Microsatellite Variation in Russian Sturgeon (*Acipenser gueldenstaedtii*) from Aquaculture

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Abstract
Most sturgeon species today are on the brink of extinction because of anthropogenic influences, but in the same time, the economical interest regarding these species was constantly increasing, especially because the great value of the roe. In order to satisfy the increasingly high-demand sturgeon farms began developing in the last decades and are nowadays the main producers of caviar and other sturgeon products. Due their characteristics (high polymorphism, high power of discrimination, codominant Mendelian inheritance), microsatellites prove themselves very usefull in population genetics studies. In our study we analyzed the cross-amplification and the polymorphism of seven microsatellite loci (LS-19, LS-54, LS-57, LS-68, Aox9, Aox23, and Aox45) in *Acipenser gueldenstaedtii* population from aquaculture. The microsatellite markers were originally designed for the American lake sturgeon, *Acipenser fulvescens* and Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*. We successfully amplified all microsatellite loci obtaining allele peaks of different sizes which were analyzed by capilary electrophoresis. The number of allele ranged beetwen 4 (Aox23) and 12 (LS-54). This technology has great potential for investigating the genetic diversity of the wild sturgeons' population and, also, might be extended to aquaculture studies aiming the monitoring of genetic variation in comercial breeding programs.

Keywords: *Acipenser gueldenstaedtii*, aquaculture, genetic diversity, microsatellites.

1. Introduction

Sturgeons are representing one of the oldest groups of fishes in the world and that has successfully survived several mass extinction events during their history. It is considered that some factors regarding their life history like long lifespan, delayed maturation, large size or anadromy which made them resilient to global environmental modifications in the past are now responsible to their high susceptibility to extinction under anthropogenic impact [1]. In order to protect this group of fish, all sturgeon species has been regulated under Convention on International Trade in Endangered Species of Wild Fauna and Flora since 1998. The genetic variability within stocks can be assessed using DNA markers such as microsatellites. Microsatellites are nuclear markers consisting in short repetitive sequence, dispersed in the entire genome. Taking into account some of their characteristics like the relatively small size, the easiness of amplification via PCR reaction, the codominant inheritance and the high level of polymorphism, these markers proven to be very useful to assess the genetic diversity and the structure of populations. The main limitation in microsatellite analysis in sturgeons is related to the complexity of their genome. Birstein et al., 1997 [2], consider that the sturgeon species with ~120 chromosomes like *Acipenser stellatus* and

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Acipenser ruthenus are tetraploids, while the one with ~250 chromosomes like *Acipenser gueldenstaedtii* are octaploids. Previous studies based on microsatellites [3] in *Acipenser stellatus* showed that some loci present a disomic profile and some are tetrasomic, which suggest that this species is in a transitional state of ploidy.
In the case of Russian sturgeon, there are few studies based on microsatellites analysis, concerning the populations of *Acipenser gueldenstaedtii* from the Black Sea, Caspian Sea and Azov Sea [4]. In Romania, there are no studies regarding the genetic diversity of sturgeon species from aquaculture.

2. Materials and methods

Sampling and DNA extraction
Samples were harvested without endangering the life of the individual. Fin clips were collected from 40 individuals of *Acipenser gueldenstaedtii* originating from aquaculture. Genomic DNA was extracted from fin tissue by a specific method [5]. The DNA concentration and quality were assessed spectrophotometrically at 260/280 nm.
PCR amplification and genotyping
We used seven pairs of primers to amplify microsatellite loci: LS-19, LS-54, LS-57, LS-68, Aox9, Aox23, and Aox45. The PCR conditions were optimized for each set of primer by varying the annealing temperature between 50˚C and 60˚C on a gradient termocycler.
For alleles detection we used the forward primers labeled with four different fluorescent dyes: PET, VIC, 6-FAM, NED (Table 1).

Table 1: Primers sequences and flourescent dyes.

