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Genetic characterization of selected Nile tilapia in Santa Catarina

Caracterização genética de tilápia-do-nilo selecionadas em Santa Catarina

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Highlights:
The study allowed the genotypic characterization of the tilapia stock used in Santa Catarina.
There is high heterozygosity within the tilapia brook stock.
There is moderate genetic differentiation between tilapia brook stocks.
All markers evaluated were polymorphic for this stock and will be used for future evaluations.

Abstract

Different Nile tilapia stocks belonging to the fish breeding program of the Epagri (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina) were characterized by microsatellite markers. A total of nine stocks (S1 to S9) were evaluated, and for each stock the caudal fin of 30 individuals were sampled. A total of 75 alleles were found at the 11 microsatellite *loci* used (UNH104, UNH108, UNH160, UNH208, UNH222, UNH848, UNH879, UNH898, UNH952, UNH998). Among the *loci* used, only UHN160 showed significance for null alleles in stocks S1, S2, S3 and S5. The average number of alleles per *loci* was 6.8, while the average number of alleles per tilapia stock was 4.4. Five unique alleles were identified between the stock S1 and S5. The observed heterozygosity values (Ho) exceeded the expected heterozygosity (He), resulting in a negative inbreeding coefficient (F_{IS} = -0.092). F_{ST} for the total population was 0.109, demonstrating moderate genetic differentiation between the stocks. According to the Euclidean distance, three groups were formed as follows: I - S6, S7 and S9; II - S2, S3 and S4; and III - S1, S5 and S8. However, the existence of two groups can be observed from the PCoA representation: I - S6, S7, S8 and S9; and II - S1, S2, S3, S4 and S5. The formation of these two genetic groups is consistent with the genealogy of stocks. The formation of group III (S1, S5 and S8) in the dendrogram can be explained by the higher average observed heterozygosity values of these stocks. Bayesian analysis revealed the formation of 16 groups with an F_{ST} value of 0.2107. This result reinforces the existence of variability existing in the Epagri breeding program, from which it is possible to form heterotic groups to enable the direction of potential crosses to obtain genetic gain. The study enabled genotypic characterization of the tilapia brood stock used in the Epagri breeding program, determining the genetic distance between the stocks, which will enable more accurate selection of individuals for mating for the next generation. It was possible to verify that there is high heterozygosity within the stocks, and moderate genetic differentiation between the stocks. Furthermore, all evaluated markers were polymorphic for this brood stock and will be used to characterize the next generations.
Key words: *Oreochromis niloticus*. Genetic variability. Microsatellite markers.

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Resumo

Diferentes plantéis de tilápia-do-nilo pertencentes ao programa de melhoramento genético de peixes da Epagri (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina) foram caracterizados por meio de marcadores microssatélites. Ao total foram avaliados nove plantéis (S1 a S9), e para cada foram amostrados nadadeira caudal de 30 indivíduos. O total de 75 alelos foram encontrados nos 11 *loci* microssatélites utilizados (UNH104, UNH108, UNH160, UNH208, UNH222, UNH848, UNH868, UNH879, UNH898, UNH952, UNH998). Entre os *loci* utilizados, apenas o UHN160 apresentou significância para alelos nulos nos estoques S1, S2, S3 e S5.A média do número de alelos por *loci* foi 6,8, enquanto a média do número de alelos por plantel de reprodutores foi 4,4. Foram encontrados cinco alelos exclusivos entre os plantéis S1 e S5. Os valores de heterozigosidade observada (Ho) foi maior do que a esperada (He), resultando em um coeficiente de endogamia (F_{IS}) médio negativo (-0,092). O F_{ST} encontrado para a população total foi de 0,109, evidenciando moderada diferenciação genética entre os plantéis. De acordo com a distância euclidiana, três grupos foram formados da seguinte forma: I - S6, S7 e S9; II - S2, S3 e S4; e III - S1, S5 e S8. Porém, a partir da representação do PCoA, observa-se a existência de dois grupos: I - S6, S7, S8 e S9; e II - S1, S2, S3, S4 e S5. A formação desses dois grupos genéticos é consistente com a genealogia dos plantéis de reprodutores. A formação do grupo III (S1, S5 e S8) no dendrograma pode ser explicada pelos maiores valores médios de heterozigosidade observados desses plantéis. A análise bayesiana mostrou a formação de 16 grupos com um valor de Fst de 0,2107. Esse resultado reforça a existência de variabilidade existente no programa de melhoramento Epagri, a partir do qual é possível formar grupos heteróticos para permitir cruzamentos potenciais para obter ganho genético. O estudo permitiu a caracterização genotípica dos plantéis de reprodutores de tilápia utilizado no programa de melhoramento Epagri, determinando a distância genética entre eles, o que permitirá a seleção mais precisa dos indivíduos para os acasalamentos da próxima geração. Foi possível verificar que há alta heterozigosidade entre os plantéis de reprodutores e moderada diferenciação genética entre eles. Além disso, todos os marcadores avaliados foram polimórficos para este estoque de matrizes e serão utilizados para caracterizar as próximas gerações.

