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GENETIC IMPLICATIONS OF RESTOCKING PROGRAMS ON WILD POPULATIONS OF STREAKED PROCHILOD *Prochilodus lineatus*

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ABSTRACT

Genetic diversity of wild and farmed populations is crucial, both for conservation of fish resources and fish culture development. To infer the genetic diversity and population structure of Streaked prochilod *Prochilodus lineatus*, individuals were sampled between 2007-2009 from four fish farms and from the Upper Uruguay River Basin, both in southern Brazil. Population structure was identified in both farmed and wild individuals through seven microsatellite loci. Bayesian analysis indicated three main groups, including two from fish farms. Pairwise genetic differentiation showed spatial structure between and within wild and farmed populations; however, the sampling design did not allow testing temporal structure according to isolation-by-time (IBT), which means that populations can breed within the same geographic distribaution, but reproduce at different times. Cultivated individuals presented lower diversity, allelic richness and effective population size, but higher inbreeding rates, compared to wild populations. These characteristics constitute warning signs against indiscriminate restocking of natural *Prochilodus lineatus* populations, a species sensitive to fragmented habitats, with farmed fish.

Key words: fishing resources; curimbatá; freshwater fish; population genetics; rebuilding.

IMPLICAÇÕES GENÉTICAS DOS PROGRAMAS DE REPOVOAMENTO DE POPULAÇÕES SELVAGENS DE CURIMBA *Prochilodus lineatus*

RESUMO

A diversidade genética das populações selvagens e cultivadas é crucial, tanto para a conservação dos recursos pesqueiros como para o desenvolvimento da piscicultura. Para inferir a diversidade genética e estrutura populacional do curimba *Prochilodus lineatus*, indivíduos foram amostrados, entre 2007-2009, em quatro fazendas de peixes e da Bacia do Alto Uruguai, ambas no sul do Brasil. A estrutura populacional foi identificada em indivíduos cultivados e selvagens, através de sete locos microssatélites. A análise bayesiana indicou três grupos principais, incluindo dois grupos oriundos de pisciculturas. A diferenciação genética par-a-par revelou estrutura espacial entre e dentro de populações selvagens e cultivadas; no entanto, o desenho amostral não permitiu testar a estrutura temporal de acordo com o isolamento por tempo (IBT), o que significa que as populações podem reproduzir dentro da mesma distribuição geográfica, mas reproduzir em diferentes momentos. Os indivíduos cultivados apresentaram menor diversidade, riqueza alélica e tamanho efetivo populacional, porém maiores taxas de endogamia, quando comparados às populações selvagens. Estas características constituem sinais de alerta contra o repovoamento indiscriminado de populações naturais de *Prochilodus lineatus*, uma espécie sensível a habitats fragmentados, com peixes oriundos de pisciculturas.

Palavras-chave: recursos pesqueiros; curimbatá; peixes de água doce; genética de populações; repovoamento.

INTRODUCTION

Genetic diversity levels and genetic composition of wild populations and farmed fish are fundamental to conservation strategies designed to improve the genetic composition of fish used in restocking projects (Bondioli et al., 2017; Duong and Scribner, 2018). However, reproduction within the confines of a commercial fish farm stands in stark contrast to the complexity of natural reproduction events that occur in large Neotropical rivers, especially in long-migration fish (Ribolli et al., 2016). More specifically, the low number of parents and non-genetic characterization of broodstock in fish farm situations can lead to genetic problems, such as inbreeding and fixation of harmful alleles. These

problems can be aggravated by the use of improper methods during the stages of reproduction like the use of imbalanced broodstock sex ratios and pooling of gametes (Tave, 1999; Ribolli and Zaniboni-Filho, 2009). In addition, domestication can involve selection of only some important alleles for aquaculture, thus losing adaptive characteristics that would be essential to the adaptation of restocked wild fish (Prado et al., 2018).

Prochilodus lineatus (Valenciennes, 1836) is a large migratory Characiformes with distribution in the Paraná-Paraguay and Paraíba do Sul River Basins (Castro and Vari, 2004) and the Uruguay River Basin (Zaniboni-Filho and Schulz, 2003). Referred to as streaked prochilod, sábalo, curimba, curimbatá and grumatão, *P. lineatus* is considered a main resource for fisheries in the Paraná River (Sverlij et al., 1993; Baigún et al., 2013) and Uruguay River (Espinach Ros et al., 1998; Schork et al., 2013). Together with other freshwater species, *P. lineatus* is an important economic resource for riverine families, mainly in tropical countries (Allan et al., 2005; Hoeinghaus et al., 2009).

Similar to other migratory species, degradation of natural river waters and damming can impose limits on its otherwise long migration of up to 1,100 km (Espinach Ros et al., 1998), thus posing the most serious threats to *P. lineatus* populations of the Upper Uruguay River Basin. In order to compensate for the decline of natural stocks, restocking practices have been urged by compensation for damming of rivers, imposing fines for environmental damage, or even voluntary restocking, to increase fishing. Notwithstanding such attempts, Brazilian legislation, as regulated by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA; Normative Instruction No. 146), does not concern itself with the genetic characteristics of the broodstock used for repopulation or the genetic composition of individuals used in restocking. *P. lineatus* is known to be a docile, easily handled species that displays dominant reproductive characteristics, high fecundity; therefore, reproduction in hatcheries can be easily achieved in small fish farms through artificial means. With only a couple of broodstock, it is possible to produce thousands of larvae of *P. lineatus* (Viveiros et al. 2010).

However, such key genetic parameters as effective population size (Ne) and genetic diversity are fundamental determinants of adaptive potential in a wild population (Hedrick, 2005).

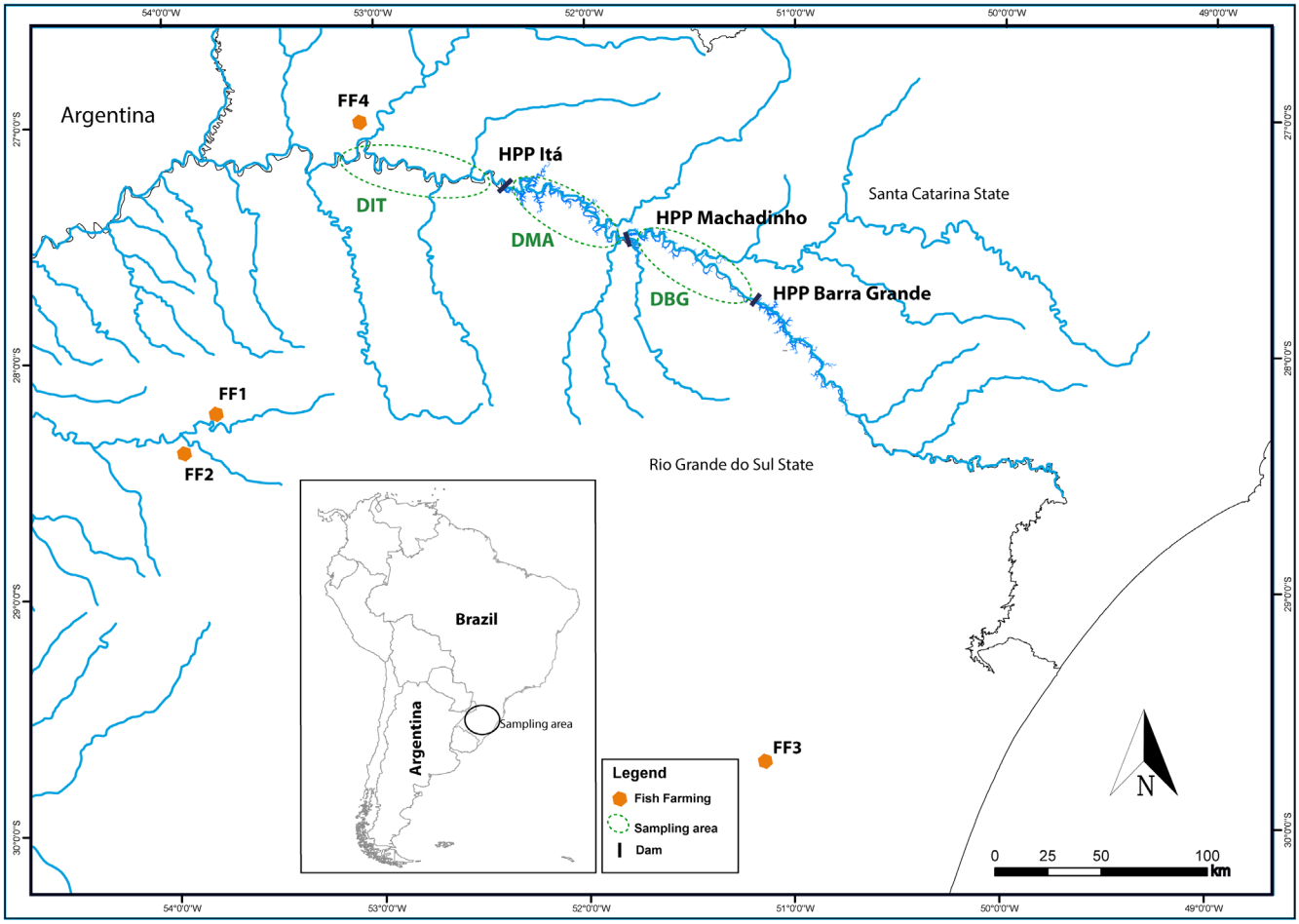


Figure 1. Map of sampling sites of wild and farmed *Prochilodus lineatus*. Wild individuals from the Upper Uruguay River: DBG (downstream from Barra Grande HPP), DMA (downstream from Machadinho HPP), and DIT (downstream from Itá HPP); fish farm stations (FF): FF1 = São Leopoldo/RS, FF2 = Ijuí/RS, FF3 = Ajuricaba/RS, and FF4 = Chapecó/SC.

