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Genetic diversity analysis of aquaculture strains of *Acipenser stellatus* (Pallas, 1771) using DNA markers

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Abstract

Acipenser stellatus (stellate sturgeon) that has been intensively captured for decades represents a sturgeon species of great importance for the scientific community and fishermen. Besides overfishing and poaching, their reproduction sites have been destroyed and their natural habitats were affected by pollution. In consequence, wild populations of stellate sturgeon have decreased dramatically and this species is being considered critically endangered. In order to conserve this species, efforts have been made to artificially reproduce native sturgeons and restock natural habitats with the broodstock. The analysis of genetic diversity of the future breeders is of great importance in order to avoid the inbreeding depression and genetic drift. Six aquaculture strains of *A. stellatus* reared in fish farms from the South of Romania were genetically analyzed. The assessment of the genetic diversity was made by using DNA markers, such as cytochrome b mitochondrial gene and microsatellite nuclear loci. The results showed a moderate genetic variability within the strains and a moderate genetic diversity between the strains. One strain was slightly inbred and is recommended not to be included in the reproduction programs. In conclusion, five out of six aquaculture strains were genetically distinct and can be used in selective breeding programs aimed to conserve stellate sturgeon from the Lower Danube River.

Keywords: Stellate sturgeon, Mitochondrial DNA, Microsatellites, Genetic diversity, Breeding programs

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Introduction

Sturgeons represent an ancient group of fish that appeared approximately 200 million years ago in Upper Cretaceous and have survived two Ice Ages and several mass extinction events. Having an extremely slow evolution, these fish have passed through insignificant morphological changes, gaining the status of living fossils (Bemis and Kynard, 1997). As a consequence, sturgeons may represent a genuine model for the study of vertebrates' evolution, a feature that makes sturgeons scientifically important. The economic value of sturgeons arises from their meat, which is considered a delicacy, and their roe used for the production of caviar, a prosperous industry all over the world (Cohen, 1997). In consequence, the sturgeons are significant both for the scientific community and the fishermen.

The high demand for sturgeon caviar and the huge prices of this delicacy have put an enormous pressure on this ancient fish group. Excessive fishing and poaching resulted in reduced fish captures every year (Billard and Lecointre, 2001). For example, sturgeon catches have decreased dramatically in Romania, from 1144 tons in 1940 to less than 8 tons in 1995 (Navodaru *et al*., 1999). Furthermore, most sturgeon species, including *A. stellatus*, are marine anadromous fishes, which migrate from the sea to freshwater in order to breed. In Romania, the construction of the Iron Gates Dams over the Danube River impaired the migration of sturgeons from the Black Sea to the Middle and Upper Danube. Sturgeon populations have been decreasing over the last decades not only because of the impaired migration and reproduction, but also due to other human activities such as pollution and river bottom-modifications which have driven to alteration of reproduction sites (Bacalbasa-Dobrovici, 1997). As a result, *A. stellatus* among other sturgeon species is facing extinction. Nowadays, stellate sturgeon is considered extinct in Upper and Middle Danube (Hensel and Holcik, 1997), while in the Lower Danube countries stellate sturgeon captures have decreased from 30 tons in 2001 to less than 10 in 2005 (Lenhardt *et al*., 2008).

Measures have been taken in order to countervail the anthropic influences on stellate sturgeon populations. *A. stellatus* has been declared a critically endangered species by the IUCN (International Union for Conservation of Nature) and by CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). In addition, sturgeon aquaculture has made significant progress in the recent years in Romania in order to satisfy the demand of consumers for meat and caviar and also to protect this species from extinction,. Thus, there are ongoing conservation projects in aquaculture aiming the reproduction of native sturgeons and restocking of their natural habitats with the obtained broodstock (Lenhardt *et al*., 2008). To avoid undesirable phenomena like inbreeding depression and genetic drift, the genitors included in the reproduction programs have to be

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genetically analyzed. Genetic analysis based on DNA markers represents a reliable support for a better management of restocking programs (Liu and Cordes, 2004). Furthermore, a genetic analysis is required for the good management of stocks in aquaculture. Therefore, breeders from strains with significant genetic diversity can be used in reproduction programs in order to maintain the genetic diversity of the broodstock. Selective breeding between unrelated individuals might be a solution for reducing the inbreeding and the negative consequences resulting from this phenomenon like loss of disease resistance and of environmental adaptability. Malformations and a high susceptibility for diseases were signaled after several generations of uncontrolled reproductions in aquaculture. Also, other aspects important for production like low growth rates and poor meat quality might be associated with the loss of genetic diversity in the cultured stocks in different fish species (Lacy, 1987; Wang *et al.*, 2012).