LS-19 F	6-FAM-catcttagccgtctgggtac
LS-19 R	caggtccctaatacaatggc
LS-68 F	NED-ttattgcatggtgtagctaaac
LS-68 R	agcccaacacagacaatatc
Aox 9 F	VIC-gatattggagctgtgcattg
Aox 9 R	acattgtttggtaggccagc
Aox23 F	6-FAM-ttgtccaatagtttccaacgc
Aox23 R	tgtgctcctgcttttactgtc
LS-54 F	NED-ctctagtctttgttgattacag
LS-54 R	caaaggacttgaaactagg
LS-57 F	PET - gcttggttgctagtttgc
LS-57 R	gtacagtatgagaccacaggc
Aox45 F	PET-ttgtccaatagtttccaacgc
Aox45 R	tgtgctcctgcttttactgtc

Amplification of the microsatellite loci was done by two multiplex PCR reactions as follows: 2-Plex reaction for Aox23 and LS-57, and 5-Plex reaction for LS-19, LS-54, LS-68, Aox9, and Aox45.
Amplification reactions were carried out in 25 µL final. 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 53/57˚C for 30 seconds, and extension at 72˚C for 60 seconds and a final extension step at 72˚C for 20 minutes. The amplified fragments were loaded with the GeneScan-500 LIZ Size Standard into ABI Prism 310 DNA Genetic Analyzer (Applied Biosystems) and the results were analyzed with the GeneScan and Genotyper Softwares (Applied Biosystems).

3. Results and discussion

Due to their ancient origin, sturgeons are considered to be genuine living fossils, being migratory fishes with a complex biology. Almost all sturgeon species are vulnerable or threatened with extinction and are included on the IUCN Red List of Threatened Species. Currently, three of the four sturgeon species from the Lower Danube, including *Acipenser gueldenstaedtii*, are considered critically endangered.
In the Lower Danube, the the Russian sturgeon populations are on the brink of extinction as a result of an „Allee" effect similar to the one that has led to the disappearance of the *Acipenser sturio* from the rivers in Western Europe [6]. In Romania, supporting stocking programs were initiated in 2005 for stellate and Russian sturgeons to compensate the effects of the intensive fishing from the last century [7].
Microsatellites are powerful markers used to assess the genetic differences on individual and population level. Thus, these markers are very suitable to investigate the genetic diversity within species and to compare wild and captive populations [8].
In our experiment we successfully amplified all seven microsatellite loci obtaining allele peaks of different sizes (Table 2).

Table 2: Characteristics of seven microsatellite loci from *Acipenser gueldenstaedtii*.

Locus	Size (bp)	Alleles number	Ploidy level
LS-19	117-153	6	Polyploid
Aox23	118-128	4	Diploid
LS-54	129- 222	12	Polyploid
LS-68	120-237	6	Polyploid
Aox9	200-240	8	Polyploid
LS-57	145-230	11	Polyploid
Aox45	124-240	6	Polyploid

The primers for the loci were originally designed for the American lake sturgeon, *Acipenser fulvescens* [9] and Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus* [10]. Genotypes for these microsatellites were determined for 40 individuals. 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 117 and 240 bp. Four to 12 alleles where observed with a mean of 7.5 alleles per locus. The most polymorphic locus was LS-54. Others loci (e.g. Aox23) presented a lower polymorphism in population. An example of electrophoregrams for the Russian sturgeon specific loci is shown in Figures 1-4.

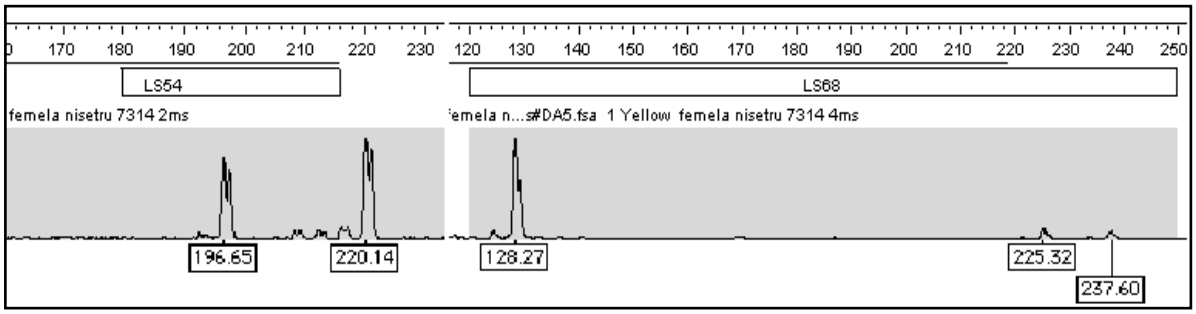

Figure 1. Genotyper Software analysis of PCR amplification product for LS-54 and LS-68 microsatellite loci.

