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Genetic and Morphometric Assessment of Sturgeon Species Bred in Closed Water Supply

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Abstract:
This article shows the results of studying genetic diversity and morphometric assessment of sturgeon species bred in closed water supply (CWS). Species belonging and microsatellite loci of sturgeon were analyzed in a sample of 100 bions. By the results of species identification, purebred in terms of genetic specimens sturgeon species were selected for forming sturgeon broodstock characterized by purity of the species, highly heterogeneous and unique in intra-species structure of sturgeon. During the analysis of correlation with microsatellite loci with the results of PCR-based diagnostics of the species, the most informative microsatellite locus was locus AfuG41.

Keywords: Sturgeon, DNA, identification, microsatellite loci, CWS.

INTRODUCTION

Sturgeons are highly valuable both in environmental context for preserving biodiversity and in food context, being the source of caviar and commercial sturgeon. However, poaching has significantly reduced their population. Today, catastrophic impoverishment of sturgeon stock has resulted in rapidly increased activity of international and national environmental organizations. Unfortunately, despite numerous programmes aimed at restoring sturgeon population, the situation continues deteriorating. Most sturgeon populations are on the verge of extinction. One of the most efficient methods for sturgeon preservation and breeding can be sturgeon growing in a closed water supply system (CWS). Some progress has been made by foreign scientists. Hungarian specialists breed the following species in CWS: sterlet; paddlefish; Russian sturgeon; Siberian sturgeon; white sturgeon, and various sturgeon hybrids. These are further works aimed at creating and operating broodstocks where Hungarian researchers see the prospects of developing sturgeon breeding [1].

Studies in the United States about the formation and operation of white sturgeon broodstocks were aimed at both obtaining caviar from the aquaculture, and at artificial reproduction of populations of this species [2].

In France, broodstock of Siberian sturgeon is formed by containing the brood in pools and feeding it with artificial feed with the aim of obtaining seeding for commercial growing; in this country there are other programmes of Atlantic sturgeon artificial reproduction [3, 4].

Significant progress in forming and operating sturgeon broodstocks was reached by Italian researchers. Thus, the Adriatic sturgeon broodstock sturgeon was created in 1988; the posterity obtained from it is used for reproduction purposes; with that, unique evidence-based programmes are implemented, including those with the participation of other European researchers, and extensive research is made [5, 6, 7, 8].

Broodstocks were formed in installations with CWS. Water temperature was maintained in the range between 20 and 24°C that was optimum for rapid growth and accelerated development of sturgeons' reproductive system.

Sturgeon breeding in the conditions of CWS is a promising and innovative method of preserving and enhancing valuable sturgeon breeds. But, along with the fact that CWS system is easily controlled, it is also a closed system, too. The isolated nature of the system results in reduced genetic diversity and undesirable genetic mutations, such as congenital deformities and offspring weakness. In this respect, it is necessary to maintain constant breeding control. Appraisal and keeping livestock documentation do not provide complete information due to the peculiarities of the subject, namely, fish (the absence of clear morphological differences and peculiarities). The use of molecular biological research methods may be promising for fish breeding. This approach would allow maintaining the genetic polymorphism of the broodstock species composition, and avoiding future congenital genetic pathologies. It is therefore advisable to perform genetic certification of sturgeons, which will allow removing the livestock with undesirable mutations from breeding lines.

The research is aimed at creating broodstock - living collections - sturgeon species; formation of a living gene bank of pure sturgeon species that are highly heterogeneous and unique in terms of intra-species structure through DNA genetic analysis. The existence of such broodstocks will allow preserving the gene pool of the Urals-Caspian population of sturgeon species in the controlled conditions of hatcheries, and ensuring annual replenishment of the sturgeon population in the Caspian Sea with juveniles.

For the purpose of obtaining the above goal, the following tasks are set:

- morphometric assessment of sturgeon species;
- sampling of the tissue of sturgeon species grown in CWS, and DNA extraction from them;
- species identification based on the DNA of the sturgeon species grown in CWS; and
- microsatellite analysis of sturgeon loci grown in CWS.

