Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v44i1.52657

Genetic diversity in Amazonian Jundiá (*Leiarius marmoratus*) stocks using heterologous primers

Laís Santana Celestino Mantovani[1*], Felipe Pinheiro de Souza[2], Eric Costa Campos[1], Vanessa Lewandowski[3], Nelson Maurício Lopera Barrero[2] and Ricardo Pereira Ribeiro[1]

[1]Departamento de Zootecnia, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. [2]Departamento de Zootecnia, Universidade Estadual de Londrina, Londrina, Paraná, Brazil. [3]Universidade Federal da Grande Dourados, Dourados, Mato Grosso do Sul, Brazil. *Author for correspondence. E-mail: lais.peixegen@gmail.com

ABSTRACT. With the rise of world fish farming, the national scenario is favorable for using native fish for intensive farming. Among the catfish, the Amazonian Jundiá (*Leiarius marmoratus*) is a robust candidate, easy to grow and with good organoleptic characteristics in its flesh. For productive success in captivity, it is necessary to consider some questions about the species, such as genetic variability, which must have an acceptable level in a breeding stock, in order to maintain a good diversity; this reduces losses due to inbreeding and low diversity. Therefore, the objective of this study was to characterize the genetic variability of commercial stocks of *L. marmoratus* from the State of Mato Grosso through microsatellite molecular markers. We analyzed 143 individuals from three stocks. The mean heterozygosity and the inbreeding coefficients observed were 0.060; 0.084; 0.141; and 0.539; 0.562; 0.514, respectively, for the stocks of Campo Verde, Juína, and Nova Mutum. The Deviation in the Hardy-Weinberg equilibrium was observed in most of the *loci* in the three populations. Considering the genetic differentiation, it is concluded that the three populations are very close genetically, which requires introduction of new genetic material in the stocks to enrich them genetically for a later reproductive program.

Keywords: DNA; molecular markers; genetic variability; fish culture; siluriformes.

Received on March 16, 2020.
Accepted on May 28, 2021.

Introduction

Brazil has shown growth in fish farming activity in the last years, and the FAO report "World State of Fisheries and Aquaculture" (SOFIA), published in June 2020, estimates that total fish production is expected to increase to 204 million tons by 2030, an increase of 15% compared to 2018, with aquaculture's share growing from the current 46% (FAO, 2020). Among the native species with great productive potential is the Jundiá of the Amazon *(Leiarius marmoratus*). The Jundiá is a migratory fish, similar to other large Amazonian catfish (Layman, Winemiller, Arrington, & Jepsen, 2005). Due to the white meat, the very favorable organoleptic characteristics and the omnivorous food habit, Jundiá presents an excellent candidate for extensive cultivation among the native species of South America (Oliveira et al., 2009). The production, management and commercialization of these animals are often carried out without reproductive control or genetic variability monitoring, which is disadvantageous not only for the fish industry, but also represents a biological risk for natural populations, since there may be a significant increase in endogamy resulting in genetic bottlenecks and consequent loss of variability of parental species in native populations.

The widely used tools nowadays in the maintenance of genetic variability are the molecular markers, which prove to be efficient in the definition of strategies in breeding programs. The microsatellite markers have been widely used in population studies, to estimate the effective size of the populations in question, and to obtain the variability of this population. It is also important for the identification of hybrids, identification of the key-population for the conservation of genetic resources and the construction of genetic maps (Chistiakov, Hellemans, & Volckaert, 2006). Thus, the use of methodologies, such as microsatellite molecular markers, becomes fundamental for the recognition and genetic characterization of an inventory. Therefore, the present study aimed to analyze the genetic variability of commercial stocks of Jundiá from the Amazon in the state of Mato Grosso.

Material and Methods

Samples

The samples were obtained from commercial stocks of Jundiá breeders from the Amazon (*Leiarius marmoratus*), belonging to the Bom Futuro group based in Cuiabá, Mato Grosso. The parents of the three stocks are sourced from capture in the Teles Pires River, located in the same state. Samples of caudal fin (0.5 cm^2) of each individual were collected from 143 individuals belonging to fish farms in the cities of Campo Verde (59 samples), Juína (60 samples) and Nova Mutum (24 samples). The samples were packed in tubes containing 70% alcohol.