Palavras-chave: *Oreochromis niloticus*. Variabilidade genética. Marcadores microssatélites.

Introduction

Nile tilapia, *Oreochromis niloticus* Linnaeus 1758 (Osteichthyes: Cichlidae), stands out as the species with the greatest potential for continental aquaculture, given its fast growth, easy adaptation to farm conditions and meat quality (Webster & Lim, 2006). In recent years, its cultivation in Brazil has increased by 14.2% per year, with higher protein production than other animals such as cattle (5.1%), pigs (2.9%) and broilers (4.1%) (Kubitza, 2015). In 2017, Brazilian production of farmed fish was around 692 thousand tons, and tilapia represented 51.7% of this weight (Associação Brasileira da Piscicultura [PeixeBR], 2018).

In Santa Catarina, the production of freshwater fish was 44,500 tons in 2017, and tilapia accounted for 74% of this weight (PeixeBR, 2018). The tilapia strain most produced in this state is GIFT, short for *Genetically Improved Farmed Tilapia* (Barroso et al., 2016). This was a strain selected for growth, reproduction, weight gain and fillet yield. However, its breeding was carried out in countries with a tropical climate, selecting animals more adapted to that condition (Bentsen et al., 2017).

In 2005, the Nile tilapia GIFT strain was introduced in Brazil by the Maringá State University (UEM) with the help of the Special Secretariat of Aquaculture and Fisheries, and later it was introduced by Epagri (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina) in Santa Catarina. Aiming at the selection of tilapia with higher weight gain, more adapted to the climatic conditions of Santa Catarina and the distribution of this genetic material to the producers of fingerlings

in the state, who are mostly family farmers, Epagri started an individual selection program for tilapia (GIFT strain) in 2011 (Halfen, Nicoletti, Appel, & Tcacenco, 2012).

Individual selection can lead to a rapid decrease of genetic variability through generations. Low genetic variability may reduce selection response in the breeding program, in addition to declining zootechnical performance (Rodriguez-Rodriguez et al., 2013). Therefore, knowledge of the genetic characteristics of the breeding stock is fundamental, making it important to evaluate polymorphism among the stock that will be used for mating in the breeding program (Gjedrem, 2010; Moraes, Saldanha, Rezende, & Sousa, 2017).

Studies of genetic variability of tilapia have been carried out mainly through microsatellite markers (Romana-Eguia, Ikeda, Basiao, & Taniguchi, 2004; Hassanien & Gilbey, 2005; Moreira, Hilsdorf, Silva, & Souza, 2007; Briñez, Caraballo, & Salazar, 2011; Petersen et al., 2012; Rodriguez-Rodriguez et al., 2013). This is a popular and versatile technique with many applications in population genetics, being a powerful tool that is capable of detecting genetic variation between individuals, species or populations (Joshi, Ram, & Lohani, 2017).

Petersen et al. (2012) evaluated the genetic variability of three tilapia varieties produced in Santa Catarina and reported a lower rate of inbreeding in GIFT tilapia. According to the authors, these results reflect the history of controlled mating in this strain, since the other strains had already been in the state for longer without this control. The results of the study by Petersen et al. (2012) reinforce the importance of periodic evaluation of the genetic variability of the brood stock for directing mating in the breeding program.

Therefore, this study aimed to carry out genotypic characterization and to determine the genetic diversity within and between the different brood stock in the breeding program of Nile tilapia conducted by Epagri.

Materials and Methods

GIFT tilapia from Epagri's breeding program of UMGEP (Unidade de Melhoramento genético de peixes da Epagri) were used in the study. All procedures were conducted in accordance with the Animal Maintenance Ethics Committee standards and procedures and approved by CEUA - No. 224/2017.

In December 2015, a total of 270 males and 450 females were selected from nine different stocks of tilapia from the second generation of the GIFT-Epagri program. From each stock of 1500 cultivated tilapia, 30 males and 50 females with the highest final weight were selected.

For each of the nine stocks (S1 to S9), 30 individuals were sampled and tagged with a passive integrated transponder (PIT). A caudal fin sample of approximately 2.0 cm^2 was collected from each animal; these were preserved in 70% ethanol at -20°C for DNA extraction.

DNA extraction was performed using the modified protocol of Aljanabi and Martinez (1997) which uses high concentrations of NaCl. Modifications included a one hour incubation in lysis buffer and the precipitation of DNA with isopropanol. DNA quality and quantity were measured in a biophotometer (Eppendorf, Germany). After quantification, the samples were standardized to 20 ng DNA per µL.