METHODS

The molecular biological research based on DNA identification and analysis of sturgeon microsatellite loci was performed with the use of special molecular genetic methods. For DNA extraction, parts of fins with the size of 1×1.5 cm were taken from 100 (n=100) sturgeons. In the process of DNA extraction from fins, standard laboratory equipment for molecular microbiology was used: automatic measurers, centrifuges, equipment for electrophoresis, visualization and documenting gels, refrigerators, freezers, vortices, etc. During DNA extraction from sturgeon tissues, PureLink Genomic DNA (K182002, Thermofisher) kit for DNA extraction was used. DNA concentration was determined on a fluorimeter (SYBR-gold dye, QUBIT device, Invitrogen) [9-18].

For species identification, primers listed in Table 1 were used.

PCR was performed with the following parameters: preheating at 95°C – 2 min, 35 cycles (92°C - 20 sec., 57°C – 30 sec., 72°C – 30 sec.), and final synthesis at 72°C for 10 minutes. In all reactions, Encyclo polymerase (Evrogen, Moscow) was used. The products of the reaction were separated in 1.5% agarose, stained with ethidium bromide and photographed in ultraviolet light [19].

Microsatellite loci were analyzed with the use of PCR with fluorescence-labeled primers followed by fragment separation on a capillary sequenator using the internal standard for each sample (Table 2). In the preliminary experiment, two most significant loci were selected. The used primers and annealing temperatures are shown in Table 2. Amplification was performed according to the following scheme: DNA pre-heating: 95°C – 10 min, synthesis of PCR products (35 cycles): melting – 95°C – 20 sec, primer annealing – 56°C – 15 sec, DNA synthesis – 72°C – 15 sec, and final chains building: 72°C – 5 min.

Fragment analysis in the conditions of capillary electrophoresis of amplification products was performed on 3500xL Genetic Analyzer (Applied Biosystems™). Cluster analysis was performed in application Bionumerics 7.0.

RESULTS AND DISCUSSION

During morphometric assessment of sturgeon species, the following parameters were measured: weight, large length, small length, caudal fin length, body height, body thickness, body girth, body girth before dorsal fin, girth at the level of the anal-and-genital opening, forehead width between eyes, head length, fatness index (Table 3).

According to the data in Table 1, the average value of body weight for great sturgeon was 9.89±2.94 kg, large length was 115.81±9.71 cm, relative body height was 17.38±1.75 cm, body thickness was 19.75±2.06 cm, forehead width between eyes was 10±1.13 cm, and head length was 26.38±5.50 cm. The variation coefficient for all morphometric indicators of great sturgeon did not exceed 32.10% (weight), standard mean square deviation ranged from 1.37 cm (forehead width between eyes) to 11.52 cm (large length).

Table 1 - Primers for sturgeon species identification

Name	Sequence (5'-3')	Used with primer	Product length (bps)	Species-specifity
AHR	TATACACCATTATCTCTATGT			All species
AGF	GCACAGACTATGTGGTATCCAGAA	AHR	420	Russian sturgeon
ABF	CAGATGCCAGTAACAGGCTGA	AHR	215	Russian sturgeon (baerii-like) and Siberian sturgeon
ABRM	TGTCTGTCTAGAACATAtG	ABF	182	Siberian sturgeon
HusF	TATCTATTACCTGCGAGCAGGCTG	AHR	374	Great sturgeon
NudF	TGTCTTTTCTGAAGGAGCTTTGC	AHR	329	Thorn sturgeon
RutF	GGGAATAACCGTTAATTTGG	AHR	190	Sterlet
SteF	GGGGTTCTTGGCATGTTGTGAGCG	AHR	266	Starred sturgeon

Table 2 - Primers for microsatellite analysis

Locus	Primer sequences	Repetitions	Annealing temperature
AfuG41	Direct: 5’-TGACTCACAGTAGTATTATTTATG-3’ Reverse: 5’-TGATGTTTGCTGAGGCTTTTC-3’	(GATA)nTA(GATA)n	56°C
AfuG51	Direct: 5’-ATAATAATGAGCGTGCTTTCTGTT-3’ Reverse: 5’- ATTCCGCTTGCGACTTATTTA-3’	(CAAA)n	56°C

