FISHERIES & AQUATIC LIFE (2020) 28: 238 - 245
Archives of Polish Fisheries
DOI 10.2478/aopf-2020-0029

sciendo

SHORT COMMUNICATION

Molecular genetic characteristics of Atlantic sturgeon (*Acipenser oxyrinchus*) cultured in Poland: Report on a bilateral project

Aliaksandr Slukvin, Nina Balashenko, Marina Sasinovich, Vladimir Kostousov, Andrzej Kapusta

Received – 13 October 2020/Accepted – 07 December 2020. Published online: 31 December 2020; ©Inland Fisheries Institute in Olsztyn, Poland
Citation: Slukvin A., Balashenko N., Sasinovich M., Kostousov V., Kapusta A. 2020 – Molecular genetic characteristics of Atlantic sturgeon (*Acipenser oxyrinchus*) cultured in Poland: Report on a bilateral project – Fish. Aquat. Life 28: 238-245

Abstract. Using 8 microsatellite markers (Spl-106, Spl-101, ATR-113, Aox23, An20, AoxD161, AoxD165, AfuG41) and mtDNA species-specific region flanked by the tRNAThr coding region and D-loop, it was found that Atlantic sturgeon stocking material planned for transport from Poland to Belarus belonged to the species *Acipenser oxyrinchus oxyrinchus* Mitchill. No interspecific hybrids were found. All of the Atlantic sturgeon individuals examined as part of this study belonged to a Canadian population.

Keywords: Molecular genetic research, Atlantic sturgeon, stocking, microsatellite markers, mtDNA tRNAThr, D-loop, site S1/G1, identification

Introduction

Wildlife conservation and environmental management is one of the most pressing issues in the world (Reid et al. 2019). Without special measures for restoration, protection, rational use, effective measures, and scientifically based decisions, some animals, in particular some species of diadromous fish, will not survive (Kolman and Kapusta 2008). To date, Belarusian scientists have not participated in the restoration of the migratory Baltic population of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus* Mitchill) because of the lack of suitable stocking material for this fish species and the lack of studies on the possibilities of its introduction. Participation in the joint Belarusian-Polish project provides a unique opportunity for the Belarusian side to join an international consortium in solving one of the most important environmental problems of restoring the Baltic population of Atlantic sturgeon in the region and the implementation of the Helcom Action Plan (AP 2019). The stage-by-stage implementation of this work will allow in the future restoring to the territory of Belarus spawning migrations of Atlantic sturgeon. Spawning migrations of this species extended up the Nemunas River as far as Belarus (Berg 1932, Zhukov 1965). The restoration of *A. oxyrinchus* has recently been initiated in the Nemunas basin in Lithuania (Stakėnas and Pilinkovskij 2019).

Historical data on the occurrence of sturgeon are few in Belarus (Zhukov 1965, Zubei 2016). The research within the framework of the project is aimed

A. Slukvin [✉], N. Balashenko, M. Sasinovich, V. Kostousov
Institute of Genetics and Cytology, National Academy of Sciences of Belarus,
27 Akademicheskaya Street, 220072 Minsk, Republic of Belarus
e-mail: a.slukvin@igc.by

A. Kapusta
Department of Ichthyology, Hydrobiology and Aquatic Ecology, Stanisław Sakowicz Inland Fisheries Institute, Olsztyn, Poland

