

MICROSATELITTE DNA VARIATION IN THE BLACK SEA STELLATE STURGEON, *ACIPENSER STELLATUS*

VARIAȚIA ADN MICROSATELIT LA PASTRUGA DIN MAREA NEAGRĂ - *ACIPENSER STELLATUS*

DUDU ANDREEA *, GEORGESCU S. E.*, LUCA CĂTĂLINA*, SUCIU R.**,
COSTACHE MARIETA*

*University of Bucharest, Molecular Biology Center

**Danube Delta National Institute, Tulcea

*Marine migratory sturgeons represent one of the most important fishery resources, both from a scientific and commercial point of view. Anthropogenic influence and other factors caused the dramatically decrease of sturgeon stocks in Romania. This required the development of conservation programs and genetic studies to distinguish different 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. In our study we evaluated the genetic diversity in stellate sturgeons, *Acipenser stellatus*, from the Black Sea, using microsatellite markers. We selected a set of seven microsatellite loci that are amplified by multiplex PCR reaction in order to estimate the differences between individuals of *Acipenser stellatus*, due to their high allelic variation. High level of polymorphism was observed for the population of sturgeons studied.*

Key words: sturgeon, microsatellite, genetic characterization, allelic variation.

Introduction

Marine migratory sturgeons, including *Acipenser stellatus*, represent one of the most important and valuable fish population both from a scientific and commercial point of view for the economy of Romania and some other riverside countries (Ciolac *et al.*, 2004).

Sturgeons (order *Ancipensiformes*) represent a very ancient group of fishes which at present time are endangered because of anthropogenic influences, such as over-exploitation, habitat alteration and pollution, loss of spawning habitat and barriers to migration. All these factors caused the dramatically decrease in the last past decades of all sturgeon stocks, including *Acipenser stellatus*, in the Romanian waters and this fact requires the development of conservation programs (Paraschiv *et.al.*2006) and studies of the genetic diversity and the evolutionary relationship 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 sturgeon's extended age to maturity (5-20 years), extended period between spawning cycles and their residence in deep waters characterize them as a difficult species for genetic investigations of behavior, selective breeding or population structure (May *et al.*, 1997). The recent developments in molecular biology allow the use of microsatellites loci for characterization of genetic variation in sturgeon populations. More than other methods, the microsatellite markers allow the evaluation of intraspecific genetic diversity and offer us the possibility to evaluate small differences between populations due to their high level of allelic variation. Microsatellite loci have a much higher mutation rate and consequently offer more alleles per locus than other markers (Grant *et al.*, 1999) and thus permit more potential genetic differences at microsatellite loci.

Materials and Methods

Microsatellite loci can be examined by using samples harvested without endangering the life of the individual. Fin clips were collected in year 2007 from 33 individuals (17 adults and 16 full sib offspring) of *Acipenser stellatus* originating in the Danube River, where they migrate for spawning. Genomic DNA was extracted from fin tissue by a specific method (Taggart *et al.*, 1992).

We used seven primer pairs to amplify seven microsatellite loci: LS-19, LS-34, LS-54, LS-57, LS-68, Aox 23, Aox 45. Initially, the PCR conditions were optimized by varying the annealing temperature between 51-60°C on a gradient thermocycler IQCycler (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 done by 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 4

Table 1: Primer sequences.

| Primer | Sequence |
|----------------------|--|
| LS-19 F LS 19 R | 5' 6-FAM-CATCTTAGCCGTCTGGGTAC CAGGTCCCTAATACAATGGC |
| LS 34 F LS 34 R | 5' VIC - TACATACCTTCTGCAACG GATCCCTTCTGTTATCAAC |
| LS 54 F LS 54 R | 5' NED - CATCTAGTCTTTGTTGATTACAG CAAAGGACTTTGAACTAGG |
| LS 57 F LS 57 R | 5' PET - GCTTGTTGCTAGTTTGC GTACAGTATGAGACCACAGGC |
| LS 68 F LS 68 R | 5' NED - TTATTGCATGGTGTAGCTAAAC AGCCCAACACAGACAATATC |
| Aox 23 F Aox 23 R | 5' 6-FAM - CAGTGTGCTAGCTTCTCAATA GTTAGCTTAACCATGAATTGTG |
| Aox 45 F Aox 45 R | 5' PET - TTGTTCAATAGTTTCCAACGC TGTGCTCCTGCTTTTACTGTC |

