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Genetic variability in *Pseudoplatystoma reticulatum* from a breeding program in Brazil[1]

Variabilidade genética de *Pseudoplatystoma reticulatum* de um programa de melhoramento genético no Brasil

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ABSTRACT - The aim of this study was to characterise genetic variability in five groups of *Pseudoplatystoma reticulatum*: Mato Grosso I (MT-I), Mato Grosso II (MT-II), Mato Grosso III (MT-III), Mato Grosso do Sul I (MS-I) and Mato Grosso do Sul II (MS-II), taken from a centre for genetic improvement. In order to determine the allelic frequency, observed heterozygosity (H_O), inbreeding coefficients (F_{IS}), Hardy-Weinberg equilibrium (H_W-*p*) and genetic differentiation, eight polymorphic microsatellite loci (*Pcor01*, *Pcor05*, *Pcor08*, *Pcor10*, *Ppu01*, *Ppu04*, *Ppu09* and *Ppu10*) were selected, in which a total of 62 alleles were observed in the individuals (n = 211). The MS-I population had the highest (*Pcor08* allele 83: 0.5000) and lowest (*Pcor10* allele 50: 0.1667) mean values for allele frequency. Higher (HO = 0.782; 0.782) and lower (F_{IS} = 0.034; 0.061) values were found in groups MS-I and MT-III respectively. The H_W-*p* showed that the five groups presented deviations for most of the loci under analysis. Finally, genetic differentiation pointed to the formation of two large genetic groups, one formed by the groups from Mato Grosso and the other by the groups from Mato Grosso do Sul. These measurements can help selection in a breeding program and maintain genetic variability.

Key words: Cachara. Inbreeding coefficient. Allelic Frequency. Population genetics. Microsatellites.

RESUMO - Objetivou-se caracterizar a variabilidade genética de cinco grupos de *Pseudoplatystoma reticulatum* Mato Grosso I (MT-I), Mato Grosso II (MT-II), Mato Grosso III (MT-III), Mato Grosso do Sul I (MS-I) e Mato Grosso do Sul II (MS-II) pertencentes a um núcleo de melhoramento genético. Para determinação das medidas de frequências alélicas, heterozigosidade observada (H_O), coeficientes de endogamia (F_{IS}), teste do equilíbrio de Hardy-Weinberg (H_W-*p*) e diferenciação genética foram selecionados oito *loci* polimórficos de microssatélites (*Pcor01*, *Pcor05*, *Pcor08*, *Pcor10*, *Ppu01*, *Ppu04*, *Ppu09*, *pu10*) nos quais observaram-se um total de 62 alelos nos indivíduos (n=211). A População MS-I apresentou o maior (*Pcor08* alelo 83:0,5000) e menor (*Pcor10* alelo 50: 0,1667) valor de frequência alélica média. Maiores (H_O= 0,782; 0,782) e menores (F_{IS}= 0,034; 0,061) foram verificados nos grupos MS-I e MT-III respectivamente e o H_W-*p* exibiu que os cinco grupos apresentaram desvios para a maioria dos *loci* analisados. Por último, diferenciação genética apontou a formação de dois grandes grupos genéticos, um conformado pelos grupos provenientes do Mato grosso e outro pelos grupos do Mato grosso do Sul. Essas medidas podem auxiliar na seleção do programa de melhoramento, permitindo a manutenção da variabilidade genética.

Palavras-chave: Cachara. Coeficiente de endogamia. Frequência alélica. Genética de populações. Microssatélites.

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INTRODUCTION

Aquaculture is the agricultural food chain with the greatest global presence among production sectors of animal origin, totalling 722,560 tons in Brazil in 2019. However, one area of research that requires further advance and consolidation in order to meet domestic demand is the area of genetic improvement of aquaculture production.

As a result, in 2008, the Aquabrasil Program (A Technological Base for the Sustainable Development of Aquaculture in Brazil), coordinated by Embrapa in partnership with the State University of Maringá, began unprecedented genetic improvement of the native species *Pseudplatystoma reticulatum*, in order to promote technological advancement and boost aquaculture production, and consolidate and foster the regional and export markets (ALBUQUERQUE; PEREIRA; RODRIGUEZ-RODRIGUEZ, 2019).

Pseudoplatystoma reticulatum, known locally as *surubim cachara*, is a catfish native to the Paraná-Paraguay basin and the central Amazon region (CREPALDI *et al*., 2006). Its feeding preference is carnivorous, and it comes at the top of the food chain in various aquatic habitats of South America's most important river basins (REID, 1983).

The aquaculture breeding project included wild pairs of *P. reticulatum* collected over four years (2008 to 2011) from breeding stations on three properties in the state of Mato Grosso and two in the state of Mato Grosso do Sul, with the aim of forming approximately 72 families through artificially induced spawning. The families from the five properties were housed on another property linked to the Aquabrasil research project, located in the municipality of Sorriso, in Mato Grosso, in the district of Primavera do Norte.