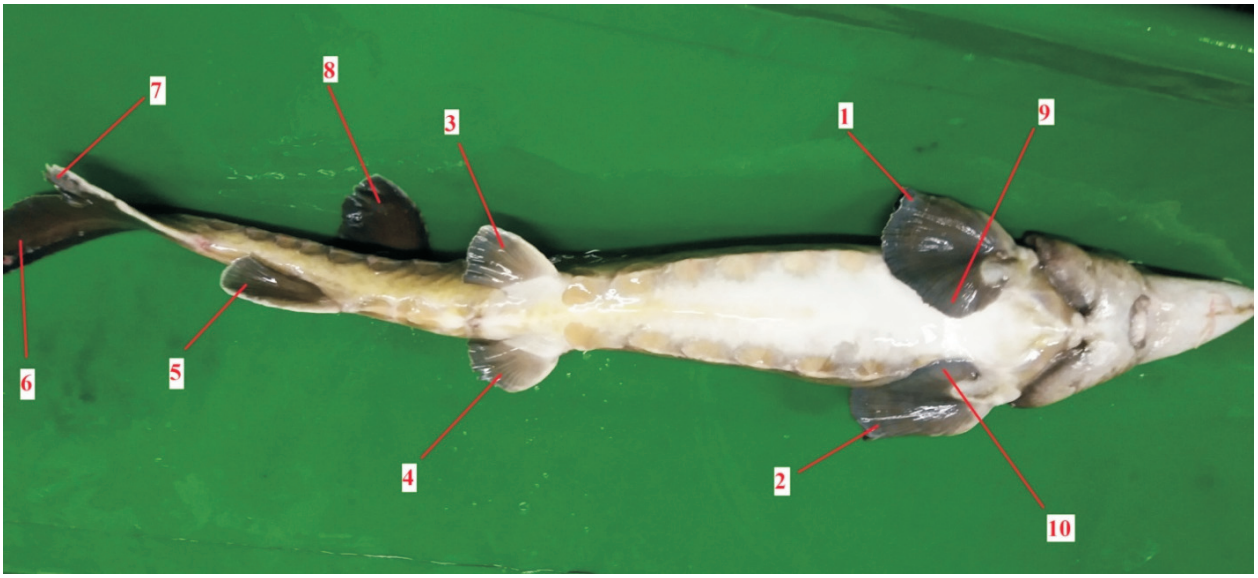

Figure 1. Marking scheme for 10 parts of Atlantic sturgeon kept in RAS at the Inland Fisheries Institute (Olsztyn, Poland)

at restoring wild populations of the Baltic Atlantic sturgeon in transboundary watercourses of the Republic of Belarus using the aquaculture capabilities of the Polish side. Fulfillment of this goal will make it possible to enrich natural watercourses on the territory of the Republic of Belarus with Atlantic sturgeon, which is aimed at preserving and increasing the biological diversity of rivers and increasing the economic stability of transboundary regions through the development of sturgeon breeding in the countries of the Baltic Sea region.

For the first time in Belarus, with the assistance of Polish partners, studies have been conducted to develop a foundation for the restoration of one of the extinct diadromous sturgeon species. The aim of this report is to preset the results of work on the joint Polish-Belarusian project to restore the Atlantic sturgeon (*A. oxyrinchus*) to Belarus. This report presents the results of the genetic testing protocol developed for sturgeon fry intended for stocking.

Materials and Methods

Biological material (fin fragments) from the seven-year-old Atlantic sturgeon was collected in March 2019. The material for performing the genetic studies were biological samples taken from fins (pectoral, ventral, anal (upper and lower lobes), dorsal-caudal region) that were collected *in vivo* using dissecting scissors from 10 seven-year-old Atlantic sturgeon specimens. The biological sampling scheme is shown in the Fig. 1. Samples were fixed in Eppendorf tubes in 96% ethanol with a three-fold change of fixative, and then stored in a freezer at -20°C. Samples with accompanying documents were delivered to the Laboratory of Animal Genetics, Institute of Genetics and Cytology, National Academy of Sciences of Belarus where the genetic analysis was performed.

DNA isolation was performed with the standard method of phenol-chloroform extraction (May et al. 1997, King et al. 2001, Henderson-Arzapalo et al. 2002). The lysis buffer contained 10 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 50 mM NaCl, 2% SDS, and 1 mM dithiothreitol (DTT). Immediately prior to lysis, a proteinase K solution (20 mg ml^{-1}) was added to the buffer to a final concentration of 100 µg ml^{-1}. Tissue samples were placed in Eppendorf centrifuge tubes (1.5 ml), poured into 500 µl of lysis buffer, and incubated overnight at 37°C, then for one hour at 65°C. The lysate was purified with a phenol-chloroform mixture, by adding 500 µl

of the phenol-chloroform mixture to the lysate. The tubes were shaken gently. They were centrifuged at 12,000 rpm for 10 minutes, and the upper aqueous phase was transferred to clean tubes. This procedure was repeated 2 times. Then, for more complete DNA purification and removal of phenol residues, 500 μl of chloroform was added to the supernatant, the samples were shaken gently, centrifuged at 12,000 rpm for 10 min, and the aqueous phase was transferred to clean tubes.