Thus, limited genetic diversity and effective population size of broodstock may pose a risk to wild populations restocked under these conditions (Small et al., 2009; Fonseca et al., 2017; Prado et al., 2018). In addition, restocking programs that avoid introduction of genotypes unrepresentative of the augmented population, low genetic diversity and inbreeding can have negative effects (Ward, 2006; Roques et al., 2018).

Therefore, this study aimed to evaluate the genetic consequences of restocking wild populations with farmed fish by comparing of genetic diversity and spatial structure between farmed and wild genetic populations of *P. lineatus* from the Uruguay River Basin, southern Brazil.

MATERIAL AND METHODS

Sampling

Adult individuals of *P. lineatus* were sampled from wild and fish farms of Santa Catarina and Rio Grande do Sul States (Figure 1). Wild individuals were sampled downstream from the Barra Grande Dam (DBG, N = 13), downstream from the Machadinho Dam (DMA, N = 44), and downstream from the Itá Dam (DIT = 49), all situated in the Upper Uruguay River Basin. Farming samples were collected from four fish farms denoted as FF1 (Fish Farm from Ajuricaba/RS, N = 10), FF2 (Fish Farm from Ijuí/RS, N = 17), FF3 (Fish Farm from São Leopoldo/RS, N = 11), and FF4 (Fish Farm from Chapecó/SC, N = 13).

Non-lethal sampling was performed between 2007 and 2009 in collaboration with the Laboratório de Biologia e Cultivo de Peixes de Água Doce (LAPAD) of the Universidade Federal de Santa Catarina (UFSC) and local fishermen. Fish samples were morphologically identified according to Castro and Vari (2003). A fragment of fin clips was removed from each individual, identified and then preserved in 96% ethanol. Voucher number MZUEL 11729 (deposited in the Ichthyological Collection of Universidade Estadual de Londrina-Parana, Brazil).

DNA extraction and amplification

Total DNA purification was performed using a CTAB protocol (2% CTAB, 20 mM EDTA, 0.1 M Tris, 1.4 mM NaCl), followed by a sodium acetate and isopropanol-induced precipitation step (Sambrook and Russell, 2001).

A portion of the mitochondrial large ribosomal subunit (16S) was amplified using primers 16SAR (CGCCTGTTTATCAAAAACAT) and 16SBR (CCGGTCTGAACTCAGATCACGT) (Kessing et al., 1989). Polymerase chain reactions (PCR) used approximately 10 ng of template DNA, 1 unit of Taq polymerase (GE Life Sciences), 200 µM each of four dinucleotides, 0.5 mM of each primer and 1.5 mM MgCl2 in 20 µL of 1x PCR buffer (GE Life Sciences). Thermocycling conditions were as follows: initial denaturation of 95 ºC for 3 min, 30 amplification cycles of 93 ºC for 1 min, 50 ºC for 1 min and 72 ºC for 1 min, followed by final elongation at 72 ºC for 5 min. Negative controls, involving template-free reactions, were included in all PCR amplifications. Both strands of PCR products were purified with a GFX™ PCR DNA and Gel Band Purification Kit (GE Life Sciences), according to the manufacturer's instructions, and sequenced in an ABI 3500 automatic sequencer with the same sets of primers as those used for the PCR reaction. All haplotype sequences obtained were deposited in GenBank (Accession Numbers MK312666 and MK31266). Additionally, sequences from GenBank were included in our phylogenetic analyses (*P. lineatus*: Z22696, Meyer et al., 1993; U34024, Orti and Meyer, 1997; *P. nigricans*: AY788075, Calcagnotto et al., 2005; *P. reticulatus*: HQ171358, Oliveira et al., 2011; *Psectrogaster rhomboides*: FJ944746, and *Semaprochilodus insignis*: FJ944756).

Polymerase chain reactions were performed using seven polymorphic microsatellite loci (SSRs) developed for *Prochilodus argenteus* (Carvalho-Costa et al., 2008) and used in *P. lineatus* (Par12 (AAAC)7, Par14 (TGTC)5, Par21 (ATGA)6, Par43 (GA)6(CA)2(CAGA)4(GA)21, Par80 (CT)37, Par82 (CT)27); Barbosa et al., 2006; Barbosa et al., 2008), and one locus developed for *Prochilodus lineatus* (Pli60; Yazbeck and Kalapothakis, 2007). PCR reactions included 15 ng template DNA, 3.6 mM of each starter primer, 200 µM dNTPs, 1.5 µL 10X buffer, 1.5 mM MgCl2, and 1 U Taq DNA polymerase (Invitrogen) in a 15 µL total volume. The amplification conditions were as follows: initial denaturing at 95 °C for 5 min, 35 amplification cycles of 94 °C for 1 min, specific annealing temperature for 1 min (Par12, 54°C; Par14 and Par21, 48°C; Par43, 50°C Par80 and Par82, 52°C; Pli60, 67°C) and 72 °C for 1 min, followed by final elongation of 72 °C for 20 min. Microsatellite amplification products were submitted to 1% polyacrylamide gel electrophoresis and visualized by silver nitrate staining.

Data analyses

The amplified 16S sequences were edited with the SEQMAN 7.0 program (DNASTAR Inc.) and aligned with the CLUSTALW algorithm (Thompson et al., 1994) provided with the MEGA 5.1 program (Tamura et al., 2011). Standard nucleotide (π) and haplotype (h) diversity indices were estimated using the DNASP 5.1 program (Librado and Rozas, 2009). Pairwise Kimura 2-parameter distances (K2P; Kimura, 1980) were used to build neighbor-joining (NJ) and maximum likelihood (ML) trees using the MEGA program.

Microsatellite genotyping was performed using the TL 100 program (TotalLab Ltd.). The presence of null alleles was investigated with the MICRO-CHECKER program (Van Oosterhout et al., 2004). GENEPOP, v.1.2 (Raymond and Rousset, 1995) was used to test for departure from Hardy-Weinberg equilibrium (HWE), as well as linkage disequilibrium. Mean number of alleles per locus (A), number of private alleles, mean observed (Ho), and expected (He) heterozygosity values were calculated in GENEALEX 6.5. The allelic polymorphism information content (PIC) was calculated using CERVUS 3.0 (Kalinowski et al., 2007). Allelic richness, inbreeding

coefficients (FIS; Weir and Cockerham, 1984) and the p-values for heterozygote excess (PL) and deficit (PS) were calculated using FSTAT 2.9.3.2 (Goudet, 2001). The percentage of population assignment into each sampled population was estimated using GENEALEX 6.5, and the effective population size (Ne) for each sampled population was calculated based on the linkage disequilibrium (LD) method (Waples and Do, 2008) using NEESTIMATOR 2.0 (Do et al., 2014).

Overall genetic structure was calculated using the analysis of molecular variance (AMOVA) within and among all populations sampled through permutation tests with 1000 replicates, using ARLEQUIN 3.5 (Excoffier and Lischer, 2010). Pairwise FST values (Weir and Cockerham, 1984) were calculated between wild and farmed sampling populations using FSTAT 2.9.3.2 (Goudet, 2001). To evaluate the population structure between wild and cultivated individuals sampled, we used a Bayesian cluster analysis implemented in STRUCTURE 2.3.3 software (Pritchard et al., 2000; Falush et al., 2003) whereby individuals were assigned to clusters without a priori information, while assuming an admixed model of population structure and correlated allele frequencies. The most likely number of genetic clusters (K) was estimated by six independent runs each for K = 1 to 9 with 600,000 Markov Chain Monte Carlo (MCMC) repetitions and using 300,000 initial interactions as the burn-in period. The optimal value of K was estimated by the Evanno method (ΔK, Evanno et al., 2005), using STRUCTURE HARVESTER (Earl, 2012), which can be found at "http://taylor0.biology.ucla.edu/structureHarvester/". The spatial population structure was also investigated to identify clusters of genetically related individuals using Discriminant Analysis of Principal Components (DAPC) available in the adegenet package (Jombart et al., 2010), implemented in R software (R Development Core Team, 2017). Evidence of genetic clusters (K) was examined in DAPC using find.clusters function and Bayesian Information Criterion (BIC). We tested values of K= 1-14, with ten runs at each value of K. The BIC values were visually examined to identify values of K (Jombart et al., 2010). The DAPC function was then executed using this grouping, retaining axes of Principal Components Analysis sufficient to explain >80% of total variance of data.