A microchip (Passive Integrated Transponder - PIT tag) was implanted in each individual, facilitating identification when obtaining zootechnical information of commercial interest, as well as making possible random rather than directed mating.

Conserving the genetic variability of the groups that make up the cachara breeding program is of paramount importance to avoid high levels of inbreeding during the formation of succeeding generations and to allow genetic gains as selections are made. Microsatellite molecular markers are a commonly used tool for monitoring the genetic variability of populations (GUICHOUX *et al*., 2011), as they allow the values of such parameters as the inbreeding coefficient, heterozygosity, allelic frequency and deviations in the Hardy-Weinberg equilibrium to be estimated, among other analyses which help to increase efficiency in defining strategies for the selection of new crosses in breeding programs.

The aim of the present study is to estimate the genetic variability of five groups of *P. reticulatum* from the satellite centre of the aquaculture breeding program in Brazil.

MATERIAL AND METHODS

A total of 211 caudal-fin samples, each approximately 0.5 cm^2, were collected and stored in absolute alcohol for further analysis at the Laboratory for Molecular Biology of the State University of Maringá. To identify the origin of each sample, five groups were formed: Mato Grosso I (MT-I), Mato Grosso II (MT-II), Mato Grosso III (MT-III), Mato Grosso do Sul I (MS-I) and Mato Grosso do Sul II (MS-II).

The methodology described by Lopera-Barrero *et al*. (2008) was used to extract the DNA, where to each fin sample were added 550 µL of lysis buffer (50 mM Tris-HCl, 50 mM EDTA, 100 mM NaCl, 1% SDS) and 10 µL proteinase K (200 µg mL^{-1}). The samples were then incubated in a double boiler at 50 °C for 12 h. The DNA was precipitated with 600 µl of 5M NaCl solution and centrifuged for 10 min at 12,000 rpm. The supernatant with DNA was transferred to new microtubes, precipitated with 700 µL absolute ethyl alcohol and incubated for 1 h at -20 °C. Following centrifugation, the DNA was washed with 700 µL 70% ethyl alcohol and resuspended in TE buffer - 10 mM Tris pH 8.0 and 1 mM EDTA (80 µL for the fin), sequentially treated with 7 µL RNase (30 µg mL^{-1}) in a double boiler at 37 °C for 1 h and stored in a freezer at -20 °C.

DNA quantification was carried using the Shimadzu UV 1601-E.U. spectrophotometer (wavelength 260 nm), and the samples diluted to a concentration of 10 ng μL^{-1}. DNA integrity was verified by horizontal electrophoresis using 1% agarose gel at 70 V for 120 min.

Image capture employed the L-PIX (LOCCUS Biotechnology) photographic system. Different DNA annealing temperatures were tested for the primers (*Loci Ppu01*, *Ppu02*, *Ppu04*, *Ppu09*, *Ppu10*, *Ppu15*, *Pcor01*, *Pcor05*, *Pcor08*, *Pcor10*) described by Revaldaves *et al*. (2005) and Saulo-Machado *et al*. (2011) for *P. corruscans* (*Pcor*) and *P. punctifer* (*Ppu*) respectively. The DNA was amplified to a total reaction volume of 11 µL using 8.1 µL Platinum® PCR SuperMix (Life Technologies, Invitrogen, Carlsbad, CA, USA), 0.9 µL of a solution containing the forward and reverse primers, and 2.0 µL of target DNA.

PCR was carried out under the following conditions: 2 min denaturation at 95 °C, 30 cycles of 2 min at 95 °C, 30 s at specific annealing temperatures for each primer (Table 1), 30 s at 72 °C and a final extension at 72 °C for 10 min. The DNA samples were then submersed in 10% polycrylamide gel (acrimylamide: bisacrylamide - 29:1) and 6 M urea, and placed in a 1X TBE (90 mM Tris-Borate, 2 mM EDTA) buffer solution at 180 V (250 mA) for between 5 and 12 h, depending on the fragment size of the primer alleles.

The microsatellite alleles were visualised using the methodology adapted by Bassam, Caetano-Anollés and Gresshoff (1991). The gel was submersed in three solutions: fixative solution (10% ethanol, 0.5% acetic acid) for 15 min, impregnation solution (6mM silver nitrate) for 30 min, and finally developer solution 0.75 M NaOh 0.22% and formaldehyde 40%. The gel images were captured with the SONY DSC HX200 digital camera. Allele size was calculated using the 100 bp and 50 bp molecular-weight marker (DNA ladder - Invitrogen®).

The number of alleles, observed heterozygosity (Ho), expected heterozygosity (He), Shannon index and Hardy-Weinberg equilibrium (H_W-*p*) were calculated using the PopGene 1.31 software (YEH; BOYLE; XIYAN, 1999).