DNA was precipitated with 96% ethanol cooled to -20°C (1 ml tube^{-1}), and the samples were left overnight at -20°C. Next, they were centrifuged at 12,000 rpm for 10 minutes. The alcohol was drained. The precipitate was washed with 70% ethanol (1 ml). The precipitate was then dried in air at room temperature and dissolved in 100 μl of deionized water. The concentration and purity of the isolated DNA was determined on a NanoPhotometer P360 Spectrophotometer (Implen, Germany).

Microsatellite (STR) Analysis

Eight microsatellite loci (STR loci) specific for different sturgeon species were selected for DNA analysis: Spl-106, Spl-101, ATR-113, Aox23, An20, AoxD161, AoxD165, and AfuG41 (McQuown et al. 2000, King et al. 2001, Rodzen et al. 2002, et al. 2015, Barmintseva and Mugue 2017). These microsatellite loci are commonly recognized as the optimal marker system for determining the level of genetic variability within study groups. Spl-106, Spl-101, ATR-113, and Aox23 were also used to determine the origin of the fish stock of the Atlantic sturgeon intended for introduction in the Belarusian section of the Viliya (Neris) River. An20, AoxD161, AoxD165, and AfuG41 markers are used commonly for genotyping sturgeon species most widely kept in captivity, and they have not yet been used for genotyping of *A. oxyrinchus*. The characteristics of the microsatellite loci used in this study and the primers used to amplify the target sites are presented in Table 1. These markers allow determining sturgeon species thanks to the difference in the length of the amplification products in the different species.

The PCR-mixture used to amplify the microsatellite loci of sturgeon, which allow confirming which species and populations individuals belonged to and determining the genetic passports of individuals, contained the components presented in Table 2. PCR was carried out on 10 individuals to test the 8 microsatellite loci. The PCR scheme was as follows: cycle 1: 94°C for 3'; cycles 2–43: 45" at 94°C, 45" at Tm according to Table 1 and 1'30" at 72°C; cycle 44 was the final elongation at 72°C during 8'. The amplification products were separated in 1.5% agarose gel stained with ethidium bromide to check the efficiency of the PCR reaction. Fragment analysis was conducted using an AppliedBiosystems 3500 GeneticAnalyzer. The preparation of the DNA samples analyzed was as follows: the PCR product obtained was diluted by 10-40 times depending on the PCR product and fluorophore accumulation intensity; 1 μL of the diluted product was added to 12.75 μL Hi-Di formamide and 0.25 μL of DSMO-100 (MCLAB) size standard. Allele lengths were identified using GeneMapper™ Software 5. The specific statistical parameters were estimated using specialized macro GenAlEx v.6.5.

Mitochondrial DNA Analysis

The genetic study of maternal sturgeon individuals was conducted by sequencing an mtDNA region flanked by the tRNAThr coding region and a D-loop. One feature of the selected pair of primers is that the DNA region is amplified only in a certain species, namely *A. oxyrinchus* (Savoy et al. 2017). The Atlantic sturgeon-specific mitochondrial DNA primers in the current study were S1 (5'ACATTAAACTATTCTCTGGC3') and G1 (5'GAATGATATAC TGTTCTACC3') (Ong et al. 1996), and they amplified an approximate 560-bp region and were also used for sequencing. PCR was conducted according to the following scheme: cycle 1: 95°C for 3'; cycles 2–32: 45" at 95°C, 45" at

Table 1
Microsatellite loci used in this study to analyze the genetic variability of sturgeon species