To guide the best crosses and crosses that should be avoided in order to maintain the genetic variability of progenies, we performed relationship assessment of farmed broodstock using COANCESTRY v.1.0.1.5 software (Wang, 2011).

RESULTS

16S sequencing

After aligning and editing the sequences, the 16S gene was much conserved and presented only one polymorphic site among *P. lineatus* individuals. The haplotype and nucleotide diversities were 0.325 and 0.0006, respectively. The average nucleotide proportions were 31:22:24:23 (A:T:C:G). Sequences from two individuals retrieved from GenBank were used to confirm the identity of *P. lineatus* specimens with sequences from two other Prochilodus species. Two species of the Curimatinae sub-family were used as outgroups. Both phylogenetic approaches retrieved trees with similar topologies; hence, only the ML tree is shown. Wild individuals from the Uruguay River formed a monophyletic group that diverged from 0 to 0.002 (K2P; Figure 2).

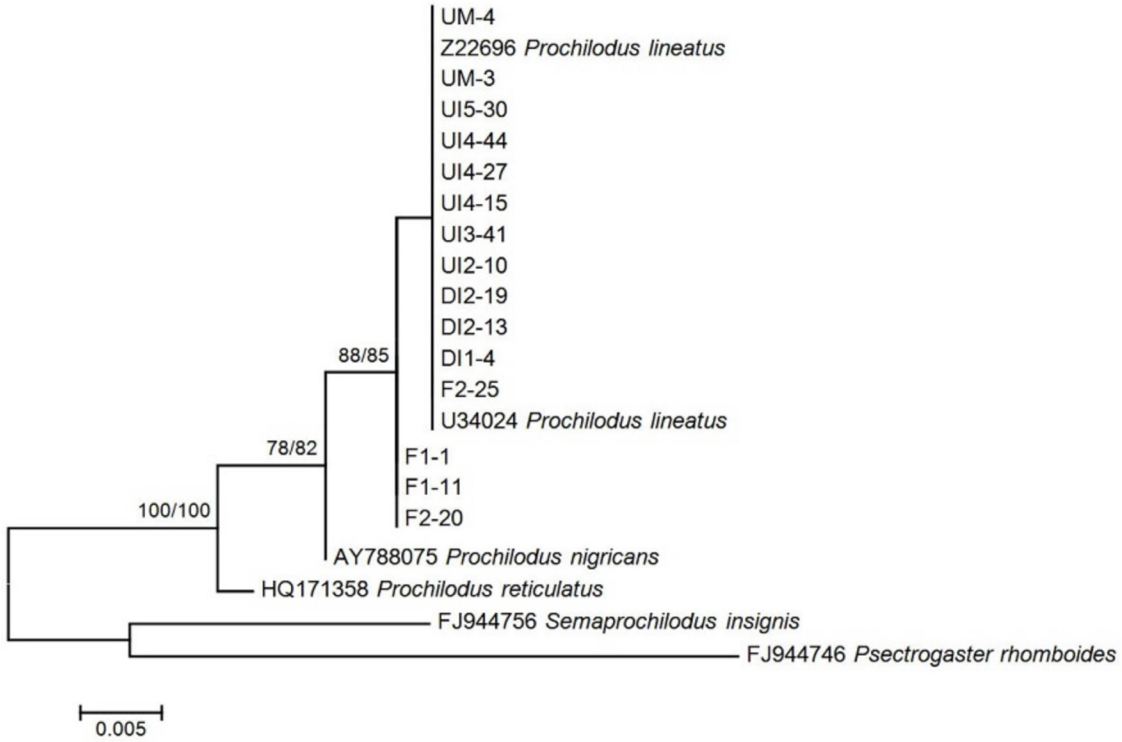


Figure 2. Maximum Likelihood phylogenetic tree based on the 16S sequences of Prochilodus spp. Bootstrap values are indicated only for the nodes exceeding 70% support for the analysis of Neighbor-Joining (1,000 replicates) and Maximum Likelihood (1,000 replicates), respectively. Tree was rooted using two Curimatinae species as outgroup.

Microsatellite analysis

The microsatellites used in this study showed no presence of large allele dropouts or other deviations when analyzed by MICRO-CHECKER. Genetic diversity estimates, number of alleles, allelic richness, effective population size, observed and expected heterozygosity and inbreeding coefficient are all shown in Table 1.

Table 1. Genetic diversity estimates for wild and farmed *Prochilodus lineatus* populations.

	Locus						
	Par12	Par14	Par21	Par43	Par80	Par82	Pli60
				FF1			
N	7	4	5	7	8	8	6
A	6.0	3.9	4.9	6.5	7.4	7.4	5.2
Ae	4.9	3.1	4.9	6.3	7.3	5.6	3.9
Ho	0.300	0.889	0.600	0.900	0.818	0.625	0.909
Ar	6.064	3.922	4.973	6.562	7.423	7.483	5.217
He	0.795	0.673	0.795	0.840	0.864	0.820	0.744
HWE	0.000*	0.020	0.259	0.414	0.102	0.163	0.224
Fis	0.654*†	-0.267	0.294	0.019	0.100	0.300	0.176
Pl	0.001	0.955	0.070	0.681	0.290	0.039	0.963
Ps	1.000	0.179	0.984	0.687	0.922	0.993	0.229
				FF2			
N	7	2	6	8	7	8	6
A	5.8	2.0	4.9	6.6	6.2	6.3	5.4
Ae	5.1	1.9	4.3	6.0	5.8	6.1	4.8
Ho	0.938	0.765	0.353	0.706	0.706	0.882	0.467
Ar	5.838	2.000	4.950	6.660	6.297	6.396	5.428
He	0.803	0.472	0.765	0.834	0.827	0.836	0.791
HWE	0.863	0.032	0.000*	0.042	0.083	0.723	0.016
Fis	-0.136	-0.600	0.560*†	0.183	0.176	-0.026	0.438*†
Pl	0.970	1.000	0.001	0.082	0.082	0.713	0.002
Ps	0.184	0.020	1.000	0.975	0.979	0.560	1.000
				FF3			
N	5	1	5	5	5	7	3
A	4.9	1.0	4.5	4.6	4.6	5.9	3.0
Ae	4.4	1.0	2.8	3.8	3.8	3.7	2.1
Ho	0.900	0.000	0.600	0.900	0.900	1.000	0.143
Ar	4.900	1.000	4.586	4.620	4.620	5.939	3.000
He	0.775	0.000	0.640	0.740	0.740	0.730	0.520
HWE	0.498	NA	0.378	0.051	0.053	0.414	0.021
Fis	-0.110	NA	0.115	-0.165	-0.165	-0.324	0.760†
Pl	0.866	NA	0.409	0.924	0.906	1.000	0.023
Ps	0.426	NA	0.864	0.318	0.330	0.023	1.000
				FF4			
N	3	3	3	6	6	6	2
A	2.9	2.7	2.9	5.2	5.2	5.5	2.0
Ae	2.9	2.3	2.9	4.7	4.7	4.6	1.9
Ho	0.167	0.923	0.769	0.923	0.923	0.769	0.462
Ar	2.998	2.797	2.999	5.288	5.288	5.539	2.000

He	0.653	0.556	0.660	0.787	0.787	0.781	0.473
HWE	0.000*	0.012	0.263	0.332	0.335	0.653	10.000
Fis	0.763*†	-0.636	-0.127	-0.134	-0.134	0.055	0.065
Pl	0.001	1.000	0.800	0.929	0.931	0.430	0.661
Ps	1.000	0.006	0.399	0.277	0.281	0.817	0.788
				DIT			
N	13	5	10	14	20	25	12
A	6.9	4.3	6.6	8.4	8.9	10.3	6.9
Ae	6.8	3,8	6.6	9.9	10.6	16.6	5,9
Ar	6.903	4.320	6.648	8.494	8.935	10.393	6.935
Ho	0.535	1.000	0.895	0.775	0,750	0.795	0.591
He	0.853	0.735	0.849	0.899	0.905	0.940	0.831
HWE	0.000	0.000	0.535	0.000	0.006	0.006	0.000
Fis	0.383	-0.352	-0.041	0.150	0.184	0.165	0.299
Pl	0.001	1.000	0.792	0.008	0.001	0.001	0.001
Ps	1.000	0.001	0.3612	0.999	1.000	1.000	1.000
				DMA			
N	8	6	10	13	12	13	9
A	6.178	4.888	5.434	8.175	7.513	7.088	6.121
Ae	5.9	3.7	4.7	9.1	7.8	7.0	4.4
Ar	6.903	4.320	6.648	8.494	8.935	10.393	6.935
Ho	0.659	1.000	0.714	0.594	0.649	0.762	0.659
He	0.831	0.726	0.788	0.890	0.872	0.857	0.775
HWE	0.004*	0.000*	0.262	0.000*	0.000*	0.000*	0.014
Fis	0.219†	-0.367*	0.108	0.347*†	0.269*†	0.122	0.162
Pl	0.004	1.000	0.132	0.001	0.001	0.032	0.022
Ps	0.999	0.001	0.928	1.000	1.000	0.987	0.994
				DBG			
N	5	5	6	8	5	8	7
A	4.021	4.065	5.503	7.093	5.000	6.462	5.413
Ae	1.7	3.3	4.7	6.9	3.9	5.1	3.3
Ar	4.021	4.065	5.503	7.093	5.000	6.462	5.413
Ho	0.400	1.000	0.750	0.750	0.714	0.583	0.538
He	0.420	0.692	0.788	0.854	0.745	0.802	0.695
HWE	0.478	0.067	0.050	0.087	0.208	0.067	0.005*
Fis	0.100	-0.412	0.092	0.165	0.118	0.313	0.263†
Pl	0.477	1.000	0.358	0.130	0.443	0.021	0.065
Ps	0.911	0.009	0.869	0.972	0.863	0.993	0.986