DNA extraction and amplification

DNA was extracted using the NaCl extraction protocol described by Lopera-Barrero et al. (2008). DNA concentration was measured using a PICODROP® Spectrophotometer (Picodrop Limited, Hinxton, United Kingdom) and the samples were standardized for a final concentration of 10 ng μL^{-1}. DNA integrity was evaluated in 1% agarose gel, stained with SYBR Safe™ DNA Gel Stain (Invitrogen, Carlsbad CA, USA), with electrophoresis conducted on TBE 0, 5X (250 mM Tris-HC1, 30 mM boric acid and 41,5 mM EDTA) for 1 hour at 70 V. The gel was visualized in a transilluminator device with ultraviolet light, and the image was photographed using the Kodak EDAS program (Kodak 1D Image Analysis 3.5).

The amplification was performed for a final reaction volume of 15 µL, using 1X Tris-KCl buffer, 2.0 mM of $MgCl_2$, 0.8 µM of each primer (forward and reverse), 4 mM of each dNTP, a Platinum Taq DNA polymerase and 20 ng DNA unit. Initially, the DNA was denatured at 94ºC for four minutes, and then 30 cycles of 30 seconds of denaturation at 94ºC were performed. Afterwards, the DNA was subjected to 30 seconds of annealing (variable temperature for each primer) and one minute of extension at 72ºC; Finally, a final extension was made at 72ºC for 10 minutes. In total, 49 *loci* microsatellites were evaluated in cross-amplification: five described by Sanches and Galleti Jr. (2006) for *Brycon hilarii* (Bh5, Bh6, Bh8, Bh13, and Bh16); eight described by Batista & Alves-Gomes (2006) for *Brachyplatystoma rosseauxii* (BR37, BR38, BR44, BR47, BR49, BR51, and BR61); nine described by Lee and Kocher (1996) for *Oreochromis niloticus* (UNH 104, UNH140, UNH 160, UNH169, UNH162, UNH190, UNH231, UNH159, and UNH163); four primers described by Calcagnotto, Russello, and DeSalle (2001) for *Piaractus mesopotamicus* (Pme2, Pme4, Pme5, and Pme28); ten described by Ríos et. al. (2013) for *Rhamdia quelen* (RHQ2, Rhq7, Rhq8, Rhq13, Rhq15, Rhq16, Rhq20, Rhq26, Rhq28, and Rhq29); two for *Pseudoplatystoma punctifer* (PPU01 and Ppu02), described by Saulo-Machado et al. (2011); two primers described by Santos, Hrbek, and Farias (2009) for C*olossoma macropomum* (Cm1A8 and Cm1A11); five described by Farias, Hrbek, Brinkmann, Sampaio, and Meyer (2003) for *Arapaima gigas* (CTm2, CTm5, CTm7, CTm13, and CTm15) and, finally, four primers described by Barbosa, Corrêa, Galzerani, Galetti, and Hatanaka (2006) for *Prochilodus lineatus* (Par12, Par14, Par15, and Par21). The primers that successfully amplified in the species *Leiarius marmoratus*, followed their respective repetitive units and annealing temperatures (ºC), as shown in Table 1. The reactions were performed in Veriti® ThermoCycler (Applied Biosystems®, Austin, TX, USA).

The amplified samples were submitted to 10% polyacrylamide gel electrophoresis (acrylamide: bisacrylamide - 29:1) denatured (6 M of urea), and carried out in TBE 0 buffer, 5X with 180 V and 250 mA for 7 hours. Silver nitrate staining was used to visualize the microsatellite alleles. The gel was subjected to a fixation solution (10% ethanol and 0.5% acetic acid) for 20 minutes; then impregnated by 6 mM silver nitrate solution for 30 minutes; revealed in solution of 0,75 M of NaOH and 0.22% of formaldehyde-40% and photographed with Nikon CoolPix 5200 camera for further analysis. The size of the alleles was calculated by the program Kodak EDAS-290, using DNA ladder (Invitrogen) of 10, 50, and 100 pb.