PCR annealing temperature	Locus	Forward primer dye label	Sequence (5'-3')	Repeat motif	NA	Allele size
52°C	Aox23	Carboxytetramethyl-rhodamine (TAMRA)	F:TTGTCCAATAGTTTCCAACGC R:TGTGCTCCTGCTTTTACTGTC	(ATT)n-(ACT)m (AAT)p	4	134 143 146 149
52°C	Atr113	Fluorescein amidite (FAM)	F: TAAACAAATACAAAACTGCGTGTC R: GGTTGGATGAGATCGGGATA	(AGAT)n	2	236 258
56°C	Spl101	Hexachlorofluorescein (HEX)	F: CCCTCCACTGGAAATTTGAC R: GCAATCAACAAGGTCTCTTTCA	(TCTA)n	3	257 261 269
56°C	Spl106	Fluorescein amidite (FAM)	F:CACGTGGATGCGAGAAATAC R:GGGGAGAAAACTGGGGTAAA	(TAGA)n-((T/G)AAA)m	2	224 228
56°C	An20	Hexachlorofluorescein (HEX)	F: AATAACAATCATTACATGAGGCT R: TGGTCAGTTGTTTTTTTATTGAT	(ATCT)n-(TG)m	3	177 189 193
56°C	AoxD161	Fluorescein amidite (FAM)	F:GTTTGAAATGATTGAGAAAATGC R:TGAGACAGACACTCTAGTTAAACAGC	(CTAT)n	4	126 138 142 146
56°C	AoxD165	Carboxytetramethyl-rhodamine (TAMRA)	F: TTTGACAGCTCCTAAGTGATACC R:AAAGCCCTACAACAAATGTCAC	(CTAT)n-CTAC-(CTAT)m	3	202 210 218
56°C	AfuG41	Fluorescein amidite (FAM)	F:TGACTCACAGTAGTATTATTTATG R:TGATGTTTGCTGAGGCTTTTC	(GATA)n TA (GATA)m	3	217 225 245

NA – total number of alleles identified in the population

Table 2
Composition of the PCR mixture and the volume of components for amplifying microsatellite loci specific to sturgeon

Components of PCR mixture	Final concentration of components (volume)
DNA template (100 ng μl^{-1})	1 µl
Dream Taq 10x buffer (2 mM $MgCl_2$)	2 µl
10x dNTP mixture	2 µl
Direct primer (F) 10 pM μl^{-1}	1 µl
Reverse primer (R) 10 pM μl^{-1}	1 µl
Taq polymerase (5 units / μl^{-1})	0.2 µl
Mili-Q water	13.8 µl
Total volume of PCR mixture	20 µl

Table 3
Temperature regime of amplification

Step	Temperature	Duration	Quantity
I	96°C	1 min	1
II			
Denaturation	96°C	10 s	40
Annealing	55°C	5 s	
Elongation	60°C	4 min	
III	12°C	Before ∞	1

57°C and 1'30" at 72°C; cycle 33 was the final elongation at 72°C during 8'. The amplification products were separated in 1.5% agarose gel stained with ethidium bromide to check the efficiency of the PCR reaction.

The next step was to process samples with enzymes (Exo 1 and FastAP) to remove nonspecific DNA fragments and to purify samples for terminal PCR. A 1.2 µl mixture of the enzymes Exo1 (0.3 µl) and FastAP (0.9 µl) was added to each sample. The samples were incubated for 30 minutes at 37°C, and then 15 minutes at 80°C. Terminal PCR was performed using a C1000TM Thermal Cycler (Bio-Rad, USA). Then, 6.5 µl of the reaction mixture and 1.5 µl of the DNA sample were added to each tube. The temperature regime of terminal amplification is presented in Table 3. After terminal amplification, the samples were cleaned and dried according to the following protocol: 1) 2 µl of 5M ammonium acetate was added and mixed by vortex; 2) 30 µl of 96% alcohol was added to each tube; 3) the mixture was kept in a freezer for 30 minutes; 4) samples were centrifuged for 10 minutes, the supernatant was removed; 5) 130 µl of 70% alcohol was added each sample; 6) samples were centrifuged for 10 minutes, the supernatant was removed; 7) open tubes were placed in a thermostat at 45°C until the precipitate was completely dry.

