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Genetic variability of tambaqui broodstocks in the Brazilian state of Pará

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ABSTRACT - The present study evaluated the genetic variability of the broodstocks of tambaqui hatcheries in the Brazilian state of Pará. Samples were obtained from the caudal fin of 110 broodstocks from one hatchery in each of four municipalities (Santarém, Peixe-Boi, Breu-Branco, and Ulianópolis), representing all the individuals of each hatchery stock. The samples were genotyped using the multiplex system microsatellite markers. Data were used to calculate observed and expected heterozygosity, number of alleles per locus, and allelic richness. The effective population size and inbreeding coefficient were also calculated. The genetic differentiation between populations was evaluated by using F_{ST}, and population structuring by the most likely number of genetically homogenous populations and Unweighted Pair Group Method (UPGMA); the arithmetic means were based on Nei's. The indices indicated a loss of genetic variability in the broodstocks from Ulianópolis, Peixe-Boi, and Breu-Branco in comparison with wild tambaqui populations, although this was not apparent in the Santarém stock. A moderate level of genetic differentiation was found among the tambaqui broodstocks based on the F_{ST} estimates, which were reinforced by the structuring found in the Bayesian analysis and UPGMA. This reflects the domestication process, given that no such structuring is found in natural tambaqui populations. This moderate genetic differentiation associated with the loss of genetic variability found in the four tambaqui broodstocks from the state of Pará provides important insights for the development of future programs of genetic improvement, as well as the conservation of the genetic diversity of these stocks.

Keywords: *Colossoma macropomum*, fish, genetic diversity, hatcheries

Introduction

Tambaqui (*Colossoma macropomum*) is the most intensively-farmed native fish species in Brazil, with a total production of 136,990 tons in 2016 (IBGE, 2016). Tambaqui farming is considered an important alternative for the exploitation of the wild stocks of this species, and, given the economic importance of the species on both regional and national levels, it has been included in the Brazilian Program of Genetic Improvement (Lopes et al., 2009).

Understanding the genetic variability of captive stocks is fundamental to any program of genetic improvement (Moreira et al., 2007) or species conservation. The loss of genetic variability in farmed

stocks has negative implications for a range of economically important parameters, such as survival and growth, as well as the loss of adaptive potential. Molecular markers such as microsatellites have proven to be efficient indicators for the measurement of genetic variation both within and among farmed fish stocks (Mojekwu and Anumudu, 2013).

Systematic data on the standard breeding practices of a hatchery are required to guarantee the long-term viability of cultivated stocks by maintaining diversity and minimizing inbreeding (An et al., 2013). In this context, the present study determined the genetic variability of tambaqui broodstocks from four hatcheries in the Brazilian state of Pará.

Material and Methods

The original project of the present study was submitted for evaluation to the Ethics Committee and received approval through case no. 031/2013 (CEUA) - 23084.008077/2013-73.

Samples were obtained from the caudal fins of 110 tambaqui broodstocks from one hatchery in each of four municipalities, representing all the individuals of each hatchery stock, that is, Santarém (2°27'41.1" S, 54°41'47.7" W), n = 30 samples; Peixe-Boi (01°09'30.01" S, 47°18'24.39" W), n = 20; Breu-Branco (04°04'04" S, 47°29'41" W), n = 21; and Ulianópolis (03°44'31" S, 47°29'41" W), n = 39, all located in the state of Pará, in northern Brazil. The commercial broodstocks of Peixe-Boi, Breu-Branco, and Ulianopólis were established from captive stocks, whereas that from Santarém was formed by native animals captured in the lower Amazon River. The broodstocks were between six and eight years old, with a mean weight of 6 kg, and have been used for reproduction since their sexual maturation at three years of age. None of the individuals had a registered pedigree or any record of their animal performance.

The DNA was extracted using a Genomic DNA Isolation kit (Norgen Biotek Corporation). The amount of DNA in the samples was determined using a NanoDrop™ ND-1000 spectrophotometer (Thermo Scientific), and once measured, the samples were standardized to a concentration of 5 ng/uL. The samples were genotyped using the multiplex system for 10 microsatellite markers (Cmacrμ01-HM579948, Cmacrμ03-HM579950, Cmacrμ04-HM579951, Cmacrμ05-HM579952, Cmacrμ07 - HM579954, Cmacrμ08 - HM579955, Cmacrμ09 - HM579956, Cmacrμ10 - HM579957, Cmacrμ12 - HM579959, and Cmacrμ13 - HM579960) developed by Hamoy and Santos (2012).