PCRs were done in 25 µL final volume with 1X PCR Buffer, Mg Cl₂, 200 µM of each nucleotide, DNA template, 0.4 µL of each primer, 2 units of AmpliTaq Gold DNA Polymerase and nuclease free water. The amplification was carried out on GeneAmp 9700 PCR System (Applied Biosystems) under the following conditions: initial denaturing step at 95°C for 10 minutes, 45 cycles of denaturing for 30 seconds, 52°C annealing for 30 seconds, 72 °C extension for 60 seconds; and a final extension at 72 °C for 60 minutes.

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

Results and Discussions

The aim of our study was to assess the genetic diversity within sturgeon species from Romania 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 that were originally designed for the American lake sturgeon, *Acipenser fulvescens*, (May *et al.* 1997). Primers for two additional microsatellite loci, Aox 23 and Aox 45, which were initially used for the Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*), were obtained from DNA sequences available in the GeneBank Database (King *et al.*, 2001).

Genotypes for these loci were determined for 33 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 95 and 200 bp. A high level of polymorphism was observed for the population of *Acipenser stellatus* studied. Seven to 13 alleles were observed with a mean of 8 alleles per locus. The most polymorphic locus is Aox 23. Others loci (LS 34) present a lower polymorphism in population.

Table 2: Characteristics of seven *Acipenser stellatus* microsatellite loci.

| Locus | Dye | Size (bp) | Alleles number | Ploidy level |
|--------|-----|-----------|----------------|--------------|
| LS 19 | FAM | 113 – 136 | 7 | Diploid |
| LS 34 | VIC | 147 | 1 | Diploid |
| LS 54 | NED | 160 – 192 | 8 | Diploid |
| LS 57 | PET | 170 – 200 | 11 | Polyploid |
| LS 68 | NED | 116 – 144 | 8 | Polyploid |
| Aox 23 | FAM | 95 – 137 | 13 | Diploid |
| Aox 45 | PET | 119 – 148 | 8 | Polyploid |

One example of electrophoregrams for the sturgeon specific loci is shown in Figures 1, 2, 3, 4.

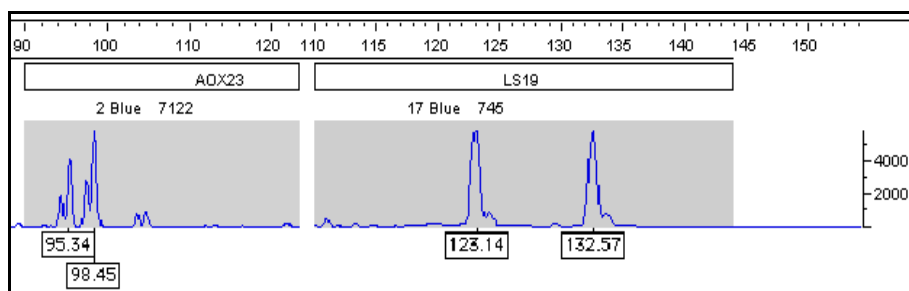


Figure 1: Genotyper software analysis of PCR amplification products for Aox 23 and LS-19 microsatellite loci (blue markers).

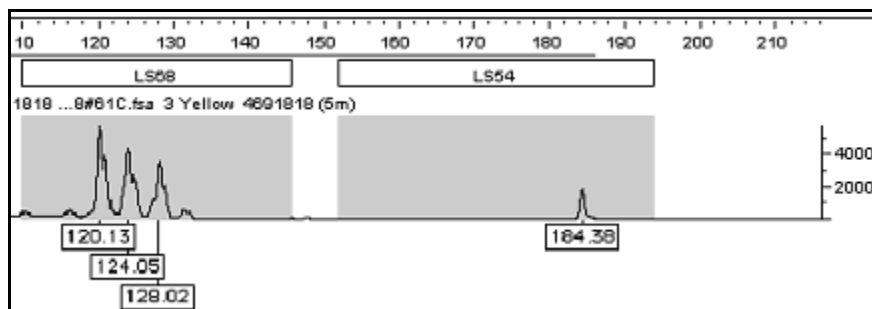


Figure 2: Genotyper software analysis of PCR amplification products for LS-54 and LS-68 microsatellite loci (yellow markers).