Statistical analysis

The number of alleles per *locus*, the effective number of alleles per *locus*, Shannon index, allelic frequencies and genetic distance of Nei (1973) were calculated using the program GenAlex version 6.5 (Peakall & Smouse, 2012). The Arlequin 3.0 (Excoffier, Laval, & Schneider, 2005) program was used for the observed and expected heterozygosity, Hardy-Weinberg equilibrium, and genetic differentiation (*F*st) values. The endogamy coefficient (*F*is) and allelic richness (Ra) were calculated for each *locus*, by using the program Fstat 2.9.3 (Goudet, 2005). In order to calculate this coefficient, the definition of Wright (1978) was used, in which the values between 0.00 and 0.05; 0.051 to 0.15; 0.151 to 0.25; and > 0.25 indicate small, moderate, high, and high

genetic differentiation, respectively. AMOVA was obtained with the program Arlequin 3.0 (Excoffier et al., 2005), while the averages' test was performed by the statistical program R (R Core Team, 2011) using Tukey at 5% significance. Using the UPGMA analysis, a dendrogram was constructed based on the genetic distance of Nei (1978), by means of the MEGA program, version 6.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The factorial analysis of correspondence (AFC) was obtained by the program Genetix (Belkhir, Borsa, Chikhi, Raufaste, & Bonhomme, 2004), by applying the function "AFC 3D sur populations". The STRUCTURE v. 2.3.3 (Pritchard, Stephens, & Donnelly, 2000) was employed to identify the formation of groups (K) of genetically similar populations, following the mixed model of clusters with a length period of 10,000 and 100,000 repetitions and the number of clusters. The estimates of K (number of clusters) were obtained from simulations performed with K ranging from one to five (K = 1-5), replaying 20 races for each hypothetical K value according to Evanno, Regnaut, and Goudet (2005).

Ethics and legal aspects

The study complied with the Brazilian guidelines for the care and use of animals for scientific and educational purposes (CONCEA – CEUA No. 9710100918).

Results and Discussion

Of the 49 primers tested in transferability to *L. marmoratus*, a good amplification of nine primers was obtained. These primers reproduced a total of 18 alleles in the Campo Verde stock, 16 in the Juína stock and 15 alleles for Nova Mutum. For the three stocks, the BR47 was monomorphic (same allelic standard for all analyzed individuals), while the other *locus* showed polymorphism, presenting 2 to 3 alleles each. The size of the alleles ranged from 136 (BR47) to 273 bp (base pairs) (BR61), as shown in Table 1.

Table 1. Description of microsatellite primers used for the analysis of variability from *Leiraius marmoratus*.

Primers	GenBank	Sequences	Annealing	Size (bp)	Reference
BR38	KC117543	F' AGTTCCTTCTCGTTCCCCTTC R' ATCTCCCACTCTCTCTGGCTC	62ºC	104-146	Batista & Alves-Gomes (2006)
BR47	KC117543	F' TCAGTGTGTGTGTGACTGTTG R' GCTCCTCTTGTTTCACTTTC	59ºC	136-150	Batista & Alves-Gomes (2006)
BR51	KC117543	F' GTTACACATGGTCGCTGGTG R' GTTCATTCTCTTCGGCTTCG	60ºC	252-257	Batista & Alves-Gomes (2006)
BR61	KC117543	F'CTGTCGAAAACATGAGGCAG R' GACATCAGAGCGAAGCACAC	65ºC	268-273	Batista & Alves-Gomes (2006)
PPU01	HQ317844	F' CAGCATCAGCGGAAAAGTTG R' CAGTGGCGCATTCTGTAATC	58ºC	205-212	Saulo-Machado et al. (2011)
PPU02	HQ317845	F' CAGAACCAGATCCAACGTCA R'CTCCCTAGACTTCCCATTTCC	62ºC	215-238	Saulo-Machado et al. (2011)
BH8	DQ408244	F' CCATGGCTCAACACAGATAT R' TGTACGAATCCTGAAATGCT	56ºC	182-193	Sanches and Galetti (2006)
RHQ2	KC117543	F' CCTCTTTCTCCTTCCCGTTT R' GCACTTGTTCTGTCTGTCC	58ºC	215-235	Ríos et al. (2013)

The expected heterozygosity ranged from 0.033 (RHQ2) to 0,477 (BR61), while the observed heterozygosity showed values of 0.000 (RHQ2) to 0.250 (BH8). The *locus* BH8 stood out in most of the evaluated parameters, being the most polymorphic among all the *locus* used. A deviation was observed in the Hard-Weinberg equilibrium ($p > 0.05$) in all analyzed *locus*, except for the *locus* BR51 in the stocks Campo Verde and Juína, *locus* RHQ2 in the stock Nova Mutum, and PPU01 in Juína.