The 11 SSR *loci* were amplified using specific primers (Table 1). These markers were synthesized by adding the 5′- M13 tail at the forward primer of each marker to enable the use of fluorescently labeled universal primers according to Schuelke (2000). Four different fluorophores were used for the primer labeling (6-FAM, VIC, NED and PET). The reaction mixture contained 40 ng of DNA, 1× PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of reverse primer, 0.4 µM of forward primer with M13 tail, 0.4 µM of the fluorescently labeled universal M13 primer and 1.0 U of *Taq* DNA polymerase (Invitrogen, Carlsbad CA, USA), in a final volume of 25 µL.

The reactions were carried out in a Veriti thermocycler (Applied Biosystems, Carlsbad CA, USA) with the following program: (i) an initial denaturation step for 5 min at 95ºC, (ii) 30 cycles of 45 s at 95ºC, 45 s of annealing at the temperature corresponding to the locus-specific primer, and 1 min at 72ºC, (iii) 8 cycles of 45 s at 95ºC, 45 s at 53ºC, and 1 min at 72ºC and (iv) a final extension step for 30 min at 72ºC.

Table 1
SSR markers, and their respective primer sequences and annealing temperature, used in the genetic characterization of selected Nile tilapia in Santa Catarina

Loci	**Annealing temperature** (ºC)	**Primer sequence (5′-3′)**	**Genbank**
UNH 104	56	F- GCAGTTATTTGTGGTCACTA R- GGTATATGTCTAACTGAAATCC	G12257
UNH 108	56	F- GGGATCAGCTGTTAAGTTT R- TGAGTTGATTATTAATTTCTGA	G12261
UNH 160	56	F - CCATTGGCTCTTACATC R- GATAGCATTTCTGTAGTTATGG	G12312
UNH 208	56	F - CTTCTTGGCCTACAATTT R - CAGATGGGTGATAGCAA	G12359
UNH 222	56	F-CTCTAGCACACGTGCAT R-TAACAGGTGGGAACTCA	G12373
UNH 848	54	F - TCCCCCGTAATAAATTAAACCA R - GCCTGTGAATAACAATGTATTTCCT	G68186
UNH 868	56	F - TCCTTGTTCAGACCTTGTGG R - AGCCAGGCTGAAAGGAAATA	G68199
UNH 879	56	F - GCATAAGGTGACTGGCTGGT R - ACAAAGGGGTCCTGCAATTT	G68206
UNH898	56	F - GATGTCCCCACAAGGTATGAA G - TAATCCACTCACCCCGTTTC	G68215
UNH 952	56	F - CAGACTGATGGCACAGAGGA R - TCTGCAATAGTGGCCATGAA	G68249
UNH 998	58	F - TCAATTGGTTTTACAGGAACACA R - GCTGAGGTCAGCTTACATGTCT	G68277

Amplified fragment pattern analysis was performed in an AB 3130 automatic gene analyzer (Applied Biosystems, Carlsbad CA, USA) with 36 cm capillary and POP7 polymer. The band amplification data were annotated in number of base pairs (bp), according to the genotyping provided by the GeneMapper 4.0 program (Applied Biosystems, Carlsbad CA, USA).

The presence of null alleles was verified using the software Micro-Checker 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). The frequency of null alleles at each locus was estimated as in Brookfield (1996), using estimator number 1:

$$\hat{r} = (He - Ho)/(1 + He)$$

The software GeneAlEx 6.5 (Peakall & Smouse, 2006) was used for statistical analysis of genotypic characterization, allele frequency and number of alleles per *loci*, observed heterozygosity (Ho) and expected heterozygosity (He), Hardy-Weinberg equilibrium (HWE), inbreeding coefficient (F_{IS}) and fixation index (F_{ST}). Effective number of alleles (Ae) was calculated using the following formula: $Ae = 1/\Sigma xi^2$, where *xi* is the frequency of each allele per *loci*. Analysis of Molecular Variance (AMOVA), with 9,999 random permutations, and Principle Coordinate Analysis (PCoA), based on Nei's genetic distances, were also performed using software GenAlEx 6.5. A dendrogram derived from Ward's method using Euclidean distance was constructed using software Past 3.15 (Hammer, Harper, & Ryan, 2001).

A Bayesian model implemented by the software Structure 2.3.4 (Pritchard, Stephens, & Donnelly, 2000; Falush, Stephens, & Pritchard, 2003) was applied to infer the number of population groups. The population inference produced was performed without incorporating predefined population information for the mixed model and with correlated frequency. Population number analysis (K) was performed for values ranging from 1 to 30 with ten independent chains, each chain having a length of 50,000 iterations, followed by 100,000 repetitions of the Markov chain Monet Carlo (MCMC) method.