Sequencing was conducted at the Center for Genomic Biotechnology, Institute of Genetics and Cytology of the National Academy of Sciences of Belarus. The results were analyzed using MEGA X. The sequences obtained were compared with known sequences posted in GenBank using the NCBI

Table 4
Frequencies of the detected alleles of 8 microsatellite DNA loci in the studied individuals of the Atlantic sturgeon

Allele size. bp	Allele frequency (%)
Aox23	
134	0.400
143	0.200
146	0.100
149	0.300
Atr113	
236	0.500
258	0.500
Spl101	
257	0.250
261	0.250
269	0.500
Spl106	
224	0.600
228	0.400
An20	
177	0.300
189	0.550
193	0.150
AoxD161	
126	0.200
138	0.150
142	0.150
146	0.500
AoxD165	
202	0.500
210	0.350
218	0.150
AfuG41	
217	0.350
225	0.150
245	0.500

BLAST tool to confirm the species affiliation of the sturgeon. Then, alignment of all sequences from each group of fish was performed in MEGA X, and the percentage of variable nucleotide sites was calculated as were the percentage of synonymous and non-synonymous nucleotide substitutions. All calculations were performed in MEGA X.

Results and Discussion

According to literature data, the ploidy of *A. oxyrinchus* is 2n (Fontana et al. 2008). Two allelic variants, therefore, must correspond to heterozygous individuals. The data on the sizes of allelic variants of the corresponding 8 microsatellite loci (Aox23, ATR-113, Spl-101, Spl-106, An20, AoxD161, AoxD165, and AfuG41) in the individuals analyzed are presented in Table 4. The resulting diversity of microsatellite locus length could indicate the close affinity of the analyzed sturgeon individuals. Therefore, at the stage of introducing these fish into the wild, it will be necessary to carefully consider the genetic diversity of the individuals selected (Ayllon et al. 2004). We established Hardy-Weinberg expectations for genotype frequencies (Table 5). The presence of 4 alleles with not significant probability of difference between the observed and expected number of allelic variation could also indicate the close relationship of the sturgeon individuals analyzed. The heterozygosity, F statistics, and polymorphism tests were performed for more detailed analyses (Table 6).

The result of the study indicated that 10 analyzed individuals demonstrated fairly high intrapopulation genetic heterozygosity (Table 6). The high occurrence of heterozygotes determines the potential of these

Table 5
Summary of chi-squared tests (Chi2) for the Hardy-Weinberg equilibrium

Locus	DF	žChi2	P	Significance
Aox23	6	13.333	0.038	P<0.05
Atr113	1	10.000	0.002	P<0.01
Spl101	3	4.000	0.261	not significant
Spl106	1	4.444	0.035	P<0.05
An20	3	2.433	0.487	not significant
AoxD161	6	10.000	0.125	not significant
AoxD165	3	0.607	0.895	not significant
AfuG41	3	10.000	0.019	P<0.05

DF – degrees of freedom = [Na(Na-1)]/2;

Table 6
Genetic characteristics of Acipenser oxyrinchus individuals analyzed for 8 microsatellite loci

Locus	Aox23	Atr113	Spl101	Spl106	An20	AoxD161	AoxD165	AfuG41
Na	4	2	3	2	3	4	3	3
Ne	3.333	2.000	2.667	1.923	2.410	2.985	2.532	2.532
I	1.280	0.693	1.040	0.673	0.975	1.238	0.999	0.999
Ho	1.000	1.000	0.900	0.800	0.800	1. 000	0.700	1.000
He	0.700	0.500	0.625	0.480	0.585	0.665	0.605	0.605
uHe	0.737	0.526	0.658	0.505	0.616	0.700	0.637	0.637
F	-0.429	-1.000	-0.440	-0.667	-0.368	-0.504	-0.157	-0.653

Na = No. of different alleles; Ne = No. of Effective Alleles = 1 / (Sum pi^2); I, Shannon's information index = -1* Sum (pi * Ln (pi)); Ho, observed heterozygosity = No. of Hets / N; He, expected heterozygosity = 1 – Sum pi^2; uHe, unbiased expected heterozygosity = (2N / (2N-1)) * He; F, fixation index = (He – Ho) / He = 1 – (Ho / He)

individuals to adapt to the changing conditions during reintroduction. On the other hand, the negative fixation index (F) estimates the indicated heterozygote excess. The fixation index (F) measures deviation from the Hardy-Weinberg expected heterozygote frequencies. So, if there would be further crossing within the group, the heterozygosity would decrease against the background of the low number of allelic variants observed. In order to be able to use the fish stock created for the reintroduction into the Viliya River, it is necessary that the introduced group can have access to other groups of reintroduced Atlantic sturgeon to replenish it with the sturgeon individuals from other regions. It is necessary because of the low number of allelic variants within the group and the possible kinship of individuals. The restoration plan must also include further stocking with individuals with predominantly different allelic variation.