Samples size, *N*; mean number of alleles, *A*; allelic richness, *Ar*; observed heterozygosity, *Ho*; expected heterozygosity, *Ae* effective number of alleles; *He*; Hardy–Weinberg equilibrium, *HWE*; population inbreeding coefficient *Fis*; *P* values for the deficit of heterozygotes, *Pl*; *P* values for the excess of heterozygotes, *Ps*. *Significant values after Bonferroni correction (α = 0.007). † Null alleles. NA = *Not analyzed*

The number of alleles per locus ranged from one (Par14; FF3) to 25 (Par82; DIT). The mean values of Ho and He ranged from 0.000 to 1.000. Significant deviations from the HWE is related to the presence of null alleles, resulting in a deficit of heterozygotes in some loci (Table 1). Private alleles, i.e., those found in a single population, were present in all wild populations (DIT = 25; DMA = 20 and DBG = 6) and only one farm population (FF1 = 5). Mean PIC values ranged from 0.800 (Par14) to 0.933 (Par82). Effective population size (Ne) ranged from 2.1 to 20.5 in farmed populations and from 13.3 to 191.4 in wild populations (Table 2). The population assignment test indicates that 83% of individuals were assigned to self-populations (Table 2).

Table 2. Effective population size (*Ne*) and population assignment for wild and aquaculture sampled populations of *Prochilodus lineatus*. Confidence interval CI = 95%.

Population	Sample Size	Ne (CI 95%)	Self Population	Other Population
FF1	11	20.5 (6.3 - inf.)	8	3
FF2	17	7.4 (3.3 - 13.1)	15	2
FF3	10	3.2 (1.7-16.1)	7	3
FF4	13	2.1 (1.4 - 4.4)	13	0
DIT	49	191.4 (70.1 - inf.)	35	14
DMA	44	31.1 (21.5 - 51.5)	42	2
DBG	13	13.3 (4.2 - 293)	11	2
Percentage		-	83%	17%

Global differentiation, including all populations sampled in the present study, by AMOVA showed low significant genetic structure in all scenarios analyzed (Table 3). Divergence was detected among wild populations (FST = 0.056, p = 0.000), among farming stations (FST = 0.051; p = 0.000), and between wild and farm *P. lineatus* (FST = 0.094; p = 0.000) (Table 4).

Table 3. Pairwise FST estimates (below of diagonal) and corresponding significance levels (above of diagonal) between sampled wild and farmed populations of *Prochilodus lineatus*.

	Farmed				**Wild**		
	FF1	FF2	FF3	FF4	DIT	DMA	DBG
FF1	**0**	0.00238	0.00476	0.00238	0.00238	0.00238	0.00238
FF2	**0.0302**	0	0.01190	0.00238	0.00238	0.00238	0.00476
FF3	0.1326	0.0674	0	0.00238	0.00238	0.00238	0.00238
FF4	**0.1319**	**0.1475**	**0.2270**	0	0.00238	0.00238	0.00238
DIT	**0.0464**	**0.0531**	**0.1112**	**0.1190**	0	0.00238	0.00238
DMA	**0.0862**	**0.0978**	**0.1762**	**0.1573**	**0.0461**	0	0.00238
DBG	**0.1277**	0.1381	**0.2381**	**0.2418**	**0.0701**	**0.0566**	0

Indicative adjusted nominal level (5%) for multiple comparisons is α = 0.002. Significance is in bold.

Table 4. Global AMOVA of *Prochilodus lineatus* from different origins (Farmed and Wild) and among all samples.

Source of variation (Percentage of variance explained)				
Origin	Among localities	Between individuals/within localities	Within individuals	Fixation index
Farm	5.657	25.053	69.289	FST = 0,056*
Wild	5.273	17.010	77.716	FST = 0.051*
All	8.165	22.594	69.239	FST = 0.094*

* $p = 0.000$

Bayesian analyses for all samples without a priori information suggest three main clusters (Figure 3). It is possible to observe a sharp structure between cultivated and wild individuals, as well as genetic structure among wild fish (Figure 4).

DAPC similarly identified a genetic structure in wild and farmed *P. lineatus*. The initial sharp decline in Bayesian Information Criterion (BIC) values was between K = 3 – 5 (Figure 5). When using the lowest K value (K = 5), it is also possible to

see the separation between individuals from FF4 (Cluster 1 in blue) and FF2 (Cluster 5 in red). For wild individuals, DAPC showed a distribution of the microsatellite genotypes into three main clusters (Clusters 2, 3 and 4; Figure 5), corroborating the genetic differentiation among wild sampled populations of *P. lineatus* from the Upper Uruguay River (Table 2). Discriminant functions based on DAPC analyses assigned most individuals to the genetic cluster where they were assigned a priori by K-means analyses used to infer the best-supported clustering (Figure 6). The low overlapping of the genetic clusters on the ordination plot indicated high degree of differentiation between FF4, and high overlapping of the genetic clusters of wild individuals (Figure 4b). Differentiation index FST similarly corroborated the results of DAPC, showing higher genetic differentiation between FF4 in relation to other farm populations (Table 4).

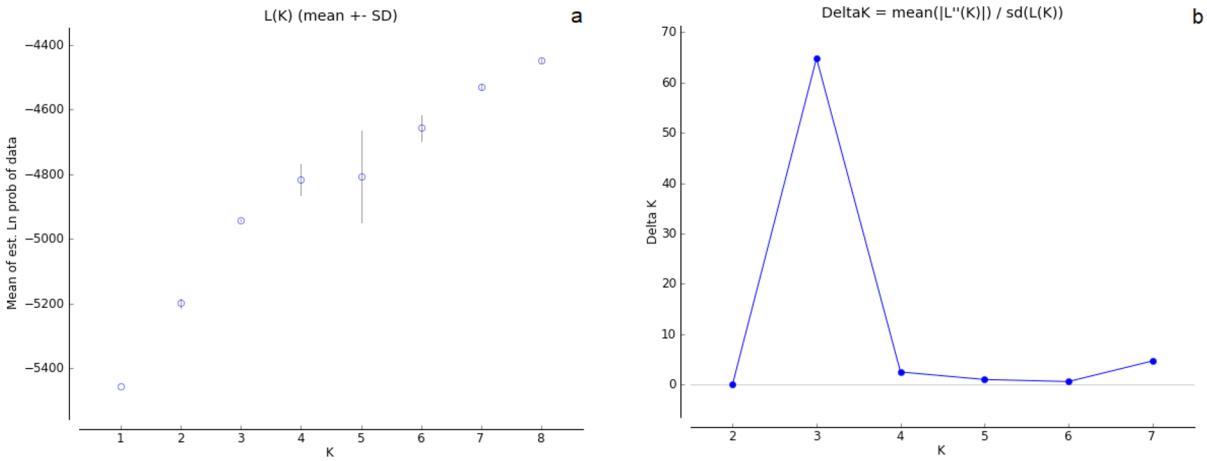


Figure 3. Plot of mean log-likelihood values (LnP (D)) (A) and Evanno´s DeltaK (B) generated in STRUCTURE HARVESTER based on wild and farmed *Prochilodus lineatus*.

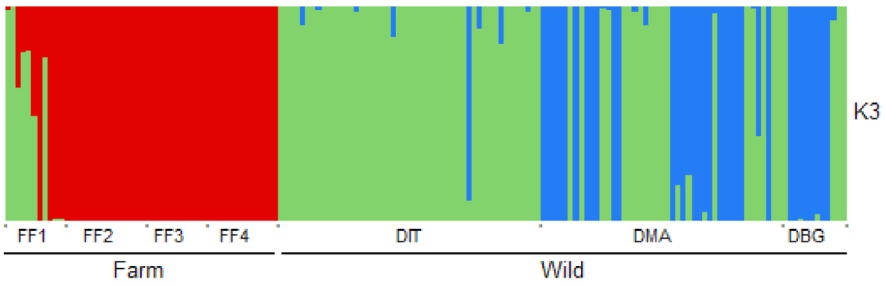


Figure 4. *Prochildous lineatus* population structure from the Bayesian cluster analysis for K = 3. Black lines separate the seven different sampled populations based on location.