Results and Discussion

Among the *loci* used, only UHN160 showed significance for null alleles in stocks S1, S2, S3 and S5 (Table 2). The presence of null alleles in the *loci* induces genotyping error, which can result in observed heterozygous deficit, causing Hardy-Weinberg equilibrium deviation. Table 3 shows the data generated from the genotyping microsatellite marker of the nine brood stock studied. Eleven *loci* analyzed showed polymorphism, with 75 alleles in total. The UNH898 and UNH952 *loci* were the microsatellite markers that showed the highest alleles numbers with ten alleles each. On the order hand the UNH108, UNH160, UNH222 and UNH868 markers showed only five alleles each. Considering all brood stock, the average of the total number of alleles per *loci* was 6.8, while the average number of alleles per brood stock was 4.4, with high similarity among the brood stock (3.7 to 5.4).

Table 2
Null allele frequency at 11 SSR *loci* for nine breed stock of Nile tilapia from the Epagri breeding program

Loci	S1	S2	S3	S4	S5	S6	S7	S8	S9
UNH104	0.029	-0.154	-0.182	-0.091	-0.114	-0.042	-0.008	-0.053	-0.035
UNH108	0.039	0.050	0.006	-0.096	-0.078	-0.012	-0.086	-0.193	-0.102
UNH160	0.248*	0.274*	0.149*	-0.159	0.118*	0.007	0.016	-0.178	-0.097
UNH208	-0.119	-0,014	-0.075	0.086	-0.003	-0.055	-0.019	0.000	-0.038
UNH222	0.050	0.001	-0.047	-0.082	-0.023	-0.002	-0.061	0.023	-0.012
UNH879	-0.063	-0,088	-0.129	-0.063	-0.041	-0.121	0.060	-0.130	-0.111
UNH898	-0.202	0.066	0.067	-0.037	-0.009	-0.060	0.056	-0.072	0.071
UNH998	-0.127	0.172	-0.016	-0.066	0.063	-0.027	-0.124	-0.061	-0.031
UNH952	-0.036	0.011	-0.066	-0.091	0.054	0.019	-0.106	-0.137	-0.085
UNH868	-0.154	-0,084	-0.055	-0.023	-0.096	-0.018	-0.020	0.002	-0.037
UNH848	-0.021	-0,141	-0.205	-0.051	-0.122	-0.010	-0.140	-0.091	-0.085

Estimator Brookfield 1 (1996); *P < 0.05.

Petersen et al. (2012) evaluated the genetic variability of different tilapia strains cultivated in Santa Catarina (GIFT, Panama and red tilapia) and reported a total of 83 alleles with four microsatellite markers. With the GIFT strain, the authors observed a total of 12 alleles for the UNH104, UNH108 and UNH160 markers, a number higher than that found in this study for these same markers (6 to 7 alleles) (Table 3). The selection process applied in the Epagri brood stock over the last five years may explain this lower number of alleles. However, other studies with GIFT tilapia in Brazil, using a smaller number of animals and markers, showed a mean number of alleles per *loci* between 4.2 and 6.1 (Rodriguez-Rodriguez et al., 2013; Baggio, Orélis-Ribeiro, & Boeger, 2016; Dias, Freitas, Arranz, Villanova, & Hilsdorf, 2016), which is lower than that found in this study.

Table 3
Number of genotyped individuals (N), total number of alleles (A), effective number of alleles (Ae), observed heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (F_{IS}) for each brood stock (S1 to S9) of Nile tilapia from the Epagri breeding program

Loci		S1	S2	S3	S4	S5	S6	S7	S8	S9	Mean ± standard deviation
UNH104	N	30	30	30	30	30	30	30	30	30	-
	A	6	6	6	5	5	5	6	5	5	-
	Ae	4.196	3.947	3.255	2.927	4.891	4.157	5.538	4.157	3.846	-
	Ho	0.900	0.867	1.000	0.633	1.000	0.833	0.833	0.867	0.800	0.859±0.110
	He	0.762	0.747	0.693	0.658	0.796	0.759	0.819	0.759	0.740	0.748±0.049
	F_{IS}	-0.182^{**}	-0.161^{ns}	-0.443^{***}	0.038^{ns}	-0.257^{*}	-0.097^{ns}	-0.017^{ns}	-0.141^{ns}	-0.081^{ns}	-0.149±0.141
UNH108	N	30	30	30	30	30	30	30	30	30	-
	A	3	4	4	3	3	3	4	3	2	-
	Ae	2.699	2.192	2.564	1.665	2.062	1.942	2.675	2.299	1.642	-
	Ho	0.567	0.467	0.600	0.533	0.633	0.667	0.767	0.867	0.533	0.626±0.042
	He	0.629	0.546	0.610	0.399	0.515	0.485	0.626	0.565	0.391	0.529±0.030
	F_{IS}	0.100^{*}	0.142^{ns}	0.016^{ns}	-0.335^{ns}	-0.230^{ns}	-0.375^{ns}	-0.224^{ns}	-0.534^{**}	-0.364^{*}	-0.200±0.078
UNH160	N	30	30	30	30	30	30	30	30	30	-
	A	4	3	3	4	5	4	6	4	3	-
	Ae	2.667	2.062	2.817	2.236	3.333	3.103	3.673	2.699	2.219	-
	Ho	0.267	0.100	0.400	0.800	0.500	0.667	0.700	0.933	0.700	0.563±0.089
	He	0.625	0.515	0.645	0.553	0.700	0.678	0.728	0.629	0.549	0.625±0.024
	F_{IS}	0.573^{***}	0.806^{***}	0.380^{***}	-0.447^{*}	0.286^{**}	0.016^{ns}	0.038^{ns}	-0.483^{***}	-0.274^{**}	0.099±0.150
UNH222	N	30	30	30	30	30	30	30	30	30	-
	A	3	4	4	4	2	2	2	3	2	-
	Ae	2.039	1.366	1.586	2.039	1.260	1.867	1.471	1.144	1.180	-
	Ho	0.433	0.267	0.433	0.633	0.233	0.467	0.400	0.100	0.167	0.348±0.168
	He	0.509	0.268	0.369	0.509	0.206	0.464	0.320	0.126	0.153	0.325±0.148
	F_{IS}	0.149^{ns}	0.004^{ns}	-0.173^{ns}	-0.243^{ns}	-0.132^{ns}	-0.005^{ns}	-0.250^{ns}	-0.207^{ns}	-0.091^{ns}	-0.059±0.162