The PCR were standardized to a final volume of 8 μL containing 6.5 μL 2X QIAGEN® Multiplex PCR Master mix (Qiagen), 0.5 μL of each primer, and 1.0 μL of DNA. The samples were amplified in a Veriti thermocycler (Applied Biosystems), based on the following protocol: initial denaturation at 95 °C for 15 min, followed by 10 cycles of 94 °C for 30 s, 60 °C for 90 s, and 72 °C for 60 s, with a final extension at 72 °C for 60 min.

The PCR products (1 μL) were mixed with 8.5 μL of Hi-Di deionized formamide (Applied Biosystems) and 0.5 μL of 500 LIZ Gene Scan (Applied Biosystems) for viewing in an Applied Biosystems 3130 automatic capillary sequencer. The individuals were genotyped in the Gene Mapper 3.7 program (Applied Biosystems).

Micro-Checker 2.2.3 was used to determine possible genotyping errors and the presence of null alleles, and the Polymorphic Information Content (PIC) was calculated in Cervus 3.0, using Botstein et al. (1980) classification system, in which values of less than 0.25 indicate low polymorphism; those of 0.25-0.5, a moderate level of polymorphism; and those higher than 0.5, a highly polymorphic locus.

Observed (H_O) and expected (H_E) heterozygosity and their possible deviations from Hardy-Weinberg equilibrium (HWE) were calculated in the Arlequin 3.5.1.3 program, followed by the determination of the P-values for the Bonferroni correction (Rice, 1989). The number of alleles per locus (N_A) and allelic richness (A_R) were all estimated using Fstat version 2.9.3.2. The effective population size (Ne) was estimated based on linkage equilibrium approach of Waples and Do (2008), run in Ne Estimator 2.01,

with a minimum allelic frequency of 0.02, and a 95% parametric confidence interval. The inbreeding coefficient was estimated as F = 1/2Ne.

The genetic differentiation between populations was evaluated using F_{ST}, run in Arlequin 3.5.1.3. To infer the most likely number of genetically homogenous populations (K), the database was analyzed using the Structure 2.2 program. This Bayesian analysis was based on 10^6 simulations with K values ranging from 1 to 4, and a burn-in of 20,000 simulations. The most likely value of K was determined by the ΔK method described by Evanno et al. (2005), which was run in Structure Harvester 0.6.94. An Unweighted Pair Group Method (UPGMA) tree was obtained from the arithmetic means based on genetic distance of Nei (1978), run in GDA 1.1.

Results

The results of the Micro-Checker analysis indicated that there were no null alleles or stutter bands in the dataset. The PIC was more than 0.5 for all markers, that is, extremely informative.

A total de 105 alleles were recorded for the 10 loci analyzed, based on the samples obtained from the 110 tambaqui broodstocks. The Santarém broodstock had the highest mean N_A (8.4), followed by Peixe-Boi (5.3), Ulianópolis (5.0), and Breu Branco (5.0). The same pattern was recorded for the mean A_R: Santarém (7.5), Peixe-Boi (5.3), Breu-Branco (4.9), and Ulianópolis (4.6) (Table 1).

Higher mean H_O values were registered in Santarém, Peixe-Boi, and Breu Branco (0.7), and lower in Ulianópolis (0.6). Mean H_E was higher in Santarém (0.8) and Peixe-Boi (0.7), and lower in Ulianópolis and Breu Branco (0.6). Most of the loci were in HWE, with two markers (Cmacrμ05 and Cmacrμ12) being out of equilibrium due to a deficiency of heterozygotes (Table 1).

The smallest Ne and highest inbreeding coefficients were recorded in Ulianópolis (Ne = 14.3 and F = 0.0350) and Breu Branco (Ne = 11.2 and F = 0.0446). The highest mean Ne (117.4) and lowest inbreeding coefficient (F = 0.0043) were recorded in Santarém, followed by Peixe-Boi (Ne = 24 and F = 0.0208) (Table 2).

The F_{ST} values indicated a differentiation of 0.07 between the broodstocks of Santarém and Peixe-Boi, 0.09 for Santarém vs. Ulianópolis, 0.10 for Peixe-Boi vs. Ulianópolis, 0.11 for Santarém vs. Breu-Branco and Breu-Branco vs. Urianópolis, and 0.12 for Peixe-Boi vs. Breu-Branco. The Bayesian analysis indicated the existence of two clusters (K = 2) in the database analyzed, one formed by the broodstocks from Santarém and Peixe-Boi, and the other by the broodstocks from Breu-Branco and Ulianópolis, a pattern corroborated by the UPGMA analysis (Figure 1).