The variable *F*is, which refers to the coefficient of inbreeding, showed a remarkable variation of -0.009 (BR51) to 1.000 (RHQ2), with negative values verified only in the *locus* BR51. In relation to the averages in the three stocks, the Juína stock presented the highest index (0.562), showing to be more endogamic. The stocks Campo Verde and Nova Mutum presented values of 0.539 and 0.514, respectively (Table 2).

AMOVA (Table 3) indicated that the largest variation is within each stock for all combinations (53.36, 53.10, and 58.24% for Campo Verde x Juína, Campo Verde x Nova Mutum, and Juína x Nova Mutum, respectively). The fixation indices (F*st*) ranged from 0.0142 to 0.049 (Table 3), demonstrating a small genetic differentiation (0.00 to 0.05) among the populations, according to Wright's (1978) definition. The genetic distance values showed high similarity among the three populations, which corroborated with the F*st* values,

since it showed slight differentiation (Table 3); also by the dendrogram (Figure 3) constructed from the genetic distance, which shows Campo Verde and Nova Mutum grouped in a first nodule at a distance of 0.003, with the inclusion of the three stocks in the second nodule at a distance of 0.006, indicating that there is low differentiation.

Table 2. Measurements of genetic variation in eight microsatellite *loci* applied to three stocks of *Leiarius marmoratus*.

Stocks	*Locus*	Na	Ne	Ra	Ho	He	*F*is	Hw
Campo Verde	BR47	3	1.071	1.931	0.017	0.066	0.747	0.000*
	BR51	2	1.070	1.749	0.068	0.065	-0.027	0.788
	BR61	3	1.554	2.288	0.085	0.357	0.766	0.000*
	PPU01	2	1.295	1.998	0.024	0.228	0.898	0.000*
	PPU02	3	1.091	2.153	0.052	0.083	0.387	0.000*
	BH8	3	1.302	2.738	0.155	0.232	0.338	0.000*
	RHQ2	2	1.053	1.651	0.017	0.050	0.663	0.000*
	Mean	2.571	1.205	2.073	0.060	0.154	0.539	0.113
Juína	BR47	1	1.000	1.000	-	-	-	-
	BR51	2	1.034	1.488	0.033	0.033	-0.009	0.896
	BR61	3	1.684	2.958	0.102	0.406	0.753	0.000*
	PPU01	2	1.180	1.988	0.100	0.153	0.360	0.058
	PPU02	3	1.144	2.359	0.033	0.126	0.740	0.000*
	BH8	3	1.942	2.871	0.233	0.485	0.525	0.000*
	RHQ2	2	1.034	1.488	0.000	0.033	1.000	0.000*
	Mean	2.286	1.288	2.022	0.084	0.206	0.562	0.159
Nova Mutum	BR47	1	1.000	1.000	-	-	-	-
	BR51	2	1.280	2.000	0.083	0.219	0.632	0.002*
	BR61	3	1.910	3.000	0.125	0.477	0.747	0.000*
	PPU01	2	1.637	2.000	0.176	0.389	0.568	0.024*
	PPU02	2	1.280	2.000	0.083	0.219	0.632	0.002*
	BH8	3	1.405	2.919	0.250	0.288	0.153	0.000*
	RHQ2	2	1.229	1.999	0.125	0.187	0.349	0.106
	Mean	2.143	1.392	2.131	0.141	0.296	0.514	0.022

Na: number of alleles per *locus*; Ne: effective number of alleles per *locus*; Ra: allelic wealth; Ho: observed heterozygosity; He: expected heterozygosity; *F*is: coefficient of inbreeding; Hw: Hardy-Weinberg equilibrium (*p < 0.05).