The allelic frequency and inbreeding coefficient (F_{IS}) for each locus was calculated using the GenePop 1.2 software (RAYMOND; ROUSSET, 1995). Linkage disequilibrium, genetic differentiation and analysis of molecular variance (AMOVA) were determined with the Harlequin 3.1 software (EXCOFFIER; SCHNEIDER, 2005) using the Markov chain method. To demonstrate the presence of null alleles, the Micro-checker 2.2.3 software was used (VAN OOSTERTER *et al*., 2004).

RESULTS AND DISCUSSION

Of the 11 primers tested in the five groups under study, eight (*Pcor01, Pcor05, Pcor08, Pcor10, Ppu01, Ppu04, Ppu09* and *Ppu10*) were polymorphic, and three did not amplify (*Ppu02*, *Ppu13* and *Ppu15*) or were monomorphic. This might be due to genetic differentiation between the species; for even when primers are from related species (heterologous primers), as in the case of *P. corruscans* and *P. punctifer* used in the analysis of *P. reticulatum*, and show some degree of conservation, differences can be found in the molecular weights of the alleles or in the non-amplification of some loci (ABREU *et al*., 2009).

Comparing various studies, such as that of Saulo-Machado *et al*. (2011) for *P. punctifer* in the Amazon basin, different sizes are found for the alleles of the *Ppu01*, *Ppu04*, *Ppu09* and *Ppu10* primers. In addition, the *Ppu02*, *Ppu13* and *Ppu15* primers presented as polymorphic, with from 7 to 18 alleles, and did not amplify for the five groups of *P. reticulatum*. However, Abreu *et al*. (2009), studying two groups of wild *P. reticulatum*, and using *P. corruscans* heterologous primers, found a different number of alleles in the *Pcor01*, *Pcor05*, *Pcor08* and *Pcor10* primers to those seen in this research.

Low values for allelic frequency were found in the *Pcor01* loci for the MT-II population, with 0.0926 and 0.0741 respectively for sizes 110 and 160 bp. In the MT-I population, a low allelic frequency was seen in the *Pcor05* primers for 85 bp; *Pcor08* for 87, 97, 109 and 118 bp; *Ppu04* for 155, 175, 195, 215 and 235 bp; *Ppu09* for 170, 220, 250 and 260 bp; and *Ppu10* for 120 and 150 bp (Table 1).

It can be seen that the MT-II population had a low allelic frequency only for the *Ppu09* and *Ppu10* loci, seen in the 110 bp fragment at a minimum frequency of 0.02.

There was an alteration in all the loci in relation to the most frequent allele in the groups, where more than two altered alleles per loci can be seen (Table 1). The allelic frequency in the MS-I population ranged from 0.0179 to 0.500 respectively for *Ppu09*/266 and *Pcor08* in fragment 83. In the MT-III population, a low frequency was seen in *Pcor01*/110/150/85 and 90, *Pcor05*/110; *Pcor08*/85 /87/90/92/106/109/118; *Pcor10*/62/70; *Ppu01*/95/155; *Ppu04*/120/155/160/205/215/255; *Pu09*/170/260; and *Ppu10*/94/110/120/140 and 160.

Analysing the data shown in Table 1, null alleles were seen in the *Pcor05* loci in all groups, *Pcor10* in MT-II, MT-III and MS-II, Ppu04 in groups MT-III and MS-II, and *Ppu10* in MT-III only. The occurrence of null alleles is mainly due to the lack of a sequence for primer annealing, which in turn may be due to point mutations (DUFRESNE *et al*., 2014) and, as reported by Kordicheva *et al*. (2010), the presence of null alleles resulting from genotyping errors is a very common phenomenon when analysing microsatellite molecular markers.

Only null alleles were seen in the *Pcor05* loci from a total of 16 samples from the MT-I population under analysis. The observed heterozygosity ranged from 0.267 for *Pcor08* to 1.00 for the *Ppu04* loci, which had a total mean value of 0.684 (Table 2).

A significant difference was found in the Hardy-Weinberg equilibrium for this population only in *Pcor05* and *Pcor10*, where the respective F_{IS} values of 0.6601 and 0.354 revealed a heterozygote deficiency. Positive results for F_{IS} may indicate a possible heterozygote deficiency, which in turn is attributed to the breeding program.

Table 1 - Allelic frequency and annealing temperatures (ºC) in cachara (*Pseudoplatystoma reticulatum*) from the aquaculture breeding project