Table 3 – Morphometric parameters of sturgeon

Indicators	Species of fish														
	Great sturgeon (6+)			Russian sturgeon (8+)			Russian sturgeon (6+)			Sterlet (5+)			Siberian sturgeon (6+)		
	X ± x	σ	Cv,%	X ± x	σ	Cv,%	X ± x	σ	Cv,%	X ± x	σ	Cv,%	X ± x	σ	Cv,%
Weight, kg	9.89±2.94	3.18	32.10	12.27±2.28	2.81	22.9	4.05±1.25	1.45	35.77	3.09±0.76	0.69	22.45	4.61±0.98	1.19	25.80
Large length, cm	115.81±9.71	11.52	9.94	122.85±6.07	7.47	6.08	97.87±9.86	11.2	11.44	78.67±3.64	5.09	6.48	102.97±5.23	6.52	6.33
Small length, cm	100.44±6.74	9.18	9.14	111.3±6.77	8.14	7.32	84.67±8	9.59	11.33	69.33±3.64	5.47	7.89	91.67±4.64	6.05	6.60
Length of caudal fin, cm	24.31±2.56	2.99	12.32	23.75±2.98	3.55	14.94	20.27±2.50	2.89	14.24	12.13±1.76	2.09	17.25	22±1.93	2.21	10.03
Body height, cm	17.38±1.75	2.03	11.67	18.55±1.90	2.25	12.11	11.32±1.07	1.34	11.83	8.50±0.93	1.14	13.41	11.83±0.94	1.24	1.54
Body thickness, cm	19.75±2.06	2.46	12.47	18.1±1.54	1.92	10.61	10.73±1.50	1.79	16.65	6.87±0.83	0.96	13.93	10.23±0.82	1.12	10.91
Body girth, cm	52.25±7.47	8.21	15.72	54±4	4.75	8.80	34.97±3.83	4.54	12.99	31.13±2.16	2.75	8.84	37.07±3.66	4.32	11.65
Body girth before dorsal fin, cm	34.56±5.26	5.74	16.62	34.95±4.26	4.83	13.83	17.83±3.57	4.52	25.37	19.93±2.06	2.41	12.08	22.40±3.07	3.95	17.66
Girth at the level of the anal-and-genital opening, cm	20.38±2.72	3.44	16.90	19.75±2.13	2.53	12.80	12.20±2.63	3.17	25.95	12.07±1.56	2.21	18.27	12.37±2.85	3.61	29.19
Forehead width between eyes, cm	10±1.13	1.37	13.69	9.2±1.34	1.57	17.05	6.83±0.89	1.16	16.93	4.67±0.44	0.47	10.10	8.47±0.56	0.62	7.30
Length of head, cm	26.38±5.50	5.59	21.19	23.1±2.72	3.14	13.61	17.40±1.76	2.23	12.82	16.13±1.09	1.41	8.73	21.47±1.65	2.05	9.53
Fatness index,%	0.64			0.66			0.43			0.63			0.42		

Note: X ± x are mean value and mean linear deviation, σ is standard deviation, CV is variation coefficient.

For Russian sturgeon (8+), the average body weight was 12.27±2.28 kg, large length was 122.85±6.07 cm with the relative body height of 18.55±1.90 cm, body thickness was 18.1±1.54 cm, forehead width between eyes was 9.2±1.34 cm, head length was 23.1±2.72 cm. The variation coefficient for all morphometric indicators of Russian sturgeon (8+) did not exceed 22.9% (weight), the standard mean square deviation ranged from 1.57 cm (forehead width between eyes) to 7.47 cm (large length). For Russian sturgeon (6+), the average body weight was 4.05±1.25 kg, large length was 97.87±9.86 cm with the relative body height of 11.32±1.07 cm, body thickness was 10.73±1.50 cm, forehead width between eyes was 6.83±0.89 cm, head length was 17.40±1.76 cm. The variation coefficient for all morphometric indicators of Russian sturgeon (6+) did not exceed 35.77% (weight), the standard mean square deviation ranged from 1.16 cm (forehead width between eyes) to 11.2 cm (large length).