Table 3. Analysis of variance for molecular data (AMOVA); distance and genetic identity of Nei in three stocks of *Leiarius marmoratus*.

		Sum of squares	Components of variance	Coefficient of variation	F_{ST}	Distance / Genetic identity of Nei
Campo Verde x Juína	Between stocks	3.740	0.0249	4.89	0.049*	0.006
	Within stocks	90.311	0.2872	56.36		
Total		23.500	0.1975	38.75		
Campo Verde x Nova Mutum	Between stocks	1.284	0.0073	1.42	0.0142	0.010
	Within stocks	63.469	0.2743	53.10		
Total		19.500	0.2349	45.48		
Juína x Nova Mutum	Between stocks	2.004	0.0154	2.54	0.0254	0.013
	Within stocks	77.508	0.3536	58.24		
Total		20.000	23.810	39.22		

*Significant p < 0.05 (1,023 permutations).

The factorial correspondence analysis (FCA) shows the distribution of genetic variability within a Cartesian plane (Figure 1), that is, it presents the distribution of genetic variability in spatial form. It is observed in the figure that there was no cluster formation (homogeneous and separate groups), but the overlap of several individuals of distinct populations, which indicates they are more genetically related, corroborating with the results obtained at AMOVA. These results are compatible with the dendrogram of genetic similarity (Figure 2), which, in general, there is no distance that shows a great genetic difference between stocks.

The graph of attribution of individuals (Figure 3) allowed the identification of individuals who are more likely to belong to a single genetic group. In addition, it is possible to observe that individuals represented by different colors have greater probability of having two or more genetic groupings defined by Bayesian analysis, which occurred in most individuals.

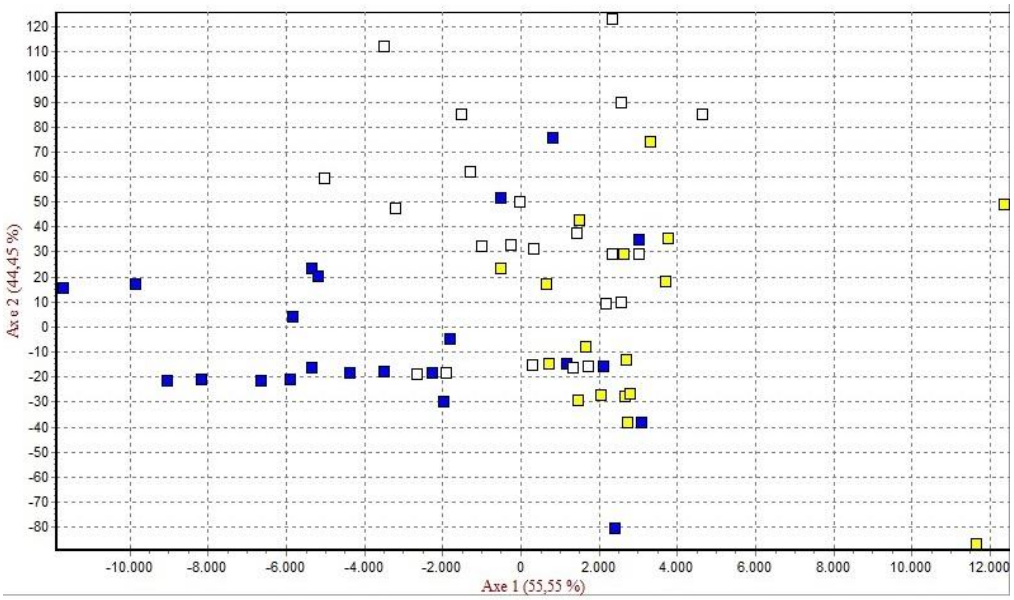


Figure 1. Factorial correspondence analysis (FCA) of three stocks of *Leiarius marmoratus*.