Loci	Ta (°C)	Group	Frequent alleles	Allelic frequency	Exclusive alleles
Pcor01	55	MT-I	160	0.1875*	-
		MT-II	125	0.3519*	-
		MS-I	125	0.4583*	-
		MT-III	140	0.4355*	-
		MS-II	125	0.3929*	-
Pcor05	56	MT-I	100	0.3333*	-
		MT-II	100	0.4524*	-
		MS-I	95	0.3333*	-
		MT-III	100	0.4052*	-
		MS-II	95	0.2891*	-
Pcor08	56	MT-I	94	0.2667*	-
		MT-II	83	0.2857*	-
		MS-I	83	0.5000*	-
		MT-III	94	0.193*	-
		MS-II	83	0.175*	-
Pcor10	58	MT-I	50	0.4286*	-
		MT-II	50	0.3500*	-
		MS-I	50/55	0.1667/0.4444	-
		MT-III	50	0.3667*	62
		MS-II	60	0.3452*	40
Ppu01	58	MT-I	105	0.4062*	-
		MT-II	120	0.4167*	-
		MS-I	105	0.3621*	-
		MT-III	120	0.3729*	155
		MS-II	105	0.2958*	-
Ppu04	50	MT-I	180	0.2857*	-
		MT-II	175	0.2333*	-
		MS-I	165	0.2857*	295
		MT-III	180	0.275*	120-201
		MS-II	165	0.1887*	153
Ppu09	59	MT-I	210/230	0.1923*/0.1923*	250
		MT-II	230	0.25*	-
		MS-I	220	0.25*	266
		MT-III	210	0.2300*	-
		MS-II	230	0.3017*	-
Ppu10	60	MT-I	100	0.300*	-
		MT-II	100	0.2600*	-
		MS-I	130/140	0.1852*/0.1852*	-
		MT-III	100	0.2951*	-
		MS-II	100	0.2574*	-

*Most-frequent values; MT-I: Mato Grosso I; MT-II: Mato Grosso II; MT-III: Mato Grosso III; MS-I: Mato Grosso do Sul I; MS-II: Mato Grosso do Sul II

Table 2 - Summarised data for the five groups of Pseudoplatystoma reticulatum from the aquaculture breeding project

Group		Loci								
		Pcor01	*Pcor05*	*Pcor08*	*Pcor10*	*Ppu01*	*Ppu04*	*Ppu09*	*Ppu10*	Meana
MT-I (n = 16)	NA	5	6	9	4	4	8	9	6	6.375
	NE	4.163	4.639	6.250	2.970	3.436	5.765	7.192	4.639	4.882
	AN	no	yes	no	no	no	no	no	no	-
	Ho	0.813	0.267	0.667	0.429	0.875	1	0.692	0.733	0.684
	He	0.784	0.812	0.869	0.714	0.732	0.890	0.895	0.812	0.813
	F_{IS}	-0.069	0.660	0.206	0.354	-0.234	-0.21	0.196	0.065	0.121
	$H_{W\text{-}p}$	0.051^{ns}	0.000**	0.000**	0.0195*	0.401^{ns}	0.952^{ns}	0.271^{ns}	0.391^{ns}	-
MT-II (n = 27)	NA	5	5	10	4	4	6	8	7	6.125
	NE	3.898	3.291	5.378	3.509	3.398	5.556	5.661	5.411	4.513
	AN	no	yes	no	yes	no	no	no	no	-
	Ho	0.704	0.429	0.714	0.3	0.708	0.667	0.864	0.760	0.643
	He	0.758	0.713	0.834	0.753	0.721	0.849	0.843	0.832	0.788
	F_{IS}	0.054	0.384	0.123	0.580	-0.004	0.187	-0.049	0.068	0.168
	$H_{W\text{-}p}$	0.885^{ns}	0.000**	0.310^{ns}	0.000**	0.408^{ns}	0.298^{ns}	0.411^{ns}	0.202^{ns}	-
MS-I (n = 30)	NA	4	7	8	4	4	10	8	8	6.625
	NE	3.032	4.861	3.419	3.177	3.649	6.438	5.723	6.813	4.639
	AN	no	yes	no	no	no	no	no	no	-
	Ho	0.708	0.542	0.857	0.444	0.862	0.762	0.960	0.741	0.735
	He	0.684	0.811	0.725	0.726	0.739	0.865	0.840	0.869	0.782
	F_{IS}	-0.057	0.318	-0.212	0.351	-0.188	0.098	-0.169	0.132	0.034
	$H_{W\text{-}p}$	0.006**	0.000**	0.111^{ns}	0.227^{ns}	0.495^{ns}	0.126^{ns}	0.282^{ns}	0.004**	-
MT-III (n = 65)	NA	5	6	10	5	5	10	8	9	7.25
	NE	3.360	3.907	7.944	3.352	3.375	5.203	6.596	5.851	4.948
	AN	no	yes	no	yes	no	yes	no	yes	-
	Ho	0.871	0.586	0.860	0.500	0.780	0.675	0.860	0.689	0.728
	He	0.708	0.751	0.882	0.714	0.710	0.818	0.857	0.836	0.784
	F_{IS}	-0.240	0.212	0.017	0.287	-0.108	0.164	-0.014	0.170	0.061
	$H_{W\text{-}p}$	0.076^{ns}	0.030*	0.002**	0.049*	0.767^{ns}	0.000**	0.092^{ns}	0.000**	-
MS-II (n = 73)	NA	5	7	11	5	4	11	7	8	7.25
	NE	3.115	5.029	7.775	3.781	3.810	7.879	4.962	5.853	5.276
	AN	no	yes	no	yes	no	yes	no	no	-
	Ho	0.700	0.438	0.817	0.333	0.817	0.755	0.845	0.750	0.682
	He	0.684	0.808	0.879	0.744	0.743	0.881	0.805	0.835	0.797
	F_{IS}	-0.031	0.454	0.063	0.547	-0.108	0.136	-0.058	0.096	0.137
	$H_{W\text{-}p}$	0.629^{ns}	0.000**	0.073^{ns}	0.000**	0.267^{ns}	0.001**	0.007**	0.002**	-