For sterlet, the average body weight was 3.09±0.76 kg, large length was 78.76±3.64 cm with the relative body height of 8.50±0.93 cm, body thickness was 6.87±0.83 cm, forehead width between eyes was 4.67±0.44 cm, head length was 16.13±1.09 cm. The variation coefficient for all morphometric indicators of sterlet did not exceed 22.45% (weight), the standard mean square deviation ranged from 0.47 cm (forehead width between eyes) to 5.47 cm (small length).

During morphometric measurement for Siberian sturgeon, the average body weight was 4.61±0.98 kg, large length was 102.97±5.23 cm with the relative body height of 11.83±0.94 cm, body thickness was 10.23±0.82 cm, forehead width between eyes was 8.47±0.56 cm, head length was 21.47±1.65 cm. The variation coefficient for all morphometric indicators of Siberian sturgeon did not exceed 25.80% (weight), the standard mean square deviation ranged from 0.62 cm (forehead width between eyes) to 6.52 cm (large length).

The dependence of the main fish parameters (weight, large length) in determining the fatness index is most clearly shown in Figure 1.

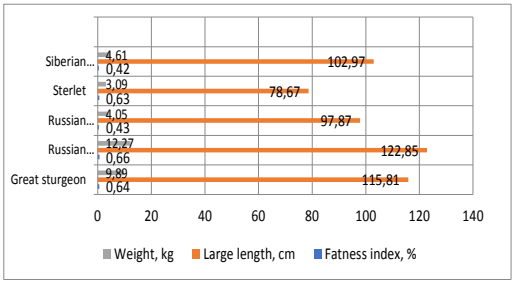

Figure 1 - Dependence of sturgeon fish basic parameters

For molecular-biological research, DNA was extracted from 100 samples of the sturgeon fins (Figure 2).

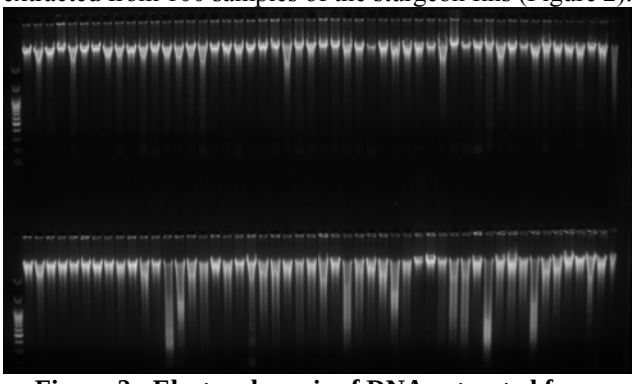

Figure 2 - Electrophoresis of DNA extracted from sturgeon fins

As a result of the species identification of samples taken from 100 bions, the following groups were identified by belonging to the genotype:

Group 1 with the genotype of Russian sturgeon and thorn sturgeon – samples No. 25 - 27, 30, 61 - 64, 66, 68, 78, 83, 95, 96;

Group 2 with the genotype of Russian sturgeon and thorn sturgeon – samples No. 21-24, 28, 29, 36-45, 67, 69, 71-75, 77, 79, 80, 94, 97, 98;

Group 3 with genotype of thorn sturgeon and great sturgeon - samples No. 31 to 35, 51-60;

Group 4 with genotype of thorn sturgeon only - samples No. 1-20, 65, 86-100;

Group 5 with genotype of thorn sturgeon only - samples No. 81, 82;

Group 6 with the genotype of Russian sturgeon and thorn sturgeon and starred sturgeon – samples No. 76;

Group 7 with the genotype of Russian sturgeon and thorn sturgeon and great sturgeon – samples No. 70;

Group 8 with genotype of thorn sturgeon and starred sturgeon - samples No. 46, 84;

Group 5 with genotype of sterlet only - samples No. 47-50, 89.