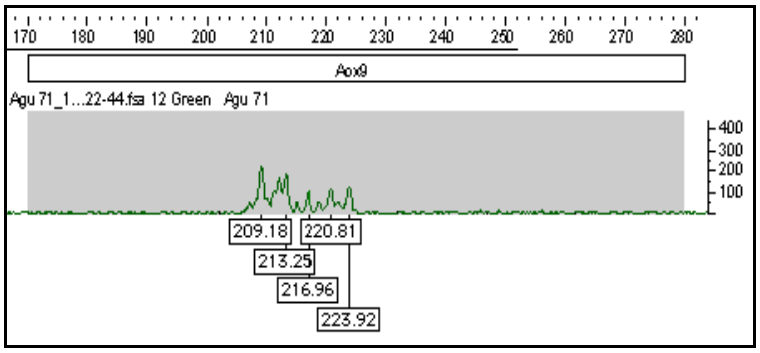

Figure 2. Genotyper Software analysis of PCR amplification product for Aox9 microsatellite locus.

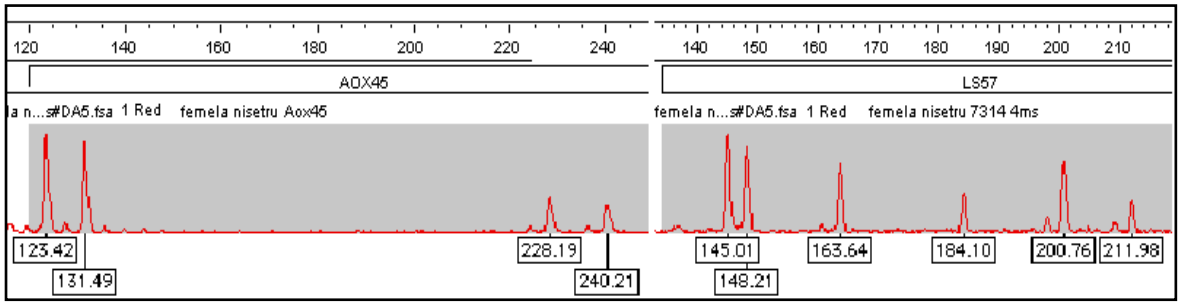

Figure 3. Genotyper Software analysis of PCR amplification product for Aox45 and LS-57 microsatellite loci.

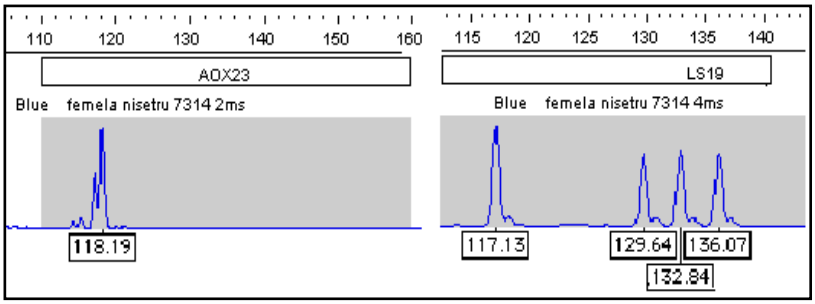

Figure 4. Genotyper Software analysis of PCR amplification product for Aox23 and LS-19 microsatellite loci.

4. Conclusions

Our results demonstrate a correct amplification of all microsatellites loci tested. This technique could represent a very easy and efficient method for evaluation of genetic diversity within sturgeon stocks. It also could provide the ability to characterize the genetic variations in sturgeon populations' individual identification and genetic distance evaluation.

This study contributes to the molecular characterization of *Acipenser gueldenstaedtii* from aquaculture using microsatellite DNA markers. Six of seven loci analyzed in this study were polymorphic confirming that the microsatellite markers used are suitable for genetic diversity studies.

Acknowledgements

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