ns: Not significant; *: Significant (P<0.05); **: Significant (P<0.01); N: Number of individuals analysed; NA: Number of alleles; NE: Effective number of alleles; NA: Null Alleles; Ho: Observed heterozygosity; He: Expected heterozygosity; F_{IS}: Inbreeding coefficient; H_{W}-*p*: Hardy-Weinberg equilibrium; MT-I: Mato Grosso I; MT-II: Mato Grosso II; MT-III: Mato Grosso III; MS-I: Mato Grosso do Sul I; MS-II: Mato Grosso do Sul II

As the families that make up the groups of *Pseudoplatystoma reticulatum* from the breeding program originated in approximately 72 families taken from five distinct regions of the Midwest, the number of families in this generation may have interfered with the parameters of genetic variability. However, it should be noted that even in natural wild stock the genetic variability of *Pseudoplatystoma reticulatum* can be affected by various attributes, such as geographical barriers or different population structures, as mentioned by Abreu *et al*. (2009) who, studying two groups of *Pseudoplatystoma reticulatum* in the Paraguay basin, supported this hypothesis.

Considering the values obtained for genetic variability in the study in question, the method of selection used in this project, based on hierarchical mating, pedigree, animal heritability, selection and crossbreeding on the basis of the genetic values of the families in the program (RIBEIRO; LEGAT , 2008) was sufficient for the maintenance and continuity of the aquaculture breeding program.

However, inbreeding in captive stock is generally to be expected, and can be controlled in advance if animals are initially correctly selected as breeding stock (LOPERA-BARRERO *et al*., 2013) so that the problem does not damage breeding programs from the outset (HILSDORF; ORFÃO, 2011). If not, it can favour the bottleneck effect and the effective size of the population, as also pointed out by Lind *et al*. (2012), Lopera-Barrero *et al*. (2010) and Ribeiro *et al*. (2016).

In animals from the MT-II population, a mean value of 0.643 was seen for observed heterozygosity, with a significant difference (P<0.01) in the Hardy-Weinberg equilibrium in the *Pcor05* and *Pcor10* loci; a heterozygote deficiency was only seen in these loci for a mean F_{IS} value of 0.168 for all loci. Analysing the population from the MS-I property, a total of 30 samples were found with a mean number of alleles of 6.625, ranging from four to ten alleles (Table 3). No null alleles were found at most of the loci under analysis. There was a significant difference in the Hardy-Weinberg equilibrium in the *Pcor01*, *Pcor05* and *Ppu10* loci only, showing an excess of heterozygotes in Pcor01, with a value of -0.057.

The mean value for observed heterozygotes was 0.735, with a minimum variation of 0.444 and a maximum of 0.964 respectively for *Pcor10* and *Ppu09*.

In the MT-III group, for a total of 65 samples, significant differences (P<0.05) were seen in the *Pcor05, Pcor08, Pcor10, Ppu04* and *Ppu10* loci, with a heterozygote deficiency revealed by positive values for F_{IS} that ranged from a minimum of 0.017 to a maximum of 0.287.

For a mean total of 7.25 alleles analysed in the MS-II population, an allelic deficiency with respective values of 0.4539, 0.5468, 0.1356 and 0.0955 was seen in the *Pcor05, Pcor10, Ppu04* and *Ppu10* loci. The deviation in the Hardy-Weinberg equilibrium (H_W-*p*) in the five groups of the above experiment can be attributed to various factors, among which heterozygote deficiency is important, a result of the interaction of several factors, such as the high number of alleles per locus, the presence of null alleles, inbreeding and the Wahlund effect (POVH *et al.*, 2010; RODRIGUEZ-RODRIGUEZ *et al*., 2013).

From the results shown in Table 3, in which ten groups are defined and analysed against each other through an analysis of molecular variance (AMOVA), it can be seen that for the group comprising the five subgroups, there was greater variability within the groups than between the groups, with values for percent variance of 98.53% (F_{ST} = 0.0147), which yielded 1.47% at a significance level of P<0.000001.

Genetic differentiation in fish is commonly seen, but with low values for F_{ST}, due possibly to the large abundance of such stock in natural environments that generally have high cosmopolitanism and *a priori* no barriers to gene flow, as explained by several authors (MELO *et al*., 2013; SANCHES *et al*., 2012).