Until now, in Romania, studies based on molecular markers assessed the phylogeographic structure of Ponto-Caspian sturgeon species (Dudu *et al*., 2014), proposed a molecular tool to identify sturgeon hybrids (Dudu *et al*., 2011) and analyzed the genetic diversity and structure of the stellate sturgeon population from North-Western Black Sea that reproduce in the Lower Danube River (Dudu *et al*., 2008). Also, the genetic variability of stellate sturgeon populations from Caspian Sea was assessed (Norouzi *et al.*, 2008; Norouzi and Pourkazemi, 2009). Molecular studies for characterizing the aquaculture stocks and strains from romanian fish farms are still at the beginning.

The purpose of this study was to analyze the genetic diversity of six *A. stellatus* aquaculture strains reared in four fish farms from the South of Romania. The genetic variability within each strain and between strains was assessed by using molecular markers like mitochondrial DNA markers (cytochrome b gene - *cyt b*) and nuclear DNA markers (microsatellites). The results of this study can be used in fish farms to properly select the future breeders implied in reproduction programs in order to avoid the inbreeding depression and the genetic drift. Consequently, this analysis is useful for conserving the genetic diversity and the fitness of aquaculture strains and to maintain aquaculture stocks ”healthy” from a genetic point of view.

Materials and methods

Sampling and DNA isolation

In this study, six aquaculture strains of *A. stellatus* totalizing 60 individuals from four fish farms from Southern Romania were genetically analyzed. A strain was considered to be a group of individuals with the same geographic origin (coming from the same sector of a river) or representing the result of the same breeding plan in aquaculture. Samples were taken from the anal fins in order to prevent harming the fish and were stored in absolute ethanol to maintain the DNA integrity until the

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extraction. DNA was isolated from fin fragments using a classic protocol with phenol-chloroform and isoamilic alcohol (Sigma Aldrich). The concentration and purity of the DNA were evaluated using NanoDrop 8000 spectrophotometer (ThermoScientific).

Mitochondrial DNA analysis

After DNA extraction, *cyt b* gene with a length of 1141 bp (base pairs) was amplified by PCR using two sets of primers: Glu F (5'-AAGAACCACCGTTGTTATTCAA-3') /Cytb R (5'TCTTTATATGAGAARTANGGGTG-3') amplifying a fragment of 714 bp and Cytb F (5'-CACGARACRGRTCNAAYAA-3')/ Thr R (5'-ACCTCCRATCTYCGGATTACA-3') amplifying a fragment of 586 bp. The PCR program used for the amplification of the *cytb* gene was the following one: 1 cycle of 95˚C for 10 minutes, 30 cycles of 95˚C for 30 seconds, 53˚C for 30 seconds, 72˚C for 1 minute and 1 cycle of 72˚C for 10 minutes. The reactions were performed in a final volume of 25 µL containing: 50 ng DNA sample, 1X PCR Gold Buffer, 25 mM $MgCl_2$, 10 mM dNTP and 1U AmpliTaq Gold DNA Polymerase (Applied Biosystems). After that, the amplicons were purified with Wizard SV Gel and PCR Clean-Up System kit (Promega).

The purified amplicons were sequenced through Sanger method, Big Dye Terminator version using Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Fragments were purified with BigDye XTerminator Purification kit (Applied Biosystems) and separated through capillary electrophoresis using ABI Prism 3130 Genetic Analyzer (Applied Biosystems).

The sequences obtained were edited using BioEdit 7.0.5.3 program (Hall, 1999) and aligned with Clustal X 2.0.9 software (Larkin *et al*., 2007). The genetic diversity in terms of number of haplotypes and haplotype diversity were analyzed with DnaSP 5.10 (Librado and Rozas, 2009). The genetic differentiation between strains based on $Gamma_{st}$ differentiation index was computed using the same software. Statistical significance was tested by permutation with 10,000 replicates.

To illustrate the relationships among the haplotypes/ strains, a haplotype network of *cyt b* sequences was inferred using median-joining algorithm implemented in NETWORK v5 (Bandelt *et al*., 1999) with default settings (ε=0) and the variable sites weighted equally (weight=10).