The range of lengths of sizes of allelic variants of the Aox23, ATR113, Spl101, and Spl106 microsatellite loci indicated that the individuals were Atlantic sturgeon (*A. oxyrinchus*) from a population that originated from Canada (Komorowo Fish Farm) with limited allelic variants (Fopp-Bayat et al. 2015; Table 7).

An mtDNA region flanked by the tRNAThr coding region and a D-loop was selected to perform genetic analysis on maternal individuals. One specific feature of this pair of primers is that the DNA region is amplified only in *A. oxyrinchus* (Savoy et al. 2017); thus, obtaining a PCR product was additional confirmation of the individual belonging to the species. A PCR product 560 bp in length was obtained through amplification with all the samples selected, which confirmed the maternal purity of the individuals studied. The MOLE-BLAST tool (National Center for Biotechnology Information, U.S. National Library of Medicine), which is available online, and the GenBank database were used to more reliably confirm the species. For the analysis, a shortened nucleotide sequence of an amplicon of 301 bp was taken. The analysis indicated a 100% identity match between the sequence used and the *A. oxyrinchus* haplotype OxyIP1 D-loop (GenBank number KT007224.1). In the course of analysis at GenBank, no similar DNA sequence belonging to any other species was identified. The results obtained once again confirm that these selected individuals belong to a non-hybrid line of *A. oxyrinchus*.

Conclusion

The data obtained from the fragment analysis (Aox23, ATR113, Spl101, and Spl106) and the results of the amplification of the mtDNA region revealed no interspecific hybrids among the individuals selected and confirmed the purity of the species, which was the Atlantic sturgeon (*A. oxyrinchus*). The data also confirmed that the fish belonged to a population originating from Canada. The diversity of microsatellite loci revealed fairly high heterozygosity among the Atlantic sturgeon individuals analyzed. The negative fixation index (F) values indicated heterozygote excess. However, with further crossing within the group, heterozygosity will

Table 7
Parameters of genetic diversity of the individuals analyzed and the range of sizes of allelic variants (bp) in the population of Acipenser oxyrinchus (origin Canada, Komorowo Fish Farm, Poland)

Locus	Number of allelic variants in individuals analyzed	Range of sizes of allelic variants (bp) in individuals analyzed	Range of sizes of allelic variants (bp) in the *Acipenser oxyrinchus* population (Canada; Komorowo Fish Farm) (Fopp-Bayat et al. 2015)
Aox23	4	134-149	94-192
ATR113	2	236-258	171-295
Spl101	3	257-269	254-268
Spl106	2	224-228	188-232

decrease because of the small number of allelic variants. If this fish stock is to be used to reintroduce Atlantic sturgeon into the Viliya River, it is necessary that the introduced group have access to other groups of reintroduced Atlantic sturgeon. In the future, it will be necessary to introduce individuals of Atlantic sturgeon with different allelic gene variants.

Acknowledgements. This research was supported financially by projects from the Belarusian Republican Foundation for Fundamental Research (Belarus) and the Ministry of Science and Higher Education (Poland). Partial financial support (AK) was provided by the Stanisław Sakowicz Inland Fisheries Institute (statutory research topic no. S-009).

Author contributions. A.K., the project manager from the Polish side, provided the initial data and wrote and edited the manuscript; A.S., the project manager from the Belarusian side, edited the manuscript; N.B. and M.S. performed the analysis; V.K., the co-leader of the project from the Belarusian side, wrote and edited the manuscript.

ORCID ID
Aliaksandr Slukvin https://orcid.org/0000-0003-0537-4450
Nina Balashenko https://orcid.org/0000-0002-4935-6513
Andrzej Kapusta https://orcid.org/0000-0001-8979-5468

References

Ayllon F., Davaine P., Beall E., Martinez J.L., Garcia-Vazquez E. 2004 – Bottlenecks and genetic changes in Atlantic salmon (*Salmo salar* L.) stocks introduced in the Subantarctic Kerguelen Islands – Aquaculture 237: 103-116.