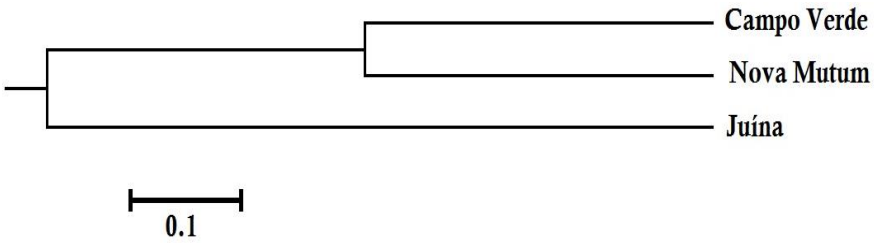
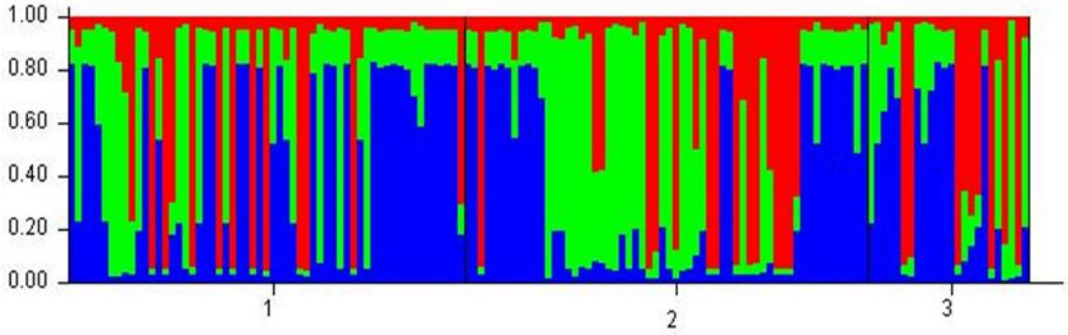


Figure 2. Dendrogram based on Nei genetic distance among individuals of *L. marmoratus*.



Individuals are represented as columns and black lines delineate stocks. Side numbers indicate the genetic proportion pertaining to each cluster. 1 = Campo Verde, 2 = Juína, and 3 = Nova Mutum.

Figure 3. Assignment of individuals through the grouping results for K = 3 of *L. marmoratus* obtained through the Structure program.

The highest value of heterozygosity observed in this study was 0.250 and occurred for the Nova Mutum stock. This value is similar to the one obtained (0.203) by Ribeiro et al. (2015) for *Piaractus mesopotamicus,* used in restocking programs, and it is inferior to the averages obtained with *Rhamdia quelen* (0.537; 0.500; 0.482) in the work conducted by Goes (2017), using the *locus* RHQ2. This means that the analysis of heterozygosity is essential for the evaluation of variability in stocks, and according to Moreira, Hilsdorf, Silva, and Souza (2007), the higher the index observed, the greater the differentiation among the individuals of the population in question. The population with the highest number of alleles was the Campo Verde stock, but the average of the observed heterozygosity for this stock was the lowest (0.060), which indicates that the majority of alleles presented in their genotype would be homozygous.

The results of the endogamy coefficient (*F*is) were significant in practically all *loci* in the three stocks, which implies a high level of consanguinity, with averages of 0.539, 0.562, and 0.514 in Campo Verde, Juína, and Nova Mutum, respectively.

The AMOVA aimed to identify whether the variation was greater among groups than within groups, and the results indicate that the variation was higher within the stocks than among them, reaching 58.24% in Juína vs. Nova Mutum. This value ratifies the AFC, which the overlapping of groups is clearly seen, while individuals from the same population are scattered, thus indicating the genetic proximity between the groups. This probably occurred due to the parents of the three stocks coming from the same river, assuming that they would have the same origin before the mating that originated the animals sampled in this study.

The domestication of wild species and their culture in captivity, where it was possible to use few specimens for the reproductive formation of the breeding stock, favored consanguineous mating due to the restricted use of same parents (Benzie, 2009). This is less likely in individuals in nature, where mating is done at random and Hardy-Weinberg's equilibrium tends to be respected, therefore generating a higher degree of polymorphism and decreased consanguinity. Moreover, it is of great importance to undertake a study of variability in the natural stocks of the Tele Pires river (Mato Grosso State, Brazil), from where the parents of the inventories analyzed in this study are originated, for the certification of the levels of heterozygosity, endogamy and balance of Hardy-Weinberg.