continue

continuation

UNH208	N	30	30	30	30	30	30	30	30	30	-
	A	6	5	5	5	6	3	2	2	3	-
	Ae	4.147	4.369	3.396	3.046	4.186	2.571	1.684	1.980	2.761	-
	Ho	0.867	0.833	0.833	0.533	0.767	0.700	0.433	0.367	0.700	0.670±0.184
	He	0.759	0.771	0.706	0.672	0.761	0.611	0.406	0.495	0.638	0.646±0.126
	F_{IS}	-0.142^{ns}	-0.081^{ns}	-0.181^{ns}	0.206***	-0.007^{ns}	-0.145^{ns}	-0.067^{ns}	0.259***	-0.098^{ns}	-0.028±0.157
UNH879	N	30	30	30	30	30	29	29	30	30	-
	A	4	5	4	4	6	5	5	6	5	-
	Ae	3.651	3.416	2.659	1.901	4.196	4.043	2.865	4.337	3.854	-
	Ho	0.833	0.833	0.833	0.567	0.833	0.966	0.552	1.000	0.933	0.817±0.053
	He	0.726	0.707	0.624	0.474	0.762	0.753	0.651	0.769	0.741	0.690±0.032
	F_{IS}	-0.148*	-0.178^{ns}	-0.336**	-0.196^{ns}	-0.094^{ns}	-0.283*	0.153^{ns}	-0.300***	-0.260*	-0.182±0.049
UNH898	N	30	30	30	30	30	30	29	30	30	-
	A	7	8	7	5	8	5	4	5	5	-
	Ae	5.085	3.774	4.688	3.789	6.691	3.704	3.121	4.138	3.186	-
	Ho	0.967	0.700	0.667	0.800	0.867	0.833	0.586	0.933	0.567	0.769±0.049
	He	0.803	0.735	0.787	0.736	0.851	0.730	0.680	0.758	0.686	0.752±0.018
	F_{IS}	-0.203***	0.048*	0.153***	-0.087**	-0.019***	-0.142^{ns}	0.137***	-0.231**	0.174***	-0.019±0.052
UNH848	N	30	30	30	30	30	30	30	29	30	-
	A	4	4	4	4	5	3	5	5	4	-
	Ae	2.517	3.468	2.723	1.963	3.622	2.410	4.072	2.427	3.571	-
	Ho	0.733	0.867	0.967	0.533	0.933	0.600	1.000	0.655	0.867	0.795±0.170
	He	0.603	0.712	0.633	0.491	0.724	0.585	0.754	0.588	0.720	0.645±0.087
	F_{IS}	-0.217^{ns}	-0.218^{ns}	-0.528***	-0.087^{ns}	-0.289*	-0.026^{ns}	-0.325***	-0.114^{ns}	-0.204^{ns}	-0.223±0.149
UNH868	N	30	30	30	30	30	30	30	30	30	-
	A	4	5	4	2	4	4	4	4	4	-
	Ae	3.082	1.779	1.827	1.260	2.791	2.025	3.617	3.364	3.046	-
	Ho	0.933	0.567	0.533	0.233	0.800	0.533	0.759	0.700	0.733	0.644±0.204
	He	0.676	0.438	0.453	0.206	0.642	0.506	0.724	0.703	0.672	0.558±0.171
	F_{IS}	-0.382***	-0.294^{ns}	-0.178^{ns}	-0.132^{ns}	-0.247^{ns}	-0.054^{ns}	-0.048^{ns}	0.004^{ns}	-0.092^{ns}	-0.158±0.128