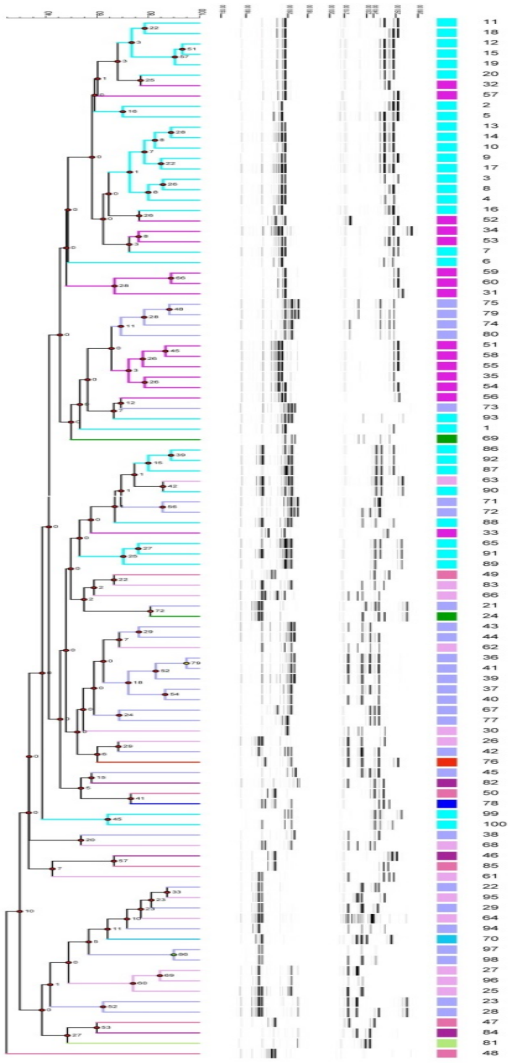

Figure 3 – The results of the combined cluster analysis by microsatellite loci AfuG41 and AfuG51

The research shows that a single bion may be the carrier of genotypes of several sturgeon species; this is the evidence of the fact that identification at the genetic level should be used during selection and breeding of purebred fish.

During the research, sturgeon species that were purebred in terms of genetic specimens were selected for forming sturgeon broodstock characterized by purity of the species, highly heterogeneous and unique in intra-specie structure of sturgeon.

During microsatellite analysis of a sampling from 100 sturgeon bions during trial fragment analysis, the variety of satellites AoxD16 and AoxD165 was negligible. At this stage of the research, satellites AfuG41 and AfuG51 were used, since they amplified better and had a larger difference in this sample.

Analysis of the results shows that it is possible to allocate 2 reliable clusters uniting related genotypes (Figure 3).

The combined cluster analysis revealed two clusters. During the identification of microsatellite loci correlation with the results of species PCR diagnostics, the maximum correlation was shown by analysis of locus AfuG41.

CONCLUSION

By the results of morphometric assessment, Russian sturgeon (8+) overrides other species by absolutely all indicators, followed by great sturgeon, Siberian sturgeon, then Russian sturgeon (6+) and sterlet. The highest variation coefficient was observed in Russian sturgeon (6+) (weight) and was 35.77%, the lowest one was observed in Siberian sturgeon (body height); the coefficient of variation for this indicator was 1.54%. As for the mean root square deviation, here the relative stability of this value ranged between 0.47 cm in starlet (forehead width between eyes) and 11.52 cm in great sturgeon (large length). The highest fatness index was observed in Russian sturgeon (8+) - 0.66%, in great sturgeon it was only 0.64%, in sterlet - 0.63%; in Russian (6+) and Siberian sturgeon it was 0.43% and 0.42%, respectively.

By the results of species identification, sturgeon species purebred in terms of genetic specimens were selected for forming sturgeon broodstock characterized by purity of the species, highly heterogeneous and unique in intra-species structure of sturgeon. During the analysis of microsatellite loci correlation with the results of PCR-based diagnostic of the species, the most informative microsatellite locus was locus AfuG41.

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