Nuclear DNA analysis

Nine microsatellite loci were used for genotyping analysis and the loci were amplified with fluorescent labeled primers (Table 1). The amplification was realized using either monoplex PCR or 3-plex PCR reactions according to the following protocol: 1 cycle of 95˚C for 10 minutes, 30 cycles of 95˚C for 30 seconds, 55˚C/ 52˚C for 30 seconds, 72˚C for 1 minute and 1 cycle of 72˚C for 30 minutes. The PCR reactions were performed in a final volume of 25 µl containing: 25 ng DNA

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sample, 1X PCR Gold Buffer, 25 mM $MgCl_2$, 10 mM dNTP and 0.5 U/1.5 U AmpliTaq Gold DNA Polymerase (Applied Biosystems).

The fluorescent labeled amplicons were separated by length through capillary electrophoresis in the ABI Prism 310 Genetic Analyzer (Applied Biosystems) along with GeneScan -500 LIZ Size Standard. The resulting data were examined with GeneMapper 3.1 software and were statistically analyzed with GENETIX 4.05 (Belkhir *et al*., 2004) and FSTAT 1.2 (Goudet, 1995) programs.

Table 1: The name of the microsatellite loci and the labeled primers used for their amplification.

Micro-satellite loci	Species	Motif	Primer sequence	Labeling
LS19 (May *et al.*, 1997)	*Acipenser fulvescens*	(TTG)n	F: CATCTTAGCCGTCTGTGGTAC R: CAGGTCCCTAATACAATGGC	FAM
LS34 (May *et al.*, 1997)		(GTT)n	F: TACATACCTTCTGCAACG R:GATCCCTTCTGTTATCAAC	VIC
LS39 (May *et al.*, 1997)		(GTT)n	F: TTCTGAAGTTCACACATTG R: ATGGAGCATTATTGGAAGG	PET
LS54 (May *et al.*, 1997)		(GATA)n (GACA)n	F: CTCTAGTCTTTGTTGATTACAG R: CAAAGGACTTGAAACTAGG	NED
AnacC11 (Forlani *et al.*, 2008)	*Acipenser naccarii*	(TCTA)n	F: AAATTTCCATTGGGGTGT R: CTTCGTTTTGAGAACCCG	VIC
AnacE4 (Forlani *et al.*, 2008)		(CA)n	F: TCAGCTACAGGGTTCTGGG R: GTTGTTACTCATTGGAACTC	FAM
Acig198 (Bork *et al.*, 2008)	*Acipenser transmontanus*	(AAAT)n	F: ACCATCCCCTCCCATATCTC R: TGGTTCTTAGCGGAGGAAGA	NED
Spl106 (McQuown *et al.*, 2000)	*Scaphirhynchus platorhynchus*	(CTAT)n	F: CACGTGGATGCGAGAAATAC R: GGGGAGAAAACTGGGGTAAA	PET
As002 (Zhu *et al.*, 2005)	*Acipenser sinensis*	(GACA)n	F: CGGACAGAATTGGAGAACAC R: TAACGCTGCCATTGCAGATA	FAM

Results

Mitochondrial data

Based on mitochondrial data, 10 polymorphic sites were identified in the *cytb* gene in the following nucleotide sites of the gene: 54, 396, 462, 465, 630, 633, 849, 900, 921 and 1089. (Fig. 1). The nucleotide sequences translated into amino acids sequences highlighted that there is no variability regarding the amino acids sequences. The number of polymorphic sites at individual level varied between 0 and 6.

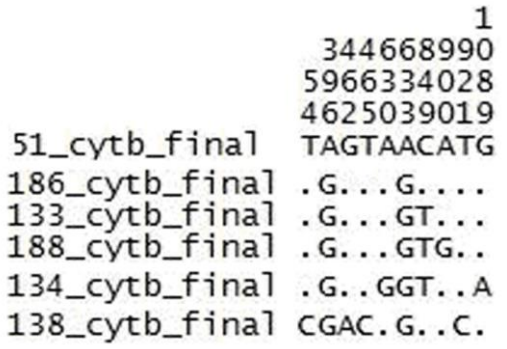

Figure 1: Variable sites in unique haplotypes of *Acipenser stellatus* strains. The numbers represent the nucleotides position within the cyt b gene.

Furthermore, six haplotypes were identified and their distribution among

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the strains was analyzed: haplotype 1 (H1) for strain 1, H2 for strains 2, 3 and 6, H3 for strains 2, 3, 4, 5 and 6, H4 for strain 6, H5 for strain 2 and H6 for strain 3. As it can be observed, strain 1 has a unique haplotype, while the rest of the strains possess common haplotypes. Also, the haplotype diversity index (Hd) was determined based on the frequency of that haplotype into a population (Table 2). It was observed that Hd value was 0 for strains 1, 4 and 5, in contrast to strains 2, 3 and 6 for whom Hd value was 0.4. These results were confirmed by the haplotype network showing the relationships between the strains and the distribution of haplotypes among strains (Fig. 2).