Barmintseva A.E., Mugue N.S. 2017 – Natural genetic polymorphism and phylogeography of Siberian sturgeon *Acipenser baerii* Brandt, 1869 – Russ. J. Genet. 53: 358-368.

Berg L.S. 1932 – Freshwater fishes of the USSR and adjacent countries – Vsesoyuznyi Institute Ozernogo i Rechnogo Rybnogo Khozyaistva, Leningrad (in Russian).

Fontana F., Lanfredi M., Kirschbaum F., Garrido-Ramos M. A., Robles F., Forlani A., Congiu L. 2008 – Comparison of karyotypes of *Acipenser oxyrinchus* and *A. sturio* by chromosome banding and fluorescent in situ hybridization – Genetica 132: 281-286.

Fopp-Bayat D., Kuciński M., Liszewski T., Teodorowicz T., Łączyńska B., Lebeda I. 2015 – Genetic protocol of Atlantic sturgeon *Acipenser oxyrinchus* (L.) fry for restocking the Vistula river, Poland – Journal of Survey in Fisheries Sciences 2: 1-10.

Henderson-Arzapalo A., King T.L. 2002 – Novel microsatellite markers for Atlantic sturgeon (*Acipenser oxyrinchus*) population delineation and broodstock management – Mol. Ecol. Notes 2: 437-439.

King T.L., Lubinski B.A., Spidle A.P. 2001 – Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and cross-species amplification in the Acipenseridae – Conserv. Genet. 2: 103-119.

Kolman R., Kapusta A. 2008 – Actual status and active protection of sturgeon fish populations endangered by extinction – MIRDRUK, Olsztyn: 310.

May B., Krueger C.C., Kincaid H.L. 1997 – Genetic variation at microsatellite loci in sturgeon: primer sequence homology in *Acipenser* and *Scaphirhynchus* – Can. J. Fish. Aquat. Sci. 54: 1542-1547.

McQuown E.C., Sloss B.L., Sheehan R.J., Rodzen J., Tranah G.J., May B. 2000 –Microsatellite analysis of genetic variation in sturgeon: new primer sequences for Scaphirhynchus and Acipenser – Trans. Am. Fish. Soc. 129: 1380-1388.

Reid A.J., Carlson A.K., Creed I.F., Eliason E.J., Gell P.A., Johnson P.T., Kidd K.A., MacCormack T.J., Olden J.D., Ormerod S.J., Smol J.P., Taylor W.W., Tockner K., Vermaire J.C., Dudgeon D., Cooke S.J. 2019 – Emerging threats and persistent conservation challenges for freshwater biodiversity – Biol. Rev. 94: 849-873.

Rodzen J.A., May B. 2002 – Inheritance of microsatellite loci in the white sturgeon (*Acipenser transmontanus*) – Genome 45: 1064-1076.

Savoy T., Maceda L., Roy N.K., Peterson D., Wirgin I. 2017 – Evidence of natural reproduction of Atlantic sturgeon in the Connecticut River from unlikely sources – PLoS ONE 12(4): e0175085.

Stakėnas S., Pilinkovskij A. 2019 – Migration patterns and survival of stocked Atlantic sturgeon (*Acipenser oxyrinchus* Mitchill, 1815) in Nemunas Basin, Baltic Sea – J. Appl. Ichthyol. 35: 128-137.

Ong T.-L., Stabile J., Waldman J.R., Wirgin I. 1996 – Genetic divergence of Atlantic and Gulf of Mexico sturgeon based on sequence analysis of the mtDNA control region – Copeia 2: 464-469.

Zubei A.V. 2016 – Commercial fish of subfossil collection of archaeological site Vitebsk, Upper Castle (r. Zapadnaya Dvina) – Proceedings of the National Academy of Sciences of Belarus, Biological series, 62(3): 33-40 (in Russian).

Zhukov P.I. 1965 – Fishes of Belarus – Nauka i Tekhnika, Minsk, p. 416 (in Russian).