continue

continuation

UNH952	N	30	30	30	30	30	30	29	30	30	-
	A	6	5	5	3	8	4	3	4	5	-
	Ae	4.036	3.015	2.473	2.965	4.932	2.715	2.116	3.550	3.232	-
	Ho	0.567	0.633	0.700	0.733	0.700	0.600	0.690	0.900	0.833	0.706±0.107
	He	0.752	0.668	0.596	0.663	0.797	0.632	0.527	0.718	0.691	0.672±0.081
	F_{IS}	0.247***	0.052ns	-0.175ns	-0.106**	0.122ns	0.050ns	-0.308ns	-0.253**	-0.207ns	-0.064±0.190
UNH998	N	30	30	30	30	30	30	30	30	29	-
	A	5	4	5	5	7	3	4	4	6	-
	Ae	3.607	3.130	2.546	1.965	4.500	2.261	3.991	3.622	3.047	-
	Ho	0.733	0.467	0.633	0.533	0.667	0.600	0.967	0.833	0.724	0.684±0.153
	He	0.723	0.681	0.607	0.491	0.778	0.558	0.749	0.724	0.672	0.665±0.095
	F_{IS}	-0.015ns	0.314*	-0.043ns	-0.086ns	0.143ns	-0.076ns	-0.290***	-0.151ns	-0.078ns	-0.031±0.173
Stock means	N	30.0	30.0	30.0	30.0	30.0	29.9	29.6	29.9	29.9	-
	A	4.7	4.8	4.6	4.0	5.4	3.7	4.0	4.1	4.0	-
	Ae	3.438	2.956	2.776	2.341	3.871	2.813	3.180	3.081	2.871	-
	Ho	0.709	0.600	0.691	0.594	0.724	0.679	0.702	0.744	0.687	0.680±0.021
	He	0.689	0.617	0.611	0.532	0.687	0.615	0.637	0.622	0.605	0.623±0.015
	F_{IS}	-0.018	0.039	-0.137	-0.134	-0.066	-0.102	-0.111	-0.160	-0.143	-0.092±0.029

ns = not significant, *P < 0.05, **P < 0.01, and ***P < 0.001 - based on equilibrium test of Hardy-Weinberg.

Regarding the Hardy-Weinberg equilibrium, it was observed that the number of *loci* per stock ranged from four to ten for populations S1 and S6, respectively. In addition, only the UNH222 locus is in equilibrium in all populations (Table 3). Given this information it is possible to make inferences about the significance (or not) of the F_{IS} values. In this regard, the UNH104, UNH208, UNH848, UNH868, and UNH879 *loci* showed exclusively negative values. As expected, from the analysis of null alleles (Table 2), stocks S1, S2, S3 and S5 showed significant values for F_{IS}, whose values were 0.573, 0.806, 0.380 and 0.286, respectively, for the *loci* UNH160 (Table 3). The average inbreeding coefficients per population ranged from -0.160 to 0.039 for stocks S8 and S2, respectively (Table 3).

In this study, the values of Ho were higher than He, with the exception of the UNH160 marker, resulting in a negative mean inbreeding coefficient (F_{IS} = -0.092). Thus, there is genetic variability within the studied brood stock, since the negative F_{IS} value indicates an excess of heterozygosity, which is interesting in a breeding program. In addition, all markers were polymorphic, showing satisfactory results for evaluating the genetic variability of this stock. In contrast to this study, Rodriguez-Rodriguez et al. (2013) found a positive F_{IS} of 0.281 and attributed the lower heterozygosity observed in the breeding stock to the selection process used. Other studies in Brazil evaluating stock of the GIFT strain, which shared the same origin through a single import of 30 GIFT tilapia families from Asia to Brazil (Halfen et al., 2012), reported F_{IS} values of 0.16 and 0.35 (Petersen et al., 2012; Dias et al., 2016). Previously, Baggio et al. (2016) reported a F_{IS} value for a stock of GIFT tilapia of -0.042, a value close to that found in our study.

The excess of heterozygotes in a stock can occur when the fitness of these genotypes provides non-random mating in the previous generation, when the stock experiences a bottleneck due to the sample size (where few individuals contribute to the next generation); or when the number of individuals sampled in the current generation is small, and with that, genetic drift may occur. The last two conditions may justify the F_{IS} values found both in this study and in other studies in the literature.