Table 2: The number of haplotypes identified for each strain and the haplotype diversity index.

Strain	Number of individuals	Farm	Number of polymorphic sites	Number of haplotypes	Hd
Strain 1	10	Farm A, Calarasi County	0	1	0
Strain 2	10	Farm B, Olt County	3	3	0.4
Strain 3	10		5	3	0.4
Strain 4	10	Farm C, Tulcea County	0	1	0
Strain 5	10	Farm D, Constanta County	0	1	0
Strain 6	10		2	3	0.4

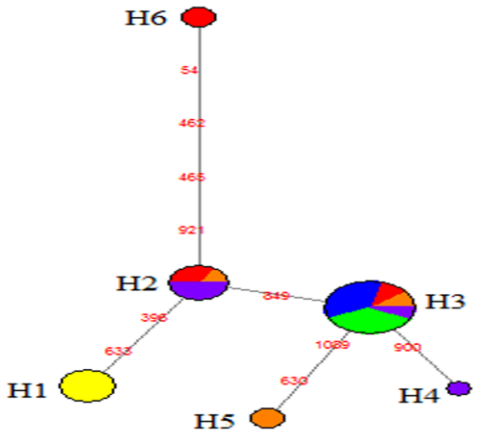

Figure 2: Median-Joining networks for cytb mtDNA haplotypes in *A. stellatus* aquaculture strains. Circle size is proportional to haplotype frequency. Branch lengths are proportional to the number of substitutions per nucleotide site and the numbers represent the point mutations. Colors indicate the strains origin of haplotypes, as follow: strain 1- yellow; strain 2- orange; strain 3 - red; strain 4 - blue; strain 5 - green; strain 6 - purple.

In addition, $Gamma_{st}$ index was determined for each pair of strains (Table 3). The values obtained in this study ranged between 0 and 1.

Table 3: The values of Gamma statistical index showing the genetic differentiation between strains.

Strain	Strain	Gamma $_{st}$
Strain 1	Strain 2	0.5
Strain 1	Strain 3	0.288
Strain 1	Strain 4	1
Strain 1	Strain 5	1
Strain 1	Strain 6	0.337
Strain 2	Strain 3	0.272
Strain 2	Strain 4	0.266
Strain 2	Strain 5	0.219
Strain 2	Strain 6	0.175
Strain 3	Strain 4	0.423
Strain 3	Strain 5	0.363
Strain 3	Strain 6	0.193
Strain 4	Strain 5	0
Strain 4	Strain 6	0.33
Strain 5	Strain 6	0.284
		Average Gamma $_{st}$ = 0.71

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Nuclear data

Regarding the nuclear data, disomic profiles were observed for seven microsatellite loci, while polysomic profiles had LS19 and As002 loci. Due to the fact that the statistical analysis software uses only disomic data, LS19 and As002 were not included in the analysis. Alleles of different sizes were identified for each locus and the allelic richness (Ar) parameter was calculated (Table 4). It was found that LS39 presented only one allele, while Spl106, LS54 and AnacC11 presented multiple alleles. Therefore, the allelic richness of LS39 was equal to 1, while the values of the other three loci ranged between 3.54 and 4.69. LS34, Acig198 and AnacE4 had intermediate values of allelic richness.

Table 4: The allelic richness parameter for each microsatellite locus and each strain.

	A	Size of alleles (bp)	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	Strain 6	Average allelic richness
Spl106	14	231-295	3.43	3.66	2.2	4.1	3.4	4	4.69
LS34	3	138-147	1.3	1.6	2.59	1.56	1.6	1.75	1.66
Acig198	2	185-189	1.98	2	2	1.99	2	2	1.97
AnacE4	5	348-358	2.74	2.83	1.96	2.47	3.25	2.7	3.01
LS54	9	161-201	2.49	4.83	3.4	3.23	3.42	3.85	4
AnacC11	7	162-186	2.22	3.06	3.06	2.9	3.5	4.75	3.54
LS39	1	114	1	1	1	1	1	1	1

A= number of alleles

The inbreeding coefficient (Fis), the observed (Ho) and expected heterozygosity (He) were determined for each microsatellite locus (Tables 5,6). Both positive and negative values were obtained for Fis coefficient, while Ho values were greater than He values, except the values of strain 2.

