Microsatelitte DNA variation in the black sea beluga sturgeon *(Huso huso)*

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Abstract

Marine migratory sturgeons represent one of the most important fishery resources, both from a scientific and commercial point of view. While the effectives of Huso huso have significantly decreased in the last decades, the economical interest has constantly increased. Under these circumstances, it is necessary to develop conservation programs for Romanian beluga sturgeon, which imply knowledge of its genetic diversity and of the evolutionary relationships among geographic populations. New molecular biology developments allowed the use of new efficient markers in the genetics of sturgeons. Thus, microsatellite markers allow the evaluation of intraspecific genetic diversity and offer us the possibility to distinguish differences between populations due to their high level of allelic variation. The aim of our study was to assess the genetic diversity within beluga sturgeon from Black Sea using a panel of seven microsatellites for this evaluation. We selected a set of microsatellite loci that are amplified by two multiplex PCR reactions in order to estimate the differences between individuals of the Huso huso species, due to their high allelic variation. Low level of polymorphism was observed for the population of sturgeons studied. This study contributes to the evaluation of genetic diversity and molecular characterization of Huso huso using microsatellite DNA markers and it was used for the first time in Romania.

Keywords: beluga sturgeon, microsatellite, genetic characterization, allelic variation.

Introduction

Sturgeons (order Ancipensiformes) represent a very ancient group of fish which appeared in the Upper Cretaceous [1]. These “living fossils” are endangered because of anthropogenic influences such as overexploitation, habitat alteration, barriers for migration, loss of spawning habitat and water pollution.

Huso huso, like other marine migratory sturgeons in Romania, swimming up the Danube River for reproduction, represents an appealing fish species from both a scientific and commercial point of view. While the effectives of *Huso huso* have significantly decreased in the last decades, the economical interest has constantly increased due to the incredible value of the roe known commercially as beluga caviar, which represents one of the most expensive food items in the world. Under these circumstances, it is necessary to develop conservation programs for Romanian sturgeon species, implying extensive knowledge of its genetic diversity and of the evolutionary relationships among geographic populations.

Genetic research on sturgeons has been limited to a few studies on chromosomal numbers and cellular DNA content, allozymes and mitochondrial DNA. The recent developments in molecular biology allow the detection of intra- and interspecific genetic differences and could provide the ability to characterize and quantify the extent of genetic variation in sturgeon populations. Multiplex polymerization chain reaction represents a technique of simultaneous amplification of multiple regions of a DNA template.

Microsatellites are efficient markers as they are evenly distributed across the genome and highly polymorphic. In addition, microsatellites can be scored from tissues non-destructively sampled [3]. They allow the evaluation of intraspecific genetic diversity, thus, microsatellite multiplexing is a powerful technique that can be successfully used in genetic studies.

Materials and methods

DNA extraction

Fin clips were collected from 23 individuals (adults and offspring) of *Huso huso* originating in the Danube River, where they migrate for spawning. Sturgeons’ fins were used for DNA extraction by a specific method [6]. The DNA concentration and quality were assessed spectrophotometrically at 260/280 nm.

PCR multiplex

We used seven pairs of primers to amplify seven microsatellite loci: LS-19, LS-34, LS-54, LS-57, LS-68, Aox 23 and Aox 45. The PCR conditions were optimized for each set of primer by varying the annealing temperature between 51-61˚C on a gradient termocycler IQ Cycler (BioRad).

For microsatellite loci detection we used the forward primers labeled with four different fluorescent dyes: PET, VIC, 6-FAM, NED (see Table 1). Amplification of the microsatellite loci was performed through two multiplex PCR reactions as follows: 2-Plex reaction for Aox 23 and LS-57, and 5-Plex reaction for LS-19, LS-34, LS-54, LS-68 and Aox 45.

Amplification reactions were carried out in 25 µL final volume and consisted of 1X PCR Buffer, $MgCl_2$, 200 µM of each nucleotide, DNA template, 0.4 µL of each primer, 0.5 units of AmpliTaq Gold DNA Polymerase and nuclease free water. Reaction mixes were amplified in GeneAmp 9700 PCR System (AppliedBiosystems) using the following program: a first denaturation step at 95˚C for 10 minutes, 40 cycles of denaturation at 95˚C for 30 seconds, annealing at 53˚C for 30 seconds, and extension at 72˚C for 60 seconds and a final extension step at 72˚C for 10 minutes.

The amplified fragments were loaded with the GeneScan-500 LIZ Size Standard into ABI Prism 310 DNA Genetic Analyzer. The results were examined with the GeneScan 3.1.2 and Genotyper 2.5.2 Softwares (AppliedBiosystems).

Table 1. Primers sequence.

