the main channel of the Amazon River. J Fish Biol. 2007; 71:33–44. https://doi.org/10.1111/j.1095-8649.2007.01514.x
- **Schlötterer C.** Evolutionary dynamics of microsatellite DNA. Chromosoma. 2000; 109:365–71. https://doi.org/10.1007/s004120000089
- **Schuelke M.** An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol. 2000; 18:233–34. https://doi.org/10.1038/72708
- **Krishan G, Singh RP, Tashi KS.** Water level fluctuation as the sum of environmental and anthropogenic activities in southeast, Punjab (India). J Environ Anal Toxicol. 2015; 5(5):1–07. http://dx.doi.org/10.4172/2161-0525.1000298
- **Sivasundar A, Bermingham E, Ortí G.** Population structure and biogeography of migratory freshwater fishes (*Prochilodus*: Characiformes) in major South American rivers. Mol Ecol. 2001; 10(2):407–17. https://doi.org/10.1046/j.1365-294X.2001.01194.x
- **Strayer DL, Dudgeon D.** Freshwater biodiversity conservation: Recent progress and future challenges. J North Am Benthol Soc. 2010; 29(1):344–58. https://doi.org/10.1899/08-171.1
- **Strungaru SA, Jijie R, Nicoara M, Plavan G, Faggio C.** Micro- (nano) plastics in freshwater ecosystems: Abundance, toxicological impact and quantification methodology. Trends Analyt Chem. 2019; 110:116–28. https://doi.org/10.1016/j.trac.2018.10.025
- **Tajima F.** Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989; 123(3):585–95. https://doi.org/10.1093/genetics/123.3.585
- **Van Leeuwen CHA, Dalen K, Museth J, Junge C, Vøllestad LA.** Habitat fragmentation has interactive effects on the population genetic diversity and individual behaviour of a freshwater salmonid fish. River Res Appl 2018; 34(1):60–68. https://doi.org/10.1002/rra.3226

- **Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P.** MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 2004; 4(3):535–38. https://doi.org/10.1111/j.1471-8286.2004.00684.x
- **Vazzoler AEAM.** Biologia da reprodução de peixes teleósteos: teoria e prática. Maringá: EDUEM; 1996.
- **Warr N, Greenfield A.** The molecular and cellular basis of gonadal sex reversal in mice and humans. WIREs Dev Biol. 2012; 1(4):559–77. https://doi.org/10.1002/wdev.42
- **Weber R, Aliyeva G, Vijgen J.** The need for an integrated approach to the global challenge of POPs management. Environ Sci Pollut Res. 2013; 20:1901–06. https://doi.org/10.1007/s11356-012-1247-8
- **Yazbeck GM, Kalapothakis E.** Isolation and characterization of microsatellite DNA in the piracema fish *Prochilodus lineatus* (Characiformes). Genet Mol Res. 2007; 6(4):1026–34. Available from: https://www.geneticsmr.com/sites/default/files/articles/year2007/vol6-4/pdf/gmr339.pdf
- **Zhang DX, Hewitt GM.** Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Mol Ecol. 2003; 12(3):563–84. https://doi.org/10.1046/j.1365-294X.2003.01773.x
- **Zhang QZ, Sun C, Zhu Y, Xu N, Liu H.** Genetic diversity and structure of the round-tailed paradise fish (*Macropodus ocellatus*): Implications for population management. Glob Ecol Conserv. 2020; 21:e00876. https://doi.org/10.1016/j.gecco.2019.e00876

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ETHICAL STATEMENT

All samples were collected in strict accordance with the regulations of the Brazilian Federal Animal Ethics Committee (SISBIO 15163/1) and authorized by the Ethics Committee on the Use of Animals (CEUA) of the Biosciences Institute at UNESP through its (protocol 971).

COMPETING INTERESTS

The authors declare no competing interests.

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