Table 1 - Genetic diversity index of tambaqui broodstocks from the Brazilian state of Pará

Loci	Santarém (N = 30)				Peixe-Boi (N = 20)				Breu Branco (N = 21)				Ulianópolis (N = 39)			
	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E
*Cmacrμ*01	5	4.6	0.6	0.6	4	4	0.4	0.5	2	2	0.4	0.4	3	3	0.6	0.5
*Cmacrμ*03	6	5.7	0.7	0.7	3	3	0.6	0.6	6	6	0.9	0.7	6	5.3	0.5	0.5
*Cmacrμ*04	10	8.9	0.9	0.9	6	6	0.8	0.8	5	5	0.6	0.6	5	4.8	0.8	0.7
*Cmacrμ*05	7	6.8	0.7	0.8	4	4	0.7	0.7	4	4	0.9	0.6	4	4	0.6*	0.7*
*Cmacrμ*07	12	10.3	0.8	0.8	9	9	0.8	0.8	9	8.7	0.9	0.7	8	6.6	0.6	0.7
*Cmacrμ*08	9	7.7	0.5	0.8	5	5	0.8	0.7	3	3	0.7	0.5	4	3.9	0.5	0.6
*Cmacrμ*09	9	7.8	0.8	0.8	4	4	0.7	0.6	7	6.9	0.9	0.7	6	5.2	0.7	0.7
*Cmacrμ*10	7	6.3	0.8	0.8	5	5	0.8	0.7	4	4	0.8	0.7	4	3.9	0.5	0.5
*Cmacrμ*12	12	10.5	0.6*	0.9*	7	7	0.3*	0.7*	5	5	0.5	0.7	7	6.5	0.5*	0.7*
*Cmacrμ*13	7	6.6	0.8	0.7	6	6	0.7	0.7	5	4.9	0.6	0.5	3	2.5	0.4	0.5
Mean	8.4	7.5	0.7	0.8	5.3	5.3	0.7	0.7	5	4.9	0.7	0.6	5	4.6	0.6	0.6

N_A - number of alleles per locus; A_R - allelic richness; H_O - observed heterozygosity; H_E - expected heterozygosity.
* Marker out of the Hardy-Weinberg equilibrium, after Bonferroni correction (adjusted P-value <0.005).

Table 2 - Sample size (N), effective population size (Ne), confidence interval (CI), and inbreeding coefficient (F) for the four *Colossoma macropomum* hatcheries analyzed in the present study

Population	N	Ne	95% Cl	$F = 1/2N_e$
Santarém	30	117.4	(56.1 - 2292.4)	0.0043
Peixe-Boi	20	24	(14.1 - 53.5)	0.0208
Breu Branco	21	11.2	(7.3 - 17.6)	0.0446
Ulianópolis	39	14.3	(10.5 - 19.6)	0.0350

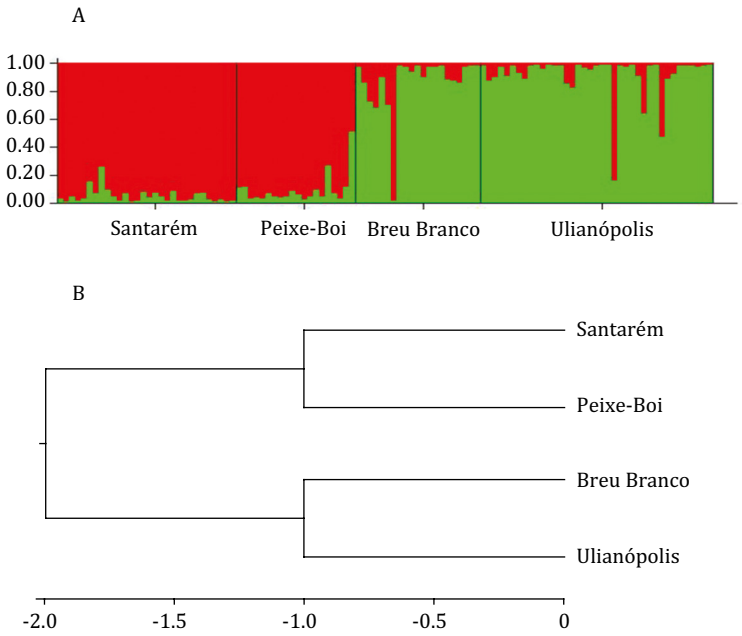

Figure 1 - Standard population structure (A) in tambaqui broodstocks from four hatcheries, supported by STRUCTURE, indicating the existence of two groups (K = 2) based on 10 microsatellite (B) patterns corroborated by Unweighted Pair Group Method (UPGMA), and arithmetic means based on Nei's.