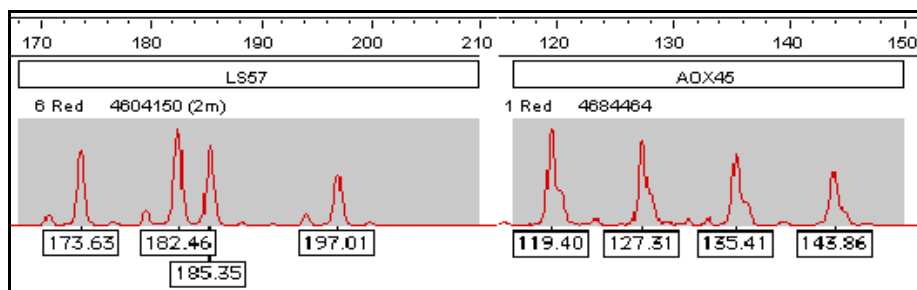


Figure 3: Genotyper software analysis of PCR amplification products for LS-57 and Aox 45 microsatellite loci (red markers).

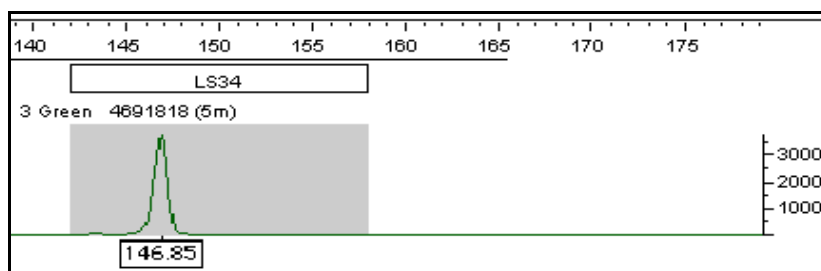


Figure 4: Genotyper software analysis of PCR amplification product for LS-34 microsatellite locus (green marker).

Conclusions

This study contributes to the evaluation of genetic diversity, and molecular characterization of *Acipenser stellatus* 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. Our results demonstrate a high level of polymorphism for all studied individuals. 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.

Bibliography

1. **Ciolac, A., N., Partiche** (2004) – *Biological aspects of main marine migratory sturgeons in Romanian Danube River*. Applied ecology and environmental research 3 (2): 101-106.
2. **Grant, W.S., J.L., Garcia –Marin, F.M., Utter** (1999) – *Defining populations' boundaries for fishery management*, Genetics in Sustainable Fisheries Management: 27- 42.
3. **King, T.L., B.A., Lubinski, A.P., Spidle** (2001) – *Microsatellite DNA variation in Atlantic sturgeon (Acipenser oxyrinchus oxyrinchus) and cross-amplification in the Acipenseridae*, Conservation Genetics 2: 103 -119.
4. **May B., C.C., Krueger, H.L., Kincaid** (1997) – *Genetic variability at microsatellite loci in sturgeon: primer sequence homology in Acipenser and Scaphirinchus*, Can. J. Fish. AquatSci 54: 1542 – 1547.
5. **Paraschiv, M., R., Suci, M., Suci**, (2006) - *Present state of sturgeon stocks in the lower Danube River, Romania*. In: Proceedings 36th International Conf. of IAD. Austrian Committee Danube Research / IAD, Vienna: 152 – 158.
6. **Pyaskowit J.D., C.C., Krueger, H.L., Kincaid, B., May** (2001) – *Inheritance microsatellite loci in polyploidy lake sturgeon (Acipenser fulvescens)*. Genome 44, 185 – 191.
7. **Taggart J.B., Hynes, R.A., Prodohl, P.A., Ferguson, A.** (1992) – *A simplified protocol for routine total DNA isolation from salmonid fishes*, Journal of Fish Biology 40: 963 - 965.
8. **Wirgin I.I., J.R., Waldman** (1994) - *What DNA can do for you*, Fisheries