Among the markers used in this study, some are already known to be regions of the genome associated with phenotypic traits of interest, known as Quantitative Trait Loci (QTL). According to Cnaani, Zilberman, Tinman, Hulata, & Ron (2004), the markers UNH848, UNH868, and UNH898 are associated with tilapia weight characteristics, program focus trait, and together explain 37.9% of the variation of this trait. The markers UNH848 and UNH868 also explain 24.4% of the variation in tilapia immunoglobulin M (IgM) data (Cnaani et al., 2004). These markers displayed greater observed heterozygosity than expected heterozygosity values, and showed the following values of inbreeding coefficient: UNH898 (F_{IS} = -0.019), UNH848 (F_{IS} = -0.223) and UNH 868 (F_{IS} = -0.158). Moreover, the UNH879 marker is associated with cold tolerance (Cnaani et al., 2003), an important trait to Santa Catarina, and also exhibited high heterozygosity and negative inbreeding coefficient (F_{IS} de -0.182). These results are important due to the need to verify how much genetic variability the base brood stock contains as the higher the genetic variability of the population, the greater the genetic gain through selection (Falconer, Mackay, & Frankham, 1996). As such, it is possible to use these data and these markers in the breeding program. Information on the allele frequency of particular *loci* in animals with better performance for the trait of interest can be used in the selection process. For example, H. P. Zhu et al. (2015) observed differential allelic segregation between cold-tolerant and cold-sensitive tilapia strains in the UNH916 and UNH999 markers and, according to the authors, this information can be used for marker-assisted selection programs in tilapia breeding.

In total, five private alleles were found, with one private allele in stock S1, and four private alleles in S5. In most cases, the private alleles had a low allele

frequency, with the exception of allele 201 of the UNH952 *loci* (S5) which had an allele frequency of 0.200 (Table 4). The presence of private alleles is due to mutation, gene flow or allele display by selecting from other stocks. Possibly, in this study, these alleles are mutations over time of S5 stock formation, as this stock has no different origin to the other stocks. From these results, it is possible to monitor animals from the next generations of tilapia in Epagri's breeding program and to avoid possible genetic contamination at mating.

Table 5 shows that the greatest genetic variation in the brood stock is between individuals within a stock (89%), not among stocks, showing what was reported in the previous paragraph, namely that the stock of GIFT tilapia in the Epagri program present a good variability between individuals (Moreira et al., 2007; Briñez et al., 2011; W. B. Zhu et al., 2017).

Table 4
Private alleles of the Nile tilapia stock from the Epagri breeding program

Stock	*Loci*	Alleles	Allelic frequency
S1	952	207	0.050
S2	-	-	-
S3	-	-	-
S4	-	-	-
S5	898 /998 / 952 / 952	282 / 119 / 201 / 234	0.067 / 0.050 / 0.200 / 0.017
S6	-	-	-
S7	-	-	-
S8	-	-	-
S9	-	-	-

Table 5
Analysis of molecular variance (AMOVA) of microsatellite markers among nine brood stock of Nile tilapia from the Epagri breeding program

Source of variation	d.f.	Sum of Squares	Variance component	Percentage of variation (%)
Among stock	8	232.943	0.427	11%
Within stock	531	1855.200	3.494	89%
Total	539	2088.143	3.921	

F_{ST}=0.109***; 9,999 permutations.

The F_{ST} found in this study for the second generation of GIFT tilapia in the Epagri program was 0.109, indicating a moderate genetic differentiation between the brood stocks. The Epagri Research Group performed the genotyping of the first generation of GIFT tilapia and reported an F_{ST} value for the brood stock of 0.239 (Silva, Pereira, & Mariguele, 2016) with only seven of the eleven microsatellite markers. These data indicate that there was a decrease in the genetic differentiation between stocks from one generation to another. This decrease in the genetic differentiation is expected because artificial selection tends to select animals with the same alleles of interest over generations. As already mentioned, the negative F_{IS} values indicate that there is good variability among the individuals in the breeding program, but genetic distances between the stocks appear to be decreasing.

According to Euclidean distance, the dendrogram presented in Figure 1 was constructed, facilitating the verification of the distance between the different brood stocks used in the tilapia breeding of Epagri. Three groups were formed as follows: I - S6, S7 and S9; II - S2, S3 and S4; and III - S1, S5 and S8. According to Nei's genetic distances, the values ranged from 0.087 to 0.461 between stocks S2 / S3 and S4 / S7, respectively. The first three PCoA coordinates explain 90.98% of the variation among the studied stocks; the first, second and third correspond to 56.75, 24.84 and 9.40% respectively. From the PCoA representation (Figure 2) the existence of two groups can be observed: I - S6, S7, S8 and S9 and II - S1, S2, S3, S4 and S5. The formation of these two genetic groups is consistent with the genealogy of stocks; stocks S1 to S5 have different origins from stocks S6 to S9. The formation of group III (S1, S5 and S8) in the dendrogram can be explained by the higher average observed heterozygosity values of these stocks (Table 3).

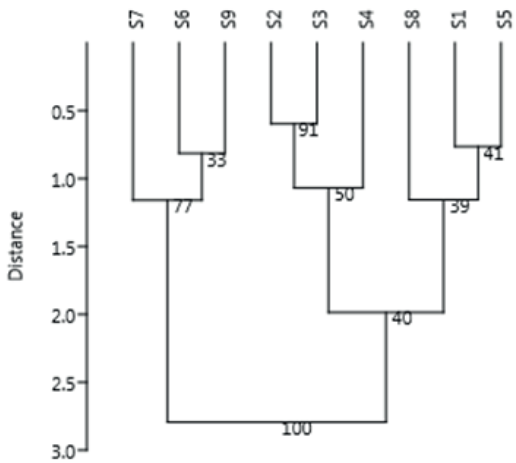

Figure 1. Dendrogram derived from Ward's method using Euclidean distance with 10,000 bootstrap samples between different brood stocks (S1 to S9) of Nile tilapia from the Epagri breeding program based on 11 SSR markers (UNH104, UNH108, UNH160, UNH208, UNH222, UNH848, UNH868, UNH879, UNH898, UNH952, UNH998).