For each combination of the groups under analysis, greater genetic variability was also seen within the groups than between groups, where the percent variance in values was greater than 96.02% (Table 3).

Based on Nei's calculation (1973), the results obtained for identity and genetic distance infer that there is strong evidence of the existence of groups, within which the groups are genetically more related. As such, it is possible to envisage a subdivision of the animals in groups MT-I and MT-III, a subdivision of group MT-II only, and one subdivision including groups MS-I and MS-II. Genetic identity for the groups in the MT-I/MT-III subdivision was 0.9768, with a small genetic distance of 0.0235. At the same time, the genetic groups of the MS-I/MS-II subdivision have a genetic identity of 0.9408 and a moderate genetic distance of 0.0610. The greatest divergence for all groups, of 0.1936, was seen between the animals from the MT-III and MS-I population.

These results can also be corroborated from the values for genetic distance (Table 3) and confirmed by the group dendogram (Figure 1). A minimum genetic distance of 0.0235 was found in group II (MT-I x MS-I) and a maximum of 0.1936 in group VIII (MS-I x MT-III).

Table 3 - Analysis of molecular variance (AMOVA), genetic distance (GD) and F_{ST} of individuals from the five groups of the aquaculture breeding project

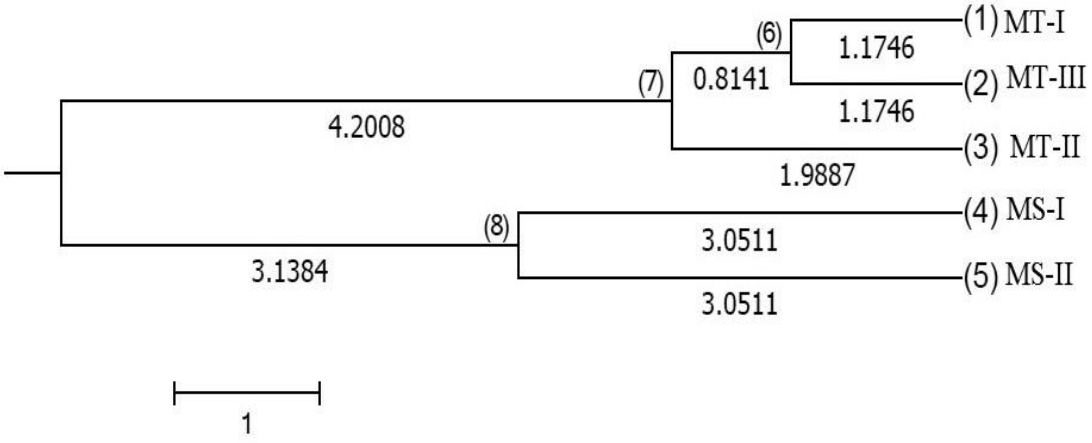
Source of variation	Degree of freedom	Sum of squares	Variance component	Percent variance	F_{ST}	GD
All groups						
Between groups	4	24.883	0.04727	1.46992*		
Within groups	417	1056.739	3.16858	98.53008	0.0147	
Total	421	1081.623	3.21585	100		
Group I: MT-I x MT-II						
Between groups	1	0.642	0.00642	1.65		
Within groups	84	32.23	0.38369	98.35	0.01646	0.0437
Total	85	32.872	0.39012	100		
Group II: MT-I x MS-I						
Between groups	1	0.339	-0.00032	-0.09		
Within groups	90	31.6994	0.35215	100.09	-0.0009	0.1315
Total	91	32.033	0.35183	100		
Group III: MT-I x MT-III						
Between groups	1	0.299	-0.00071	-0.21		
Within groups	160	53.695	0.33559	100.21	-0.0021	0.0235
Total	161	53.994	0.33488	100		
Group IV: MT-I x MS-II						
Between groups	1	1.24	0.01064	1.54		
Within groups	176	120.007	0.68186	98.46	0.01536	0.0819
Total	177	121.247	0.6925	100		
Group V: MT-II x MS-I						
Between groups	1	7.211	0.09037	2.79352*		
Within groups	112	267.773	3.14456	97.20648	0.02794	0.1575
Total	113	274.984	3.23493	100		
Group VI: MT-II x MT-III						
Between groups	1	0.833	0.00648	1.88*		
Within groups	182	61.613	0.33853	98.12	0.01879	0.0359
Total	183	62.446	0.34501	100		
Group VII: MT-II x MS-II						
Between groups	1	0.659	-0.00008	-0.01		
Within groups	198	131.636	0.66483	100.01	-0.0001	0.0866
Total	199	132.295	0.66475	100		
Group VIII: MS-I x MT-III						
Between groups	1	11.604	0.12989	3.97451*		
Within groups	188	465.505	3.13819	96.02549	0.03975	0.1936
Total	189	477.505	3.26808	100		
Group IX: MS-I x MS-II						
Between groups	1	0.743	0.00462	1.31		
Within groups	204	71.282	0.34942	98.69	0.01306	0.061
Total	205	72.024	0.35404	100		