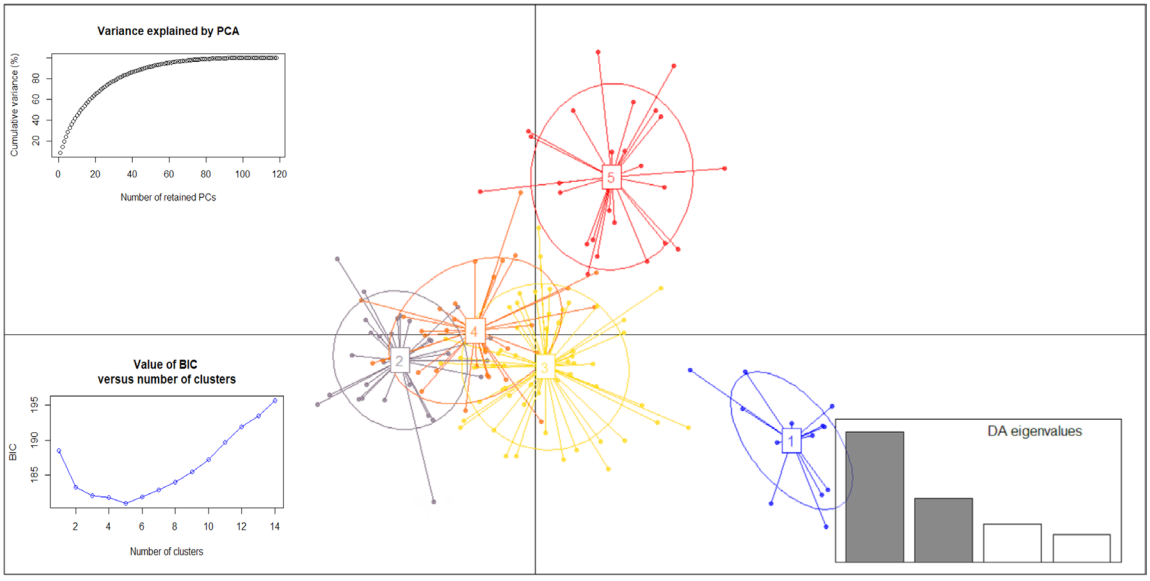


Figure 5. Discriminant Analysis of Principal Components (DAPC) for 30 retained PC axes and two discriminate functions. Five clusters were recovered with this model (BIC scores indicating K = 5). The bottom right graphic shows eigenvalues of the two principal components. Cluster 1 (blue) = *Prochilodus lineatus* from FF4; Cluster 2 (lavender) = individuals from DMA and DBG; Cluster 3 (yellow) = FF1, DIT and DMA; Cluster 4 (orange) = DBG, DMA and DIT; and Cluster 5 (red) = FF2 and FF3.

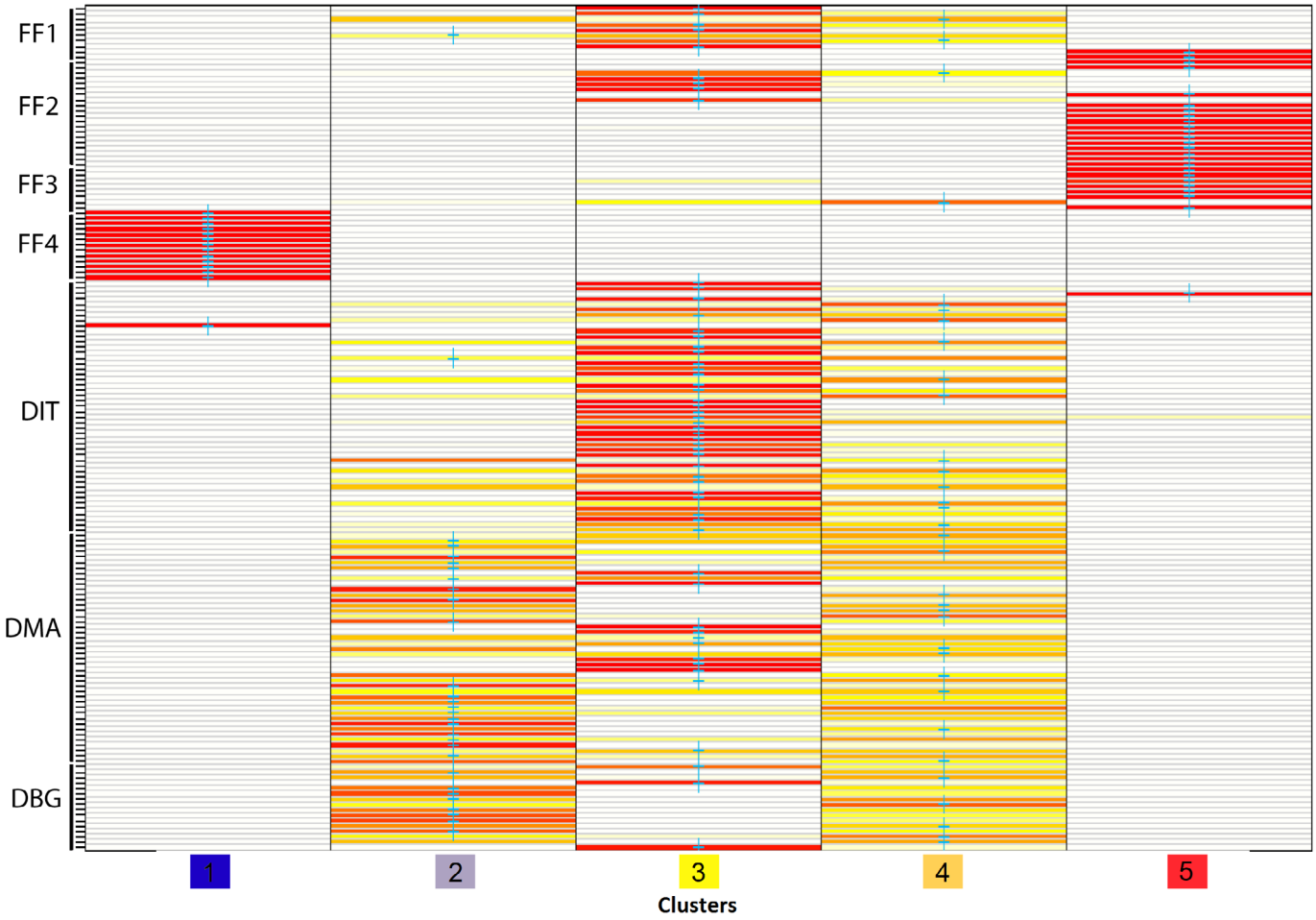
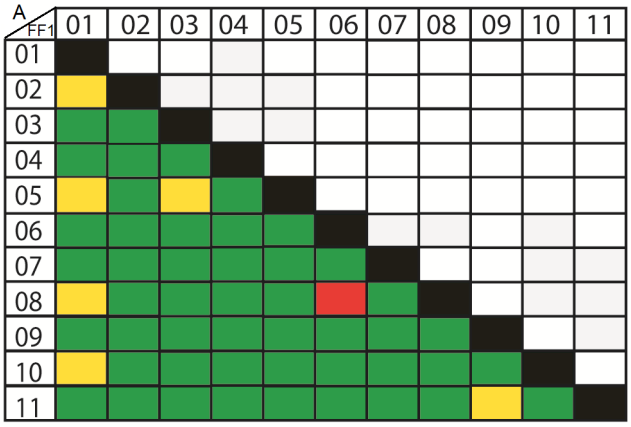
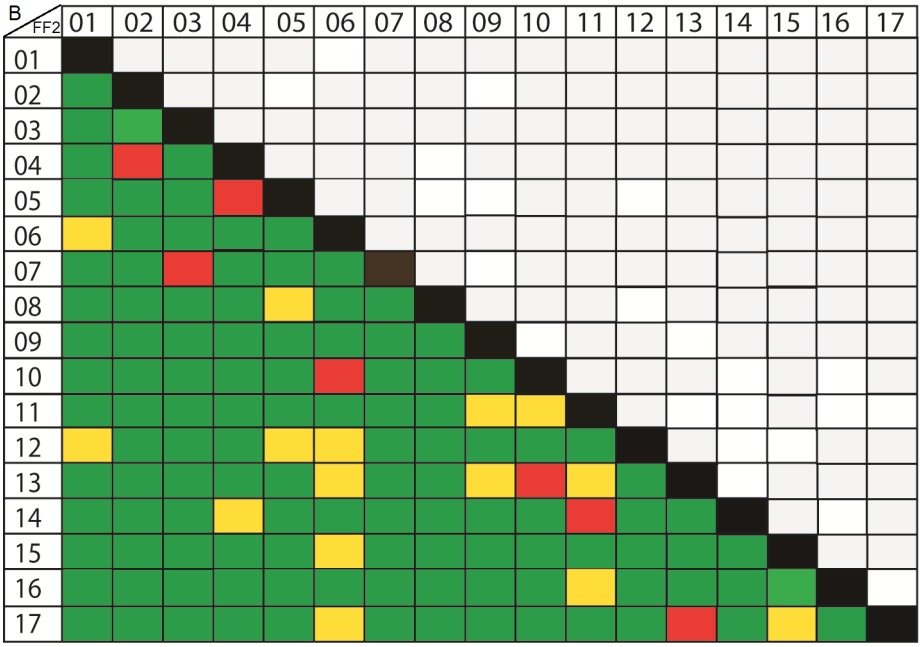
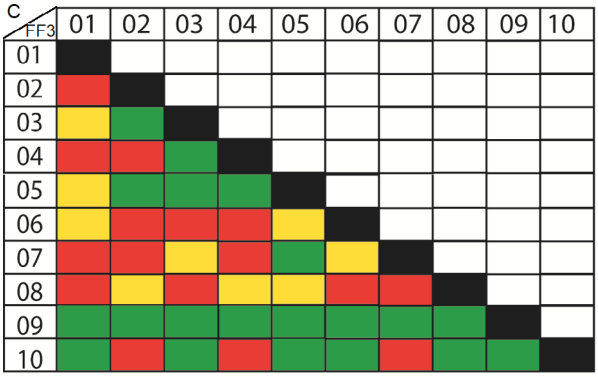