Table 5: The expected (He) and observed (Ho) heterozygosity determined for each strain.

Strain	He	Ho
Strain 1	0.43	0.57
Strain 2	0.48	0.45
Strain 3	0.43	0.55
Strain 4	0.47	0.60
Strain 5	0.48	0.59
Strain 6	0.49	0.66

Table 6: The values of inbreeding coefficient (Fis) for each microsatellite locus and each strain.

Locus	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	Strain 6
Spl106	- 0.304	0.226	- 0.067	- 0.19	- 0.333	0.2
LS34	0	0	0.077	- 0.067	0	0
Acig198	- 1	-1	- 1	- 1	- 1	- 1
Anac E4	- 0.2	0.733	- 0.2	0.429	- 0.2	- 0.286
LS54	- 0.514	0.135	- 0.032	- 0.067	0.25	- 0.2
Anac C11	0.621	0. 407	0. 077	- 0.407	0.273	- 0.143
Average	- 0.268	0. 163	- 0.158	- 0.209	- 0.122	- 0.204
Average Fis =- 0.154						

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The genetic distances between each pair of strains were determined based on Cavalli-Sforza and Edwards Method (Table 7). The greatest genetic distances were obtained for the following pairs of strains: strains 1 and 4, strains 1 and 6, strains 2 and 3. In contrast, the smallest genetic distances were determined between strains 2 and 5, strains 4 and 5 and strains 5 and 6.

Table 7: Cavalli-Sforza and Edwards genetic distances for each pair of strains.

Strain	Strain	Genetic distance
Strain 1	Strain 2	0.251
Strain 1	Strain 3	0.214
Strain 1	Strain 4	0.272
Strain 1	Strain 5	0.204
Strain 1	Strain 6	0.274
Strain 2	Strain 3	0.299
Strain 2	Strain 4	0.174
Strain 2	Strain 5	0.149
Strain 2	Strain 6	0.168
Strain 3	Strain 4	0.232
Strain 3	Strain 5	0.235
Strain 3	Strain 6	0.255
Strain 4	Strain 5	0.145
Strain 4	Strain 6	0.206
Strain 5	Strain 6	0.145

Discussion

Based on mitochondrial data, there were three strains with no polymorphisms, in contrast to the other three that presented several polymorphisms, illustrating the nucleotide variability within these strains. The fact that all amino acid sequences encoded by *cyt b* gene were identical suggests that all the identified polymorphisms were represented by silent mutations.

According to the haplotype distribution among strains, it was observed that strain 1 is completely differentiated from the other strains possessing a unique haplotye, while the rest of the strains are maternally genetically related, sharing common haplotypes.

The haplotype network have a star-like shape showing also the uniqueness of strain 1 which possesses one distinct haplotype in comparison to the other strains. The most frequent haplotypes are H2 and H3 which are shared by all the strains except strain 1. H3 is shared by five strains and is the most frequent among the individuals, while H2 is found in three of the analyzed strains. The strains 2, 3 and 6 are the most heterogeneous regarding the number of haplotypes within population, while strains 1, 4 and 5 have only one haplotype.

The haplotype diversity index, which measures the uniqueness of a haplotype into a population, was determined based on the frequency of that haplotype among a particular strains and the number of the individuals from the strain. The values of Hd can range between 0 indicating a lack of haplotype diversity and 1 indicating total haplotype diversity. Strains 1, 4 and 5 that have no polymorphic sites, presented only one haplotype and therefore have no haplotype diversity (Hd value is 0). In contrast, strains 2, 3 and 6 possess multiple polymorphic sites, each have three haplotypes and therefore moderate haplotype diversity (Hd value is 0.4). By comparing with strains 1, 4 and 5, strains 2, 3 and 6 are more suitable for reproduction

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programs. $Gamma_{st}$ index shows the genetic differentiation between populations and is determined for each pair of strains. This parameter represents an unbiased estimate of F_{st} that corrects errors associated with incomplete sampling of populations and is more suitable for mitochondrial haplotype data (Weir and Cockerham, 1984). The higher is the value of $Gamma_{st}$, the more significant is the genetic differentiation between two populations. Generally, any value of $Gamma_{st}$ index between 0.15 and 1 indicates a high differentiation between two populations. The highest value of this index obtained in this study was 1 and indicates an absolute differentiation between strains 1 and 4 and between strains 1 and 5, respectively. The smallest value of $Gamma_{st}$ was 0 and indicates the lack of differentiation between strains 4 and 5. Except these two strains, all values were higher than 0.15 for the rest of the pairs, suggesting that the analyzed strains show a great genetic differentiation and could be used in reproductive purpose.