Primer	Sequence	Color
LS-19 F LS-19 R	6-FAM CATCTTAGCCGTCTGGGTAC CAGGTCCCTAATACAATGGC	Blue
LS-34 F LS-34 R	VIC TACATACCTTCTGCAACG GATCCCTTCTGTTATCAAC	Green
LS-54 F LS-54 R	NED CATCTAGTCTTTGTTGATTACAG CAAAGGACTTTGAAACTAGG	Yellow
LS-57 F LS-57 R	PET GCTTGGTTGCTAGTTTGC GTACAGTATGAGACCACAGGC	Red
LS-68 F LS-68 R	NED TTATTGCATGGTGTAGCTAAAC AGCCCAACACAGACAATATC	Yellow
Aox 23 F Aox 23 R	6-FAM CAGTGTGCTAGCTTCTCAATA GTTAGCTTAACCATGAATTGTG	Blue
Aox 45 F Aox 45 R	PET TTGTTCAATAGTTTCCAACGC TGTGCTCCTGCTTTTACTGTC	Red

Results and discussions

The genetics of beluga sturgeon is poorly understood because of their unique life history and sampling difficulties. Sexual maturation takes 10–15 years and subsequent spawning occurs at intermittent intervals of 1–2 years. Sampling enough individuals as juveniles or adults for population genetic studies is difficult because of their scarcity and low distribution over large geographic areas during most of their life. Only during the spawning season are the adults vulnerable to sampling. In addition, nonlethal genetic techniques are required to study beluga sturgeon because the species is endangered.

Techniques recently developed to examine variation at microsatellite loci facilitate genetic studies of beluga sturgeon in three ways. First, microsatellite loci can be examined with nonlethal tissue sampling. DNA for microsatellite studies can be extracted from fin, barbell, or scale tissue [8]. Second, microsatellite loci provide greater discriminatory power than other genetic markers, especially when genetic variation exists at low levels in other genetic characters. Microsatellite loci have a much higher mutation rate and consequently often more alleles per locus than allozymes [2] and thus permit more potential genetic differences at microsatellite loci to accumulate among populations. However, confirmation of Mendelian inheritance of microsatellite variation is important, especially for polyploid derived species, to correctly analyze allelic variation.

The aim of our study was to assess the genetic diversity within the beluga sturgeon species from the Black Sea and a panel of seven microsatellites was used for this evaluation.

In our experiment we successfully amplified all seven microsatellite loci obtaining allele peaks of different sizes (see Table 2). These involved tri- (LS-19, LS-34, LS-57) and tetra- (LS-68, LS-54) nucleotide microsatellite markers originally designed for the American lake sturgeon, *Acipenser fulvescens* [4]. Primers for

two additional microsatellite loci, Aox 23 and Aox 45, initially used for the Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*), were obtained from DNA sequences available in the GenBank Database [3].

Table 2. Characteristics of seven *Huso huso* microsatellite loci.

Locus	Dye	Size (bp)	Alleles Number	Ploidy Level
LS-19	6-FAM	136 – 156	3	Diploid
LS-34	VIC	140 – 150	2	Diploid
LS-54	NED	233 – 253	5	Diploid
LS-57	PET	193 – 211	4	Polyploid
LS-68	NED	148 – 152	2	Diploid
Aox 23	6-FAM	89 – 136	5	Diploid
Aox 45	PET	151 – 155	2	Diploid

Genotypes for these loci were determined for 23 offspring and adult sturgeons. The number of allele peaks depends on the level of ploidy of the analyzed species and on whether the individual tested is heterozygote or homozygote. The size of the alleles at individual loci varied between 89 and 253 bp. A low level of polymorphism was observed for the *Huso huso* population studied. Only 2 to 5 alleles where observed with a mean of 3.3 alleles per locus. The most polymorphic loci are Aox 23, LS-54 and LS-57 with only 5, respectively 4 different alleles per locus. The others loci present a low level of polymorphism in the population.

Examples of electrophoregrams for sturgeon specific loci are shown in Figures 1, 2, 3, 4.

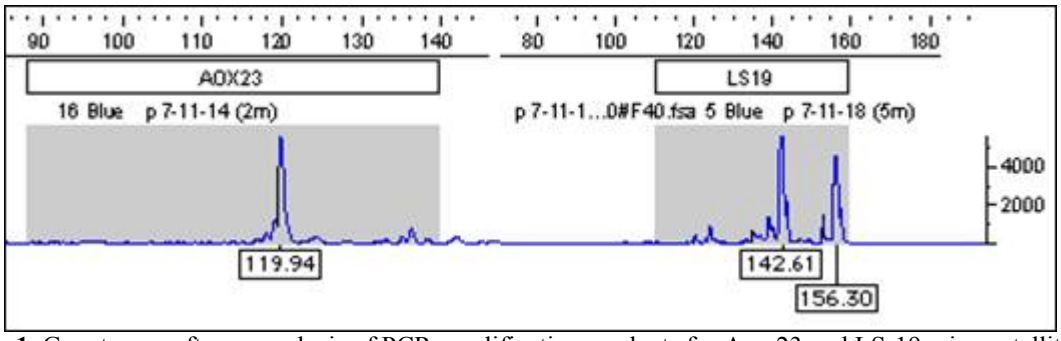

Figure 1. Genotyper software analysis of PCR amplification products for Aox 23 and LS-19 microsatellite loci.