Figure 6. Panel represents whether the individuals (rows) were correctly assigned (based DAPC; Figure 5) to the genetic cluster where they were included a priori (columns). Colors represent membership probabilities to each genetic cluster (red = 1, orange = 0.75, yellow = 0.25, white = 0) and blue crosses indicate the cluster where the individuals were originally assigned.

The lowest variance for the kinship estimators was found in the triadic likelihood estimator (TrioML). Sampling variances for the kinship estimators (KE) ranged from 0.0000 to 0.5709 in FF1, from 0.000 to 0.1890 in FF2, from 0.0000 to 0.8095 in FF3, and from 0.0000 to 0.8231 in FF4. The relatedness values for simulated pairs were split into three categories, according Wang (2011): high (>0.5; full-sib and parent-offspring), intermediate (>0.25 and <0.5; half-sib or other kinship), and low (<0.25; unrelated). Considering all possible crosses, advisable matings were 90.4% (FF1), 84% (FF2), 56.9% (FF3) and 62% (FF4). Results for indicated mating crossings are showed in green, crosses to be avoided are showed in yellow, and prohibitive mating are showed in red (Figure 7).


A
FF1
01 02 03 04 05 06 07 08 09 10 11


B
FF2
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17


C
FF3
01 02 03 04 05 06 07 08 09 10

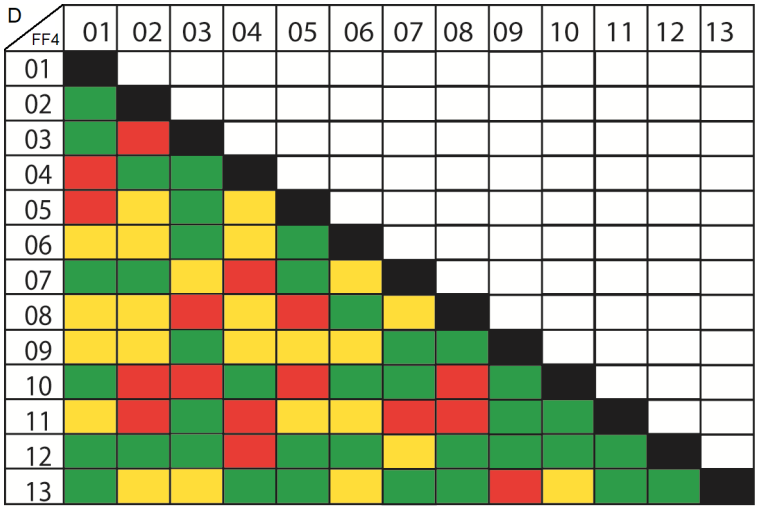


Figure 7. Consensus pairwise relatedness of farmed broodstock of *Prochilodus lineatus* estimated using COANCESTRY program. Crosses in red are prohibitive, in yellow should be avoided and in green are unreasonable. A = Fish Farm FF1; B = FF2; C = FF3; D = FF4.

DISCUSSION

In this study, we identified genetic divergence between and within wild and farmed populations of *P. lineatus* in southern Brazil. Wild populations showed moderate to high genetic diversity, a characteristic that seems to be shared among most Prochilodus species (Sivasundar et al., 2001; Rueda et al., 2013; Braga-Silva and Galleti Jr., 2016; Ferreira et al., 2017). On the other hand, the cultivated populations showed smaller values of genetic diversity, especially when compared to wild populations of *P. lineatus*. Farmed populations similarly displayed lower diversity and allelic richness, but higher inbreeding rates, when compared to wild individuals. These confined populations are more likely to present a reduction in genetic variability as a result of genetic drift (de Oliveira et al., 2018). Among other problems, the low values of genetic diversity increase susceptibility to disease in aquaculture programs (Doyle, 2016) and would, therefore, compromise the viability of natural populations in any restocking initiative (Allendorf and Luikart, 2009).

Farmed fish presented genetic structure distinct from that of wild populations in addition to genetic differentiation among the farmed groups studied. In particular, individuals from FF4, which is located in southeastern Rio Grande do Sul State, exhibited substantially distinct genetic composition in comparison to individuals from other fish farms studied. In general, all fish farms presented some crosses not indicated, due to the high degree of kinship among some broodstock. According to Fonseca et al. (2017), the successful restocking programs depends upon keeping levels of inbreeding low and optimizing genetic variability, resultant of genetic information of broodstock and breeding designs

The genetic composition of the freshwater broodstock is generally formed by exchange of matrices between fish farmers, regardless of the river basin where they are found, and guidelines are available for this commercialization. In addition to compromising the genetic composition of natural populations (Vaini et al., 2016), crosses among fish from different hydrographic basins may result in non-intentional, as well as intentional, interspecific crosses, as detected in several species of fish in previous studies (Hashimoto et al., 2014a,b; Scaranto et al., 2018).

Different from recent studies that found genetic structure associated with temporal experimental design (e.g., Braga-Silva and Galleti Jr., 2016; Ribolli et al., 2017; Ribolli et al., 2018), our sample arrangement did not allow for testing IBT (isolation-by-time) in the wild populations. Nevertheless, as reported in many studies of population genetics, population structure in Neotropical migratory fish species is not rare (Pereira et al., 2009; Garcez et al., 2011; Ashikaga et al., 2015). The genetic structure of *P. lineatus* identified in the present study may be related to the geographical features of the Upper Uruguay River, which is characterized by stretches of rapids and canyons (e.g., Canyon Augusto César), forming a semipermeable barrier in periods of drought that may have contributed to genetic differentiation of populations downstream from the Machadinho Dam. The installation of Itá Dam probably preserves the genetic isolation of the natural barrier flooded by the Itá Reservoir. Additionally, since wild fish samples were collected in different years, the possible relationship between population structure and reproductive organization, as already reported in potamodromous migrating fish, cannot be ruled out (Braga-Silva and Galetti Jr., 2016, Ribolli et al., 2017).

Genetic differentiation between and within wild and farmed fish is a clear signal that restocking using farmed fish may, indeed, compromise the genetic diversity of wild stocks, resulting in genetic introgression, which occurs when exogenous genetic material is different from that of the wild population (Ryman et al., 1995; Prado et al., 2018). Furthermore, the genetic constitution of the wild population can be permanently altered by the loss of important genetic material. This happens when genes or gene complexes favored by artificial selection are not adaptable to the natural environment, thus causing erosion

of the wild gene pool, decreasing the reproductive capability of wild fish and introducing diseases in the wild populations, or even leading to loss of the natural population (Ryman et al., 1995).Therefore, the identification of population structure is an essential precondition for developing recommendations for genetic management because the populations present in different fragments can be completely, or partially, isolated, or even be a single population (Frankham, 2008).

P. lineatus from fish farms showed lower genetic variability and effective population size compared to their counterparts in natural populations. Moreover, farmed individuals presented a genetic composition completely different from that of wild individuals. Thus, if these farmed fish were used for restocking purposes, the genetic variability of recipient populations could be compromised since wild populations undergo continual natural selection, selecting specific genotypes that maximize the fitness of individuals to survive in particular natural environments (Ward, 2006), in this case, *P. lineatus*.