Regarding the nuclear data, disomic profiles were observed for seven microsatellite loci, while polysomic profiles had LS19 and As002 loci. This finding could be explained by the process of functional reduction of the genome from a tetraploid state to a diploid state. Although it is considered a diploid species, *A. stellatus* similar to other sturgeon species has suffered a genome reduction process (Ludwig *et al.*, 2001; Vasiliev, 2009). Apparently, this process is not completed and while the majority of loci are disomic, some still remained with tetrasomic profile (up to four alleles for a locus in an individual) from the previous 4n state.

Allelic richness (Ar) parameter was calculated as a measure of the polymorphism level of the loci. The number of alleles found for a locus determines the allelic richness of the particular locus. If a locus presents many alleles, then it is characterized by a great allelic richness and it is suited for the genetic diversity analysis. It was found that LS39 presented only one allele, while Spl106, LS54 and AnacC11 presented multiple alleles. Therefore, the allelic richness of LS39 was equal with 1, indicating a fixed allele in the population and the allelic richness values of the other three loci ranged between 3.54 and 4.69 indicating a high level of polymorphism. LS34, Acig198 and AnacE4 had intermediate values of allelic richness indicating a moderate level of polymorphism. Based on the allelic richness, overall the microsatellite loci, except LS39 which had only one allele, were characterized by moderate genetic diversity and proved to be informative for genetic analysis. Consequently, LS39 data were not taken into account when assessing the genetic diversity.

The inbreeding coefficient (Fis), the observed (Ho) and expected heterozygosity (He) were determined for each microsatellite locus. If the Ho value is greater than the He value, the strain is characterized by a high degree of heterozygosity and genetic diversity. Fis coefficient values can range between -1 and +1. Positive values for

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Fis coefficient indicate that the individuals are related and the strain is inbred, while negative values indicate that the individuals are genetically different and the strain is not affected by the inbreeding. The results showed that the Ho value is smaller than the He value for strain 2, suggesting that this one has a low genetic diversity, which is confirmed by the positive value of Fis coefficient which indicates inbreeding. With the exception of strain 2, the values obtained for the rest of the strains indicate that the individuals within the strains are not related and have heterozygous genotypes. Overall, the values indicate a moderate degree of genetic diversity within each strain, except for strain 2.

Genetic distances were determined in order to estimate the genetic differentiation between the strains. The greater the genetic distance is, the higher the genetic differentiation between the strains is. According to this parameter, strains 1 and 4, strains 1 and 6 and strains 2 and 3 are very different genetically. In contrast, strains 2 and 5, strains 4 and 5 and strains 5 and 6 are genetically related. Overall, the genetic differentiation between the aquaculture strains is moderate. The nuclear data confirms the mitochondrial data.

The results of this study can be used to recommend particular strains for selective breeding schemes that aim to have valuable aquaculture stocks for several generations. Therefore, if the reproduction is realized with individuals from different strains, the most recommended strains are strain 1 - strain 4, strain 1 - strain 6 and strain 2 - strain 3, because there is a high genetic differentiation between these pairs. The following pairs, strain 2 - strain 5, strain 4 - strain 5 and strain 5 - strain 6 are not recommended because the individuals are genetically related, according to both genetic distances and the values of $Gamma_{st}$ index.

If the reproduction is realized with individuals from the same aquaculture strain, strains 3 and 6 are recommended because these strains have high genetic diversity according to the mitochondrial data and are not affected by inbreeding according to nuclear data. Strains 1, 4, and 5 are not recommended for this type of reproduction because they have low haplotype diversity, although they are not inbred. Strain 2 should be excluded from the reproduction programs because it is affected by inbreeding and has low genetic diversity according to nuclear data. Strain 2 could be included in restocking programs only if the individuals are crossed with individuals from other strains in order to realize a gene flow.

In conclusion, the genetic diversity analysis based on DNA markers represents a powerful tool for making recommendations regarding aquaculture strains involved in artificial reproduction. The mitochondrial and nuclear DNA markers can be used to assess the genetic diversity within a strain and between strains. Both types of genetic data should be taken into account when selecting the best - suited breeders involved in aquaculture reproduction. This analysis will help future broodstock to have a proper

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genetic diversity in order to be used for natural habitat restocking.

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