Continuation Table 3

Group X: MT-III x MS-II						
Between groups	1	1.031	0.00519	1.6*		
Within groups	274	87.114	0.31793	98.4	0.01605	0.0917
Total	275	88.145	0.32312	100		

F_{ST}: fixation index; AMOVA: Analysis of Molecular Variance; *: Significant (P<0.05); MT-I: Mato Grosso I; MT-II: Mato Grosso II; MT-III: Mato Grosso III; MS-I: Mato Grosso do Sul I; MS-II: Mato Grosso do Sul II

Figure 1 - Dendogram of the five groups under analysis from the aquaculture breeding project



It is extremely important to characterise and maintain genetic variability between the groups in the breeding program. Animals that make up the parental generation of these five groups were captured from different sub-basins of the River Paraguay, and a genetic analysis was first carried out to ensure the necessary genetic variability and divergence to initiate the *Pseudoplatystoma reticulatum* breeding program. The results from the differentiation and genetic distance of the five groups prove that they are subdivided into two main groups, which in turn consist of two and three subgroups (Figure 1).

These results can support and indicate strategies for animal selection in the genetic improvement program. In order to conserve the number of families over the generations and maintain genetic variability, thereby avoiding inbreeding, these data can aid in carrying out crosses between families of the same subgroup (MT-I, II and II) and (MS-I and II), or even in crosses between the two subgroups, as described in Figure 1.

Animals inserted in the same genetic group should be considered when forming a restricted genetic base and in selection analysis, so that future crosses between related animals can maintain genetic variability and generate an increase in the characteristics of zootechnical interest for the next generations of genetically superior animals, as highlighted in several studies (BRIÑEZ; CARABALLO; SALAZAR, 2011; MACHADO-SCHIAFFINO *et al.*, 2007; PONZONI *et al.*, 2010, 2011).

With the data obtained, selections can be made to preserve the genetic variability of *Pseudoplatystoma reticulatum* from the families of the genetic groups in the breeding program, with the possible strategy of mating between individuals of the two subgroups or between the groups under analysis. Finally, it is considered that microsatellite molecular markers can play a role in decision-making when monitoring the genetic variability of groups in breeding programs, helping to maintain low levels of inbreeding, and is considered a viable tool of molecular biology for aquaculture.

CONCLUSIONS

1. The five groups under study are distinct, and form two genetic groups composed of three and two subgroups respectively;

2. Based on the data obtained, selections can be made with a view to conserving the genetic variability of *Pseudoplatystoma reticulatum* in the genetic improvement program of aquaculture species.

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REFERENCES

ABREU, M. M. *et al*. Genetic variability of two populations of *Pseudoplatystoma reticulatum* from the Upper Paraguai River Basin. **Genetic and Molecular Biology**, v. 32, n. 4, p. 868-873, 2009.

ALBUQUERQUE, D. M.; PEREIRA, R. P.; RODRIGUEZ-RODRIGUEZ, M. P. Avanços no programa de melhoramento genético do cachara no Brasil. **Ciência Animal**, v. 29, n. 1, p. 109-120, 2019.

BASSAM, B. J.; CAETANO-ANOLLÉS, G.; GRESSHOFF, P. M. Fast and sensitive silver staining of DNA in polyacrylamide gels. **Analytical Biochemistry**, v. 196, p. 80-83, 1991.

BRIÑEZ, R. B.; CARABALLO, O. X.; SALAZAR, V. M. Genetic diversity of six populations of red hybrid tilapia, using microsatellites genetic markers. **Revista MVZ Córdoba**, v. 16, p. 2491-2498, 2011.

CREPALDI, D. V. *et al*. O surubim na aquacultura do Brasil. **Revista Brasileira de Reprodução Animal**, v. 30, n. 3/4, p. 150-158, 2006.

DUFRESNE, F. *et al.* Recent progress and challenges in population genetics of poyploid organism: an overview of current state-of-the-art molecular and statistical tools. **Molecular Ecology**, v. 23, p. 40-69, 2014.

EXCOFFIER, L. G.; SCHNEIDER, S. Arlequin version 3.0: an integrated software package for population genetics data analysis. **Evolutionary Bioinformatics Online**, v. 1, p. 47-50, 2005.

GUICHOUX, E. *et al.* Current trends in microsatellite genotyping. **Molecular Ecology Resources**, v. 11, p. 291-611, 2011.

HILSDORF, A. W. S.; ORFÃO, L. H. Aspectos gerais do melhoramento genético em peixes no Brasil. **Revista Brasileira de Zootecnia**, v. 40, p. 317-324, 2011.

KORDICHEVA, S. Y. *et al.* A search for null alleles at the microsatellite locus of chum salmon (*Oncorhynchus keta* Walbaum). **Russian Journal of Genetics**, v. 46, n. 8, p. 1019-1022, 2010.