CONCLUSION

The collective results of the present study should serve as a warning against restocking of natural fish populations that occur in just about all Neotropical river basins using fish, either larvae or juveniles, from commercial fish farms. Our results also show that the stock of fish farms, FF1-FF4, as analyzed in the present study, should not be used for restocking as an alternative for the recovery and maintenance of wild *P. lineatus* stocks of the Upper Uruguay River Basin. It is clear from our results that any programs that propose to set up breeding stock for restocking purposes should be cognizant of the genetic diversity of the stock to be managed, both wild and cultivated. It is also recommended that policymakers consider adding measures to legislation that include genetic analyses to any current or proposed restocking programs in order to properly preserve and/or recover wild stocks of freshwater fish, including *P. lineatus*.

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REFERENCES

Allan, J.D.; Abell, R.; Hogan, Z.E.B.; Revenga, C.; Taylor, B.W.; Welcomme, R.L.; Winemiller, K. 2005. Overfishing of inland waters. AIBS Bulletin, 55(12): 1041-1051. **https://doi.org/10.1641/0006-3568 (2005)055[1041:OOIW]2.0.CO;2**

Allendorf, F.W.; Luikart, G. 2009. Conservation and the genetics of populations. John Wiley and Sons. N575.8. 602p.

Ashikaga, F.Y.; Orsi, M.L.; Oliveira, C.; Senhorini, J.A.; Foresti, F. 2015. The endangered species Brycon orbignyanus: genetic analysis and definition of priority areas for conservation. Environmental Biology of Fishes, 98(7): 1845-185. **http://dx.doi.org/10.1007/s10641-015-0402-8**

Baigún, C.; Minotti, P.; Oldani, N. 2013. Assessment of sábalo (*Prochilodus lineatus*) fisheries in the lower Paraná River basin (Argentina) based on hydrological, biological, and fishery indicators. Neotropical Ichthyology, 11(1): 199-210. **http://dx.doi.org/10.1590/S1679-62252013000100023**

Barbosa, A.C.; Galzerani, F.; Corrêa, T.C.; Galetti Jr, P. M.; Hatanaka, T. 2008. Description of novel microsatellite loci in the Neotropical fish *Prochilodus argenteus* and cross-amplification in *P. costatus* and *P. lineatus*. Genetics and Molecular Biology, 31(1): 357-360. **http://dx.doi.org/10.1590/S1415-47572008000200032**

Barbosa, A.C.; Correa, T.C.; Galzerani, F.; Galetti Jr, P.M.; Hatanaka, T. 2006. Thirteen polymorphic microsatellite loci in the Neotropical fish *Prochilodus argenteus* (Characiformes, Prochilodontidae). Molecular Ecology Notes, 6(3): 936-938. **http://dx.doi.org/10.1590/S1415-47572008000200032**

Bondioli, A.C.V.; Marques, R.C.; Toledo, L.F.A.; Barbieri, E. 2017. PCR-RFLP for identification of the pearl oyster *Pinctada imbricate* from Brazil and Venezuela. Boletim do Instituto de Pesca, 43(4): 459-463. **http://dx.doi.org/10.20950/1678-2305.2017v43n3p459**

Braga-Silva, A.; Galetti, P.M. 2016. Evidence of isolation by time in freshwater migratory fish *Prochilodus costatus* (Characiformes, Prochilodontidae). Hydrobiologia, 765 (1): 159-167. **https://doi.org/10.1007/s10750-015-2409-8**

Calcagnotto, D.; Schaefer, S. A.; DeSalle, R. 2005. Relationships among characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences. Molecular Phylogenetics and Evolution, 36(1): 135-153. **https://doi.org/10.1016/j.ympev.2005.01.004**

Carvalho-Costa, L.F.; Hatanaka, T.; Galetti Jr, P.M. 2008. Evidence of lack of population substructuring in the Brazilian freshwater fish *Prochilodus costatus*. Genetics and Molecular Biology, 31(1): 377-380. **http://dx.doi.org/10.1590/S1415-47572008000200036**

Castro, R.M.P.; Vari, R.P. 2004. Detritivores of the South American fish family Prochilodontidae (Teleostei: Ostariophysi: Characiformes): a phylogenetic and revisionary study. Smithsonian Contributions to Zoology, 622: 1–90. **https://doi.org/10.5479/si.00810282.622**

Castro, R.M.C.; Vari, R.P. 2003. Family Prochilodontidae. Check List of the Freshwaters of South and Central America. EDIPUCRS, 1: 65-70.

de Oliveira, R.C.; Santos, M.D.C.F.; Bernardino, G.; Hrbek, T.; Farias, I.P. 2018. From river to farm: an evaluation of genetic diversity in wild and aquaculture stocks of *Brycon amazonicus* (Spix and Agassiz, 1829), Characidae, Bryconinae. Hydrobiologia, 805(1): 75-88. **https://doi.org/10.1007/s10750-017-3278-0**

Do, C.; Waples, R.S.; Peel, D.; Macbeth, G.M.; Tillett, B.J.; Ovenden, J.R. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources, 14(1:09-214. **https://doi.org/10.1111/1755-0998.12157**

Duong, T.Y.; Scribner, K.T. 2018. Regional variation in genetic diversity between wild and cultured populations of bighead catfish (*Clarias macrocephalus*) in the Mekong Delta. Fisheries Research, 207: 118-125. **https://doi.org/10.1016/j.fishres.2018.06.012**

Doyle, R.W. 2016. Inbreeding and disease in tropical shrimp aquaculture: a reappraisal and caution. Aquaculture research, 47(1): 21-35. **https://doi.org/10.1111/are.12472**

Earl, D.A. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4(2): 359-361. **https://doi.org/10.1007/s12686-011-9548-7**

Espinach Ros, A.; Sverlij, S.; Amestoy, F.; Spinetti, M. 1998. Migration pattern of the sábalo *Prochilodus lineatus* (Pisces, Prochilodontidae) tagged in the lower Uruguay River. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen, 26(5): 2234-2236. **https://doi.org/10.1080/03680770.1995.11901143**

Evanno, G.; Regnaut, S.; Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14(8): 2611-2620. **https://doi.org/10.1111/j.1365-294X.2005.02553.x**

Excoffier, L.; Lischer, H.E. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular ecology resources, 10(3): 564-567. **https://doi.org/10.1111/j.1755-0998.2010.02847.x**

Falush, D.; Stephens, M.; Pritchard, J.K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 164(4): 1567-1587. **https://doi.org/10.1111/j.1471-8286.2007.01758.x**

Ferreira, D.G.; Souza-Shibatta, L.; Shibatta, O.A.; Sofia, S. H.; Carlsson, J.; Dias, J.H.P.; Makrakis, S.; Makrakis, M.C. 2017. Genetic structure and diversity of migratory freshwater fish in a fragmented Neotropical river system. Reviews in Fish Biology and Fisheries, 27(1): 209-231. **https://doi.org/10.1007/s11160-016-9441-2**

Fonseca, F. S.; Domingues, R.R.; Hallerman, E.M.; Hilsdorf, A.W. 2017. Genetic diversity of an imperiled Neotropical catfish and recommendations for its restoration. Frontiers in genetics, 8: 196. **http://dx.doi.org/10.3389/fgene.2017.00196**

Frankham, R. 2008. Genetic adaptation to captivity in species conservation programs. Molecular Ecology, 17(1): 325-333. **https://doi.org/10.1111/j.1365-294X.2007.03399.x**

Garcez, R.; Calcagnotto, D.; De Almeida-Toledo, L.F. 2011. Population structure of the migratory fish *Prochilodus lineatus* (Characiformes) from Rio Grande basin (Brazil), an area fragmented by dams. Aquatic Conservation: Marine and Freshwater Ecosystems, 21(3): 268-275. **https://doi.org/10.1002/aqc.1176**

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9. 3. Disponível em: **http://www2.unil.ch/popgen/softwares/fstat.htm**.

Hashimoto, D.T.; Prado, F.D.; Senhorini, J.A.; Foresti, F.; Porto-Foresti, F. 2014. Aquaculture of Neotropical catfish hybrids: genetic strategies for conservation and management. In Carp and Catfish: Biology, Behavior and Conservation Strategies (Regan, B., ed), pp. 1-10. Nova Science Publishers, New York.