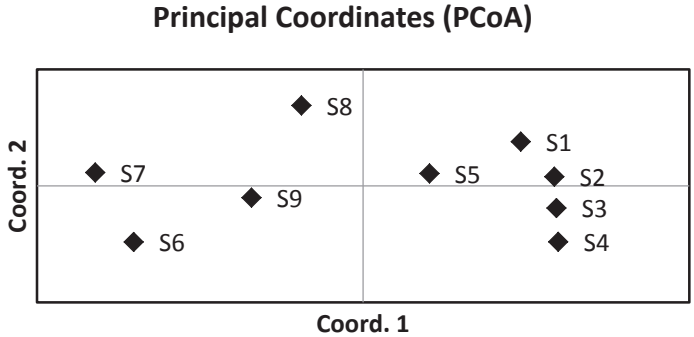

Figure 2. Principal Coordinate Analysis of genetic distances of nine breed stock of Nile tilapia from the Epagri breeding program.

Based on studies with different tilapia strains in Brazil and the rest of the world, it is possible to observe that, although the F_{ST} value has decreased between generations, the F_{ST} value found in this study is of considerable value to determine genetic differentiation between brood stocks.

Rodriguez-Rodriguez et al. (2013), after genetic characterization of different generations of tilapia (GIFT strain) derived from a family-based breeding program, also observed moderate values for genetic differentiation (F_{ST}) among the generations of GIFT tilapia (0.014 to 0.081). Other studies comparing different strains cultivated in Brazil found moderate values for genetic differentiation, with F_{ST} values of 0.130 and 0.131, respectively, in the studies of Petersen et al. (2012) and Moreira et al. (2007). The approximate values of F_{ST} in different studies comparing different stocks of tilapia rearing in Brazil is understandable, since much of the GIFT tilapia produced in the country so far have derived from a single import, as was the case with red tilapia and Chitralada tilapia.

The implemented Bayesian analysis was applied in order to generate genetically homogeneous groups among 270 pre-defined individuals in nine stocks (Figure 3). From this analysis, the formation of 16 groups with an F_{ST} value of 0.2107 was established. This result reinforces the existence of variability existing in the Epagri breeding program, from which it is possible to form heterotic groups to enable the direction of potential crosses to obtain genetic benefits.

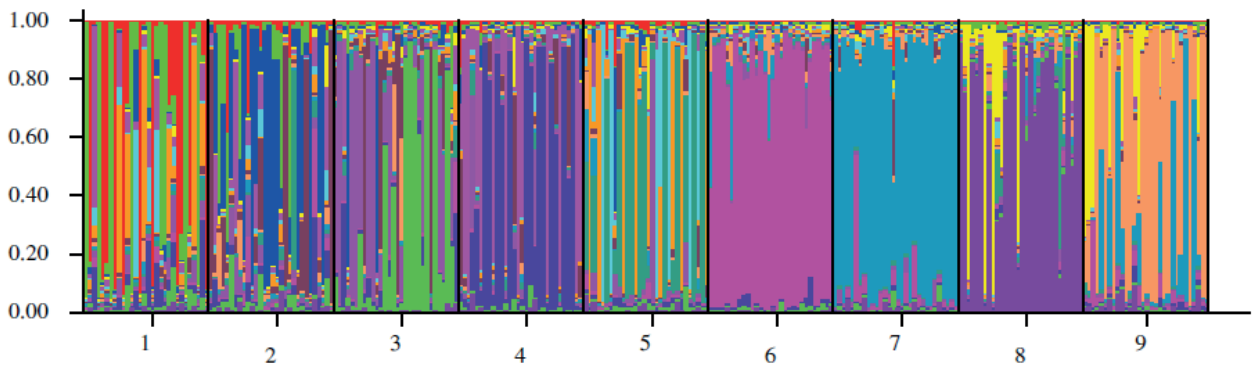

Figure 3. Allocation of 270 tilapia GIFT individuals of nine breed stock (K=16) of Nile tilapia from the Epagri breeding program.

Conclusions

The study enabled genotypic characterization of the tilapia brood stock used in the Epagri breeding program, determining the genetic distance between the stocks, which will enable more accurate selection of individuals for mating for the next generation. It was possible to verify that there is high heterozygosity within the stocks, and moderate genetic differentiation between the stocks. Furthermore, all markers evaluated were polymorphic for this brood stock and will be used to characterize the next generations.

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