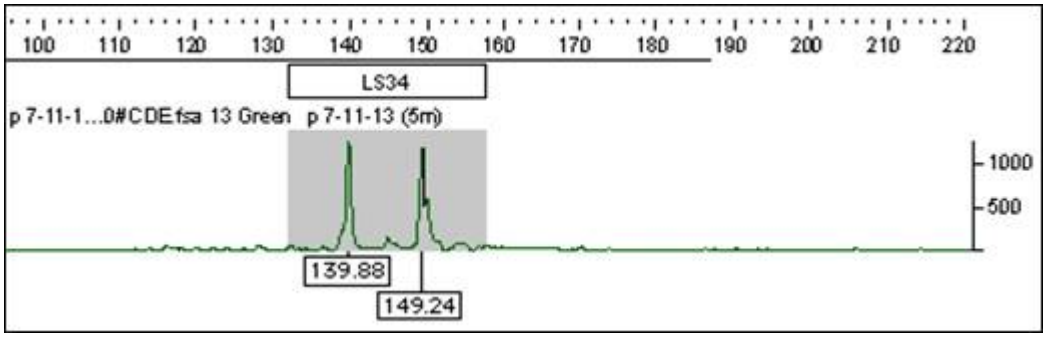

Figure 2. Genotyper software analysis of PCR amplification product for LS-34 microsatellite locus.

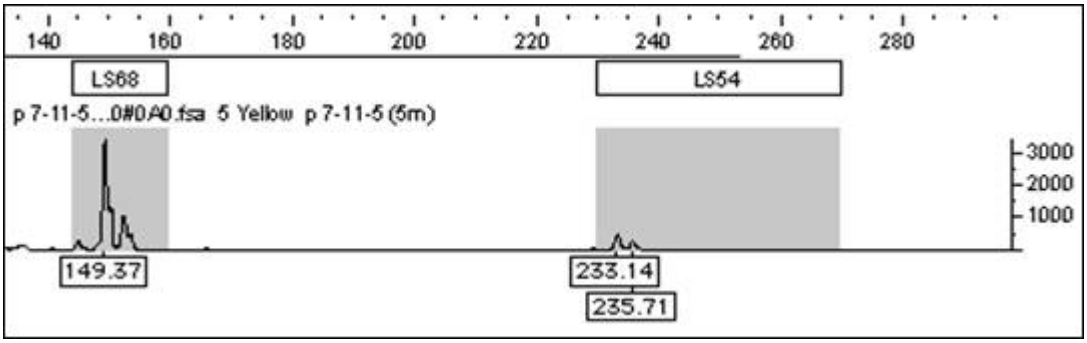

Figure 3. Genotyper software analysis of PCR amplification products for LS-68 and LS-54 microsatellite loci.

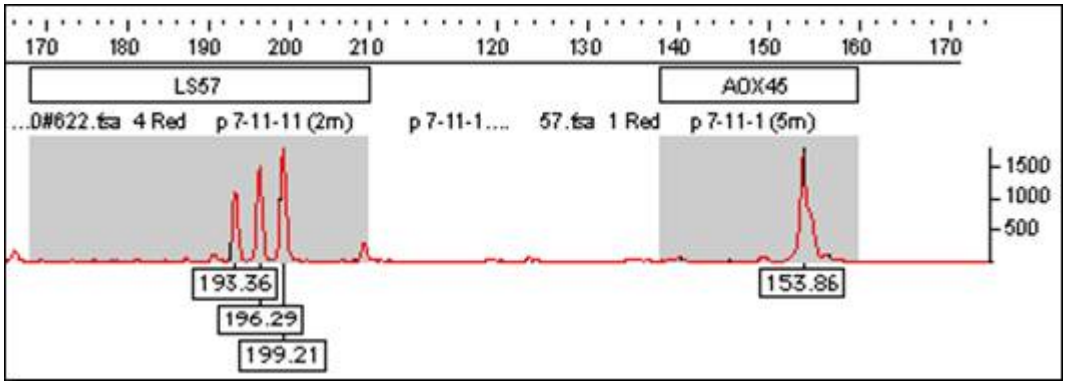

Figure 4. Genotyper software analysis of PCR amplification products for Aox 45 and LS-57 microsatellite loci.

Conclusions

Microsatellite loci have a much higher mutation rate and consequently offer more alleles per locus than other markers, thus permitting more potential genetic differences to be identified.

Our results demonstrate a correct amplification of all seven microsatellites tested. This technique could represent a very good method for individual identification and genetic distance evaluation within sturgeon stocks. It also could allow the evaluation of intra-specific genetic diversity and provide the ability to characterize the genetic variations in sturgeon populations.

This study contributes to the evaluation of genetic diversity, and molecular characterization of *Huso huso* using microsatellite DNA markers. The co-amplification of the microsatellites by PCR multiplex reaction is a very good method allowing the evaluation of intraspecific genetic diversity used for the first time in Romania. It will allow us to characterize the genetic variations in Black Sea sturgeon species and populations.

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