The Bayesian analysis indicated that most individuals have a "mixture" of genetic groups, as a consequence of crosses of individuals from the same site. The results corroborate with the UPGMA analysis presented in dendrogram.

Conclusion

This study identified that the populations of *Leiarius marmoratus* in captivity of the evaluated region are very close genetically, presenting a low variability and disfavoring the use of these animals to initiate a process of selection and improvement. Thus, it is essential to conduct a reproductive management program that aims to introduce new genetic material in stocks, in order to genetically enrich the animals destined to the stock of commercial breeders.

Acknowledgements

The authors are grateful to Peixegen Group for providing laboratorial support in this study. This work was conducted during a scholarship supported by the Program of Academic Excellence - PROEX at the State University of Maringá, which is financed by CAPES – Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Brazil Education Ministry.

References

Barbosa, A. C. D. R., Corrêa, 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. DOI: https://doi.org/10.1111/j.14718286.2006.01406.x

Batista, J. S. & Alves-Gomes, J. A. (2006). Phylogeography of *Brachyplatystoma rousseauxii*-(Siluriformes: Pimelodidae) in the Amazon Basin offers preliminar evidence for the first case of "homing" for an Amazonian migratory catfish. *Genetics and Molecular Research*, *5*, 723–740.

Belkhir, K., Borsa, P., Chikhi, I. L., Raufaste, N., & Bonhomme, F. (2004). GNETIX 4.05. *Logiciel sous windows tm pour la génétique des populations* (Laboratoire génome, populations, interactions, cnrs umr 5000). Retrieved on Aug. 30, 2017 from http://kimura.univ-montp2.fr/genetix/

Benzie, J. A. H. (2009). Use and exchange of genetic resources of penaeid shrimps for food and aquaculture. *Reviews in Aquaculture*, *1*(34), 232-250. DOI: https://doi.org/10.1111/j.1753-5131.2009.01018.x

Calcagnotto, D., Russello, M., & DeSalle, R. (2001). Isolation and characterization of microsatellite *loci* in Piaractus mesopotamicus and their applicability in other Serrasalminae fish. *Molecular Ecology Resources*, *1*(4), 245-247. DOI: https://doi.org/10.1046/j.1471-8278.2001.00091.x

Chistiakov, D. A., Hellemans, B., & Volckaert, F. A. (2006). Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. *Aquaculture*, *255*(1-4), 1-29. DOI: https://doi.org/10.1016/j.aquaculture.2005.11.031

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. DOI: https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, *1*, 47-50. DOI: https://doi.org/10.1177/117693430500100003

Farias, I. P., Hrbek, T., Brinkmann, H., Sampaio, I., & Meyer, A. (2003). Characterization and isolation of DNA microsatellite primers for *Arapaima gigas*, an economically important but severely over-exploited fish species of the Amazon basin. *Molecular Ecology Notes*, *3*(1), 128-130. DOI: https://doi.org/10.1046/j.1471-8286.2003.00375.x

Food and Agriculture Organization [FAO]. (2020). *The State of World Fisheries and Aquaculture (SOFIA)*. Rome, IT: FAO yearbook.

Goes, M. D., Goes, E. S. R., Ribeiro, R. P., Lopera-Barrero, N. M., Castro, P. L., Bignotto, T. S., & Bombardelli, R. A. (2017). Natural and artificial spawning strategies with fresh and cryopreserved semen in *Rhamdia quelen*: Reproductive parameters and genetic variability of offspring. *Theriogenology*, *88*, 254-263. DOI: https://doi.org/10.1016/j.theriogenology.2016.09.029

Goudet, J. (2005). *FSTAT*: *A Program to Estimate and Test Gene Diversities and Fixation Indices (version 2.9.3.2)*. Retrieved on Sep. 09, 2017 from http//www.unil.ch/izea/softwares/FSTat.html.