LIND, C. E. *et al.* Selective breeding in fish and conservation of genetic resources for aquaculture. **Reproduction in Domestic Animals**, v. 47, n. 4, p. 255-263, 2012.

LOPERA-BARRERO, N. M. *et al*. Comparación de protocolos de extracción de ADN com muestras de aleta y larva de peces: extracción modificada con sal (NaCl). **Ciencia e Investigación Agraria**, v. 35, n. 1, p. 15-24, 2008.

LOPERA-BARRERO, N. M. *et al*. Diversidad genética de *Piaractus mesopotamicus* utilizado em programas de repoblación. **Archivos de Zootecnia**, v. 59, p. 51-62, 2010.

LOPERA-BARRERO, N. M. *et al.* Genetic diversity of *Prochilodus lineatus* stocks using in the stocking program of Tietê River, Brazil. **Revista MVZ Córdoba**, v. 18, n. 13, p. 3759-3766, 2013.

MACHADO-SCHIAFFINO, G.; DOPICO, E.; GACIA-VAZQUEZ, E. Genetic variation losses in Atlantic salmon stocks created for supportive breeding. **Aquaculture**, v. 624, p. 59-65, 2007.

MELO, B. F. *et al.* The roles of marginal lagoons in the maintenance of genetic diversity in the Brazilian migratory fishes *Prochilodus argentus* and *P. costatus*. **Neotropical Ichthyology**, v. 11, n. 3, p. 625-636, 2013.

NEI, M. Analysis of gene diversity in subdivided populations. **Proceedings of the National Academy of Science**, v. 70, n. 12, p. 3321-3323, 1973.

PONZONI, R. W. *et al*. Genetic improvement of Nile tilapia (*Oreochromis niloticus*) with special reference to the work conducted by the WorldFish Center with the GIFT strain. **Aquaculture**, v. 3, n. 1, p. 27-41, 2011.

PONZONI, R. W. *et al*. Inbreeding and effective population size in the Malaysian nucleus of the GIFT strain of Nile tilapia (*Oreochromis niloticus*). **Aquaculture**, v. 302, p. 42-48, 2010.

POVH, J. A. *et al*. Microsatellite analysis of the parental contribution of *Piaractus mesopotamicus* to the production of offspring in the semi-natural system of reproduction. **Brazilian Archives of Biology and Technology**, v. 53, n. 2, p. 389-396, 2010.

RAYMOND, M.; ROUSSET, F. Genepop version 1.2: population genetics software for exact tests and ecumenicism. **Journal of Heredity**, v. 86, p. 248-249, 1995.

REID, S. La biologia de los bagres rayados *Pseudoplatystoma fasciatum* y *P.tigrinus* en la cuenca del Rio Apure, Venezuela. **Revista Unellez de Ciencia y Tecnología**, v. 1, p. 13-41, 1983.

REVALDAVES, E. *et al*. Isolation and characterization of microsatellite loi in *Pseudoplatystoma corruscans* (Siluriformes, Pimelodidae) and cross-species amplification. **Molecular Ecology Notes**, v. 5, n. 3, p. 463-465, 2005.

RIBEIRO, R. P. *et al*. Caracterização genética de estoques de Tambaqui do Estado de Rondônia (Brasil): implicações na produção e na conservação. **Semina**, v. 37, n. 4, p. 2375-2385, 2016.

RIBEIRO, R. P.; LEGAT, A. P. **Delineamento de programas de melhoramento genético de espécies aquícolas no Brasil**.

Teresina: Embrapa Meio-Norte, 2008. 25 p. (Embrapa Meio-Norte. Documentos, 184).

RODRIGUEZ-RODRIGUEZ, M. P. *et al*. Caracterização genética de gerações de tilápia Gift por meio de marcadores moleculares microssatélites. **Pesquisa Agropecuária Brasileira**, v. 48, n. 10, p. 1385-1393, 2013.

SANCHES, A. *et al*. Genetic population structure of two migratory freshwater fish species (*Brycon orthotaenia* and *Prochilodus argentus*) from the São Francisco River in Brazil and it's significance for conservation. **Latin American Journal of Aquatic Research**, v. 40, n. 1, p. 177-186, 2012.

SAULO-MACHADO, A. C. *et al*. Polymorphic microsatellite DNA markers for the Amazonian catfish *Pseudoplatystoma punctifer* (Siluriformes: Pimelodidae). **Conservation Genetic Resources**, v. 3, p. 307-310, 2011.

VAN OOSTERHOUT, C. *et al*. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. **Molecular Ecology Notes**, v. 4, p. 535-538, 2004.

YEH, F. C.; BOYLE, T. Y. Z.; XIYAN, J. M. **PopGene version 131**: Microsoft Window-based freeware for population genetic analysis. Alberta: University of Alberta: Center for International Forestry Research, 1999. 29 p.