Hashimoto, D.T.; Senhorini, J.A.; Foresti, F.; Martínez, P.; Porto-Foresti, F. 2014. Genetic identification of F1 and post-F1 *Serrasalmid juvenile* hybrids in Brazilian aquaculture. PloS one, 9(3): e89902. **https://doi.org/10.1371/journal.pone.0089902**

Hedrick, P.W. 2005. A standardized genetic differentiation measure. Evolution, 59(8): 1633-1638. **https://doi.org/10.1111/j.0014-3820.2005.tb01814.x**

Hoeinghaus, D.J.; Agostinho, A.A.; Gomes, L.C.; Pelicice, F.M.; Okada, E.K.; Latini, J.D.; ... Winemiller, K.O. 2009. Effects of river impoundment on ecosystem services of large tropical rivers: embodied energy and market value of artisanal fisheries. Conservation Biology, 23(5): 1222-1231. **https://doi.org/10.1111/j.1523-1739.2009.01248.x**

IBAMA. Instituto brasileiro do meio ambiente e dos recursos naturais renováveis. Instrução Normativa Nº- 146, De 10 de Janeiro De 2007. Disponível em: **http://www.icmbio.gov.br/sisbio/images/stories/instrucoes_normativas/IN146_2007_Empreendimentos.pdf**

Jombart, T.; Devillard, S.; Balloux, F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC genetics, 11(1): 94.

Kalinowski, S.T.; Taper, M. L.; Marshall T. C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16: 1099–1106. **http://dx.doi.org/10.1111/j.1365-294X.2007.03089.x**

Kessing, B.; Croom, H.; Martin, A.; McIntosh, C.; Mcmillan, W.O.; Palumbi, S. 1989. The simple fool's guide to PCR. University of Hawaii, Honolulu. 45p.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16(2): 111-120.

Librado, P.; Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25(11): 1451-1452. **http://dx.doi.org/10.1093/bioinformatics/btp187**

Meyer, A.; Biermann, C.H.; Orti, G. 1993. The phylogenetic position of the zebrafish (*Danio rerio*), a model system in developmental biology: an invitation to the comparative method. Proceedings of the Royal Society of London. Series B: Biological Sciences, 252(1335): 231-236. **http://dx.doi.org/10.1098/rspb.1993.0070**

Oliveira, C.; Avelino, G.S.; Abe, K.T.; Mariguela, T.C;, Benine, R.C.; Ortí, G.;...; Castro, R.M. C. 2011. Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. BMC Evolutionary Biology, 11(1): 275. **https://doi.org/10.1186/1471-2148-11-275**

Pereira, L.H.G.; Foresti, F.; Oliveira, C. 2009. Genetic structure of the migratory catfish *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae) suggests homing behaviour. Ecology of Freshwater Fish, 18(2): 215-225. **https://doi.org/10.1111/j.1600-0633.2008.00338.x**

Prado, F.D.; Vera, M.; Hermida, M.; Blanco, A.; Bouza, C.; Maes, G.E.; ...; AquaTrace Consortium. 2018. Tracing the genetic impact of farmed turbot *Scophthalmus maximus* on wild populations. Aquaculture Environment Interactions, 10: 447-463. **http://dx.doi.org/10.3354/aei00282**

Pritchard, J.K.; Stephens, M.; Rosenberg, N.A.; Donnelly, P. 2000. Association mapping in structured populations. The American Journal of Human Genetics, 67(1): 170-181. **https://doi.org/10.1086/302959**

Raymond, M.; Rousset, F. 1995. An exact test for population differentiation. Evolution, 49(6): 1280-1283.

Ribolli, J.; Zaniboni-Filho, E. 2009. Individual contributions to pooled-milt fertilizations of silver catfish *Rhamdia quelen*. Neotropical Ichthyology, 7(4): 629-634. **http://dx.doi.org/10.1590/S1679-62252009000400011**

Ribolli, J.; Mino, C.I.; Zaniboni-Filho, E.; de Souza Guerreiro, T.C.; Reynalte-Tataje, D.A.; de Freitas, P.D.; Galetti, P.M. 2016. Preliminary insights into the genetic mating system of Neotropical Salminus brasiliensis: kinship assignment and parental reconstruction reveal polygynandry. Ichthyological Research, 63(1): 187-191. **http://dx.doi.org/10.1007/s10228-015-0487-2**

Ribolli, J.; Scaranto, B.M.; Shibatta, O.A; Bombardelli, R.A.; Zaniboni-Filho, E. 2017. DNA barcoding confirms the occurrence of *Rhamdia branneri* and *Rhamdia voulezi* (Siluriformes: Heptapteridae) in the Iguaçu River Basin. Neotropical Ichthyology, 15(1). **http://dx.doi.org/10.1590/1982-0224-20160147**

Ribolli, J.; Zaniboni-Filho, E.; Freitas, P.D.; Galetti, P.M. 2018. Genetic evidences of non-reproductive shoaling in the freshwater fish *Salminus brasiliensis*. Hydrobiologia, 815(1): 65-72. **https://doi.org/10.1007/s10750-018-3550-y**

Roques, S.; Berrebi, P.; Rochard, E.; Acolas, M.L. 2018. Genetic monitoring for the successful re-stocking of a critically endangered diadromous fish with low diversity. Biological Conservation,221: 91-102. **http://dx.doi.org/10.1016/j.biocon.2018.02.032**

Rueda, E.C.; Carriquiriborde, P.; Monzón, A.M.; Somoza, G.M.; Ortí, G. 2013. Seasonal variation in genetic population structure of sábalo (*Prochilodus lineatus*) in the Lower Uruguay River. Genetica, 141(7-9): 401-407. **https://doi.org/10.1007/s10709-013-9739-0**

Ryman, N.; Utter, F.; Laikre, L. 1995. Protection of intraspecific biodiversity of exploited fishes. Reviews in Fish Biology and Fisheries, 5(4): 417-446.

Sambrook, J.; Russell, D. W.; Maniatis, T. 2001. Molecular cloning, vol. 1-3. Cold Spring Habour Laboratory Press, New York. 2100p.

Scaranto, B.M.S.; Ribolli, J.; Zaniboni-Filho, E. 2018. DNA barcoding reveals blend of silver catfish Rhamdia species from fish farms in Southern Brazil. Aquaculture Research, 49(5): 1907-1913. **https://doi.org/10.1111/are.13646**

Schork, G.; Hermes-Silva, S.; Zaniboni-Filho, E. 2013. Analysis of fishing activity in the Itá reservoir, Upper Uruguay River, in the period 2004-2009. Brazilian Journal of Biology, 73(3): 559-571. **http://dx.doi.org/10.1590/S1519-69842013000300014**

Sivasundar, A.; Bermingham, E.; Ortí, G. 2001. Population structure and biogeography of migratory freshwater fishes (Prochilodus: Characiformes) in major South American rivers. Molecular Ecology, 10(2): 407-417. **https://doi.org/10.1046/j.1365-294X.2001.01194.x**

Small, M.P.; Currens, K.; Johnson, T.H., Frye, A.E.; Von Bargen, J.F. 2009. Impacts of supplementation: genetic diversity in supplemented and unsupplemented populations of summer chum salmon (*Oncorhynchus keta*) in Puget Sound (Washington, USA). Canadian Journal of Fisheries and Aquatic Sciences, 66(8): 1216-1229. **https://doi.org/10.1139/F09-068**

Sverlij, S. B. 1993. Sinopsis de los datos biológicos y pesqueros del sábalo, *Prochilodus lineatus* (Valenciennes, 1847) (No. 154). Food and Agriculture Org. 64p.

Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M.;Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28(10): 2731-2739.

Tave, D. 1999. Inbreeding and brood stock management (No. 392). Food and Agriculture Org.

Thompson, J.D.; Higgins, D.G.; Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22(22): 4673-4680.

Vaini, J.O.; do Amaral Crispim, B.; dos Santos Silva, D.B.; Benites, C.; Russo, M.R.; Grisolia, A.B. 2016. Genetic variability of pure *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum* individuals in the Paraná and Paraguay River basins. Fisheries Science, 82(4): 605-611. **http://dx.doi.org/10.1007/s12562-016-0999-3**

Van Oosterhout, C.; Hutchinson, W.F.; Wills, D.P.; Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4(3): 535-538. **https://doi.org/10.1111/j.1471-8286.2004.00684.x**

Viveiros, A.T.M.; Nascimento, A.F.; Orfão, L.H.; Isaú, Z.A. 2010. Motility and fertility of the subtropical freshwater fish streaked prochilod (*Prochilodus lineatus*) sperm cryopreserved in powdered coconut water. Theriogenology, 74(4): 551-556.

Waples, R.S.; Do, C. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. MolecularEecology Resources 8:753–756. **https://doi.org/10.1111/j.1755-0998.2007.02061.x**

Ward, R.D. 2006. The importance of identifying spatial population structure in restocking and stock enhancement programmes. Fisheries Research, 80(1): 9-18.

Weir, B.S.; Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution, 38(6): 1358-1370.

Yazbeck, G.M.; Kalapothakis, E. 2007. Isolation and characterization of microsatellite DNA in the piracema fish *Prochilodus lineatus* (Characiformes). Genetics and Molecular Research, 6(4): 1026-1034.

Zaniboni-Filho E.; Schulz, U.H. 2003. Migratory fishes of the Uruguay River. In: Carolsfeld J.; Harvey,B.; Baer, A.; Ross,C. (eds). In: Migratory fishes of South America: biology, fisheries and conservation status. International Development Research Centre and the World Bank. Victoria, Canada. p. 157-194.