Layman, C. A., Winemiller, K. O., Arrington, D. A., & Jepsen, D. B. (2005). Body size and trophic position in a diverse tropical food web. *Ecology*, *86*(9), 2530-2535. DOI: https://doi.org/10.1890/04-1098

Lee, W. J., & Kocher, T. D. (1996). Microsatellite DNA markers for genetic mapping in Oreochromis niloticus. *Journal Fish Biololy*, *49*(1), 169-171. DOI: https://doi.org/10.1111/j.1095-8649.1996.tb00014.x

Lopera-Barrero, N. M., Povh, J. A., Ribeiro, R. P., Gomes, P. C., Jacometo, C. B., & Lopes, T. S. (2008). Comparison of DNA extraction protocols of fish fin and larvae samples: modified salt (NaCl) extraction. *Ciencia e Investigación Agraria*, 35(1), 77-86. DOI: http://dx.doi.org/10.4067/S0718-16202008000100008

Moreira, A. A., Hilsdorf, A. W. S., Silva, J. V., & Souza, V. R. (2007). Variabilidade genética de duas variedades de tilápia nilótica por meio de marcadores microssatélites. *Pesquisa Agropecuária Brasileira*, *42*(4), 521-526. DOI: https://doi.org/10.1590/S0100-204X2007000400010

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the Unidet State of America*, *70*(13), 3321-3323. DOI: https://doi.org/10.1073/pnas.70.12.3321

Oliveira, M. D. S., Luiz, D. D. B., Santos, V. R. V., Oliveira, E. H. S., & Martins, G. D. S. (2019). Aspectos de qualidade e segurança do tambaqui (*Clossoma macropomum*) e pintado da Amazônia (*Pseudoplatystoma reticulatum x Leiarius marmoratus*). R*evista Desafios*, *6*, 10-16. DOI: http://dx.doi.org/10.20873/uft.2359365220196Especialp10

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics*, *28*(19), 2537-2539. DOI: http://dx.doi.org/10.1093/bioinformatics/bts460

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multi*locus* genotype data. *Genetics*, *155*(2), 945-959. DOI: https://doi.org/10.1093/genetics/155.2.945

R Core Team (2011). *R: A language and environment for statistical computing*. Vienna, AU: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Ribeiro, R. P., Lopera-Barrero, N. M.; Santos, S. C. A.; Rodriguez-Rodriguez, M. D. P.; Oliveira, S. N.; Vargas, L. & Poveda-Parra, A. R. (2015). Genetic diversity of pacu for restocking programs in the Tietê and Grande rivers, Brazil. *Semina*: *Ciências Agrárias*, *36*(6). DOI: http://dx.doi.org/10.5433/1679-0359.2015v36n6p3807

Ríos, N., Bouza, C., Pardo, B. G., Guerra-Varela, J., Gutierrez, V., Martinez, P., & García, G. (2013). Pyrosequencing for microsatellite discovery and validation of markers for population analysis in the non-model Neotropical catfish *Rhamdia quelen*. *Molecular Ecology Resources*, *13*, 546-549. DOI: http://dx.doi.org/10.1111/1755-0998.12095

Sanches, A., & Galetti Jr., P. M. (2006). Microsatellites *loci* isolated in the freshwater fish *Brycon hilarii*. *Molecular Ecology Notes*, *6*(4), 1045-1046. DOI: https://doi.org/10.1111/j.1471-8286.2006.01427.x

Santos, M. D., Hrbek, T., & Farias, I. P. (2009). Microsatellite markers for the tambaqui (*Colossoma macropomum*, Serrasalmidae, Characiformes), an economically important keystone species of the Amazon River floodplainn. *Molecular Ecology Resource*, *9*(3), 874-876. DOI: https://doi.org/10.1111/j.1755-0998.2008.02331.x

Saulo-Machado, A. C., Formiga, K. M., Ortiz, M. F., Sousa, A. C. B., Alves-Gomes, J. A., & Batista, J. S. (2011). Polymorphic microsatellite DNA markers for the Amazonian catfish *Pseudoplatystoma punctifer* (Siluriformes: Pimelodidae). *Conservation Genetics Resources*, *3*(2), 307-310. DOI: https://doi.org/10.1007/s12686-010-9349-4

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725-2729. DOI: https://doi.org/10.1093/molbev/mst197

Wright, S. (1978). *Evolution and genetics of populations*. Chicago, US: University of Chicago Press.