Discussion

A loss of genetic variability was detected in the Ulianópolis, Peixe-Boi, and Breu-Branco broodstocks in comparison with those of the wild tambaqui populations from the Amazon basin described by Hamoy et al. (2011), based on the analysis of 20 individuals from the lower Amazon, and by Fazzi-Gomes et al. (2017), who analyzed 247 individuals from Manaus and Santarém, based on the set of microsatellite markers analyzed in the present study. As these broodstocks were formed from captive individuals, it seems likely that this loss of genetic variability reflected the low variability of the stocks from which these broodstocks were derived, reflecting the loss of the natural genetic variation of the population through the domestication process.

The stock from Santarém analyzed in the present study presented relatively high indices of genetic variability, as recorded by Aguiar et al. (2018) for a second broodstock in the same region, which according to the authors, indicates that these broodstocks were established, and possibly also restocked, with wild-caught individuals. This is an important factor that should be considered for the establishment and maintenance of farm broodstocks.

The loss of variability is emphasized most clearly by the A_R values, given that A_R is not biased by sample size (Spencer et al., 2000), when the values recorded in the present study are compared with those found in a wild population by Hamoy et al. (2011), who recorded an A_R value of 9, while

Fazzi-Gomes et al. (2017) registered A_R of 8.6, indicating higher levels of genetic variability in the wild populations.

Gonçalves et al. (2018), Aguiar et al. (2018), and Santos et al. (2016) also found evidence of the loss of genetic variability in tambaqui broodstocks from a number of different regions of Brazil. The authors attributed these low values to a process of genetic drift, resulting from the small effective size of the breeding populations found in the tambaqui farms, a practice adopted to minimize production costs, and the lack of any genetic management.

Effective population size is an important parameter for the evaluation of farmed fish stocks, because it determines the potential for genetic drift in a population which, in turn, influences the rate of loss of genetic diversity, rate of fixation of deleterious alleles, and potential for the maintenance of beneficial alleles through selective processes (Berthier et al., 2002). The effective numbers recorded in Ulianópolis, Breu Branco, and Peixe-Boi were lower than expected, i.e., 50-100, as observed by Aguiar et al. (2018), a value that would require improvement, given the risks associated with low Ne.

The inbreeding coefficient was within the limits recommended by Tave (1999), that is, below 0.05, for the avoidance of inbreeding depression, which was probably due to the fact that the broodstocks had been formed only a short time prior to the study. Except for Santarém, none of the broodstocks appears to have levels of genetic variability adequate for the avoidance of inbreeding depression over the long term.

The tambaqui broodstocks analyzed here presented a moderate degree of genetic differentiation, based on the F_{ST} values, which were all higher than 0.05 (see Hartl and Clark, 2010). This moderate level of differentiation is supported by the structuring recorded in the Bayesian analysis (K = 2) and UPGMA, which reflects the domestication process, given that structuring is not found in the natural tambaqui populations, as confirmed by Fazzi-Gomes et al. (2017), using the same microsatellite markers as those used in the present study.

Conclusions

This moderate genetic differentiation associated with the loss of genetic variability found in the four tambaqui broodstocks from the Brazilian state of Pará provide important insights for future programs of genetic improvement, as well as the conservation of the genetic diversity of these stocks. These findings indicate that an increase in gene flow would be advisable, in particular through the integration of wild-caught specimens in the breeding stocks of the tambaqui farms analyzed in the present study. In addition to being a highly effective tool for the evaluation of the genetic diversity of broodstocks, molecular microsatellite markers may also contribute to the genetic management of stocks, helping to avoid the loss of genetic variability through the identification of less closely related breeders, in genetic terms. This would ensure the maximum possible genetic variability of the broodstocks, while also minimizing the levels of endogamy.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: L.A. Ferreira, P.F. Fazzi-Gomes and I. Hamoy. Data curation: L.A. Ferreira and P.F. Fazzi-Gomes. Funding acquisition: A.K. Ribeiro-dos-Santos, S. Santos and I. Hamoy. Investigation: L.A. Ferreira and P.F. Fazzi-Gomes. Methodology: L.A. Ferreira, P.F. Fazzi-Gomes, S. Guerreiro and M.D.N. Rodrigues. Project administration: P.F. Fazzi-Gomes, S. Santos and I. Hamoy. Resources: M.D. N. Rodrigues, A.K. Ribeiro-dos-Santos, S. Santos and I. Hamoy. Supervision: A.K. Ribeiro-dos-Santos, S. Santos and

I. Hamoy. Writing-original draft: L.A. Ferreira and P.F. Fazzi-Gomes. Writing-review & editing: L.A. Ferreira, P.F. Fazzi-Gomes, M.D.N. Rodrigues, S. Santos and I. Hamoy.

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