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Genetic health and diversity assessment of Sturgeon species in Kazakhstan's aquaculture and natural habitats

Vadim Ulyanov1, Indira Beishova1, Tatyana Ulyanova1, Aziza Sidarova1, Nurbek Ginayatov¹∗**, Alexandr Kovalchuk1, Gulzhagan Chuzhebaeva2, Kuantar Alikhanov3, Bekbol Sariyev1, Ulbolsyn Kuzhebayeva1, Farida Nurzhanova1, Rustem Beishov2, Arman Sabyrzhanov¹ and Ayaulym Bexultan¹**

¹Zhangir Khan West-Kazakhstan Agrarian Technical University, Zhangir Khan Str. 51, 090009 Uralsk, Kazakhstan

² Akhmet Baitursynuly Kostanay Regional University, Baytursynov Str. 47, 110000 Kostanay, Kazakhstan

³ Kazakh National Agrarian Research University, Abay Avenue, 8, 050022 Almaty, Kazakhstan

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Abstract

Sturgeon species hold significant commercial and ecological value, with many listed as endangered. Understanding their genetic structure is crucial for developing effective conservation strategies. The main aim of this study is to examine the genetic structure of sturgeon, which can provide a foundation for creating strategies to conserve these species. A total of 121 sturgeon individuals were analyzed using microsatellite molecular markers (STR) to conduct the study on the genetic structure of sturgeon fish species. DNA was extracted from fin tissues using a commercial kit, and genotyping was conducted using seven microsatellite loci. Cluster analysis and principal coordinates analysis (PCoA) were performed to identify genetic differentiation among populations. The analysis showed differences in genetic diversity between the Siberian sturgeon, Russian sturgeon, and beluga samples. A deficiency of heterozygotes was discovered in artificial samples of sturgeon, indicating possible inbreeding. Genetic analysis has also revealed genetic differentiation between populations, possibly due to geographic structure or other factors. The study allows us to recommend increasing genetic diversity by introducing individuals from natural environments into sturgeon populations in aquaculture. Regular monitoring of genetic parameters in aquaculture populations and monitoring diversity dynamics are also crucial for the conservation and sustainability of sturgeon populations. The findings could help shape conservation strategies, especially in managing genetic diversity in aquaculture and reducing the risks associated with inbreeding and genetic drift.

Keywords: *Acipenser baerii, Acipenser gueldenstaedtii, Huso huso,* Western Kazakhstan, Genetic structure, STR

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Introduction

Sturgeons are a family of valuable commercial fish; many species are included in the Red Book and Appendix II CITES (Convention International Trade in Endangered Species of Wild Fauna and Flora) and have a protected status [\(Osadchy et al.,](#page-10-0) 2024). At the same time, high demand for their products remains on the world market [\(Abdullayev et al.,](#page-9-0) 2023). *Acipenser baerii* and *Acipenser gueldenstaedtii* are the most common sturgeon fish farmed worldwide [\(Fopp-](#page-10-1)Bayat [et al., 2022;](#page-10-1) [Sergaliev et al., 2021;](#page-10-2) [Kozhanov et al., 2023\)](#page-10-3). The natural range of the Siberian sturgeon, *Acipenser baerii*, is extensive. In Baikal and Zaisan, it forms lacustrine residential forms [\(Bakiyev et al., 2022;](#page-9-1) [Fopp-](#page-10-1)[Bayat et al., 2022\)](#page-10-1).

Genetic analysis of sturgeon has some difficulties due to the peculiarities of the genome and different ploidy since independent duplications of their complete genome occurred [\(Korotkih et al., 2023;](#page-10-4) [Shevchenko et al., 2023;](#page-10-5) [Beishova et al., 2023\)](#page-9-2). The morphological identification of sturgeon can present specific

difficulties due to the similarity of their morphological characteristics [\(Sattarova et al.,](#page-10-6) [2023\)](#page-10-6). For example, some sturgeon species, such as beluga (*Huso huso*) and Kaluga (*Huso dauricus*), have similar appearances and sizes, which can make it difficult to distinguish them based on morphological characteristics alone [\(Sarsembayeva et al., 2021\)](#page-10-7).

Russian sturgeon (*Acipenser gueldenstaedtii*) is an anadromous species [\(Spesivtseva et al.,](#page-11-0) [2023\)](#page-11-0). The natural habitat of the Russian sturgeon is the Caspian, Black, and Azov Seas, with large rivers flowing into them. *A. gueldenstaedtii*, a species with ~250 chromosomes, is considered by most authors to be a tetraploid species with an octoploid ancestor. Some of these ancestral octoploid characteristics are still evident in its microsatellite loci [\(Fopp-Bayat et al., 2022\)](#page-10-1).

Beluga (*Huso huso*) is one of the largest freshwater fish and an anadromous species. The habitat is similar to that of the Russian sturgeon [\(Cortés-Sánchez et al., 2023\)](#page-9-3). Unlike the two species described above, the beluga is a diploid [\(Fazli et al., 2020\)](#page-10-8).

In addition, frequent hybridization between different species may occur in sturgeons. This may be caused by cross-fertilization in the wild or artificial cross-breeding during captive breeding. Hybridization can lead to the emergence of hybrid forms that may have mixed morphological characters and genetic structure, thus complicating their identification [\(Linhartová et al., 2018;](#page-10-9) [Sergeev, 2020;](#page-10-10) [Nasiyev](#page-10-11) [et al., 2023\)](#page-10-11).

Studies of sturgeon fish based on microsatellite analysis began with North American species, and currently, there is data on the genetic diversity of many sturgeon

populations, but Kazakhstani sturgeon populations have been little studied, and for some, there is no information at all [\(Karmaliyev](#page-10-12) [et al., 2023;](#page-10-12) [Mazina et al., 2022\)](#page-10-13).

Understanding the genetic structure and diversity of sturgeon populations is crucial for developing conservation strategies [\(Oleinikova et](#page-10-14) [al., 2024\)](#page-10-14). It is important to expand research on the genetic structure and evolutionary history of this species and to conduct further studies using additional genetic markers [\(Kabylbekova et al.,](#page-10-15) [2024\)](#page-10-15) and a larger sample size. These steps are necessary to obtain a comprehensive understanding of the population structure and genetic diversity of sturgeon fish species [\(Beishova et al., 2023\)](#page-9-2). Therefore, this study aims to investigate the genetic makeup of sturgeons, providing a foundation for crafting conservation strategies for these species.

Materials and methods

A total of 121 individuals were analyzed in our study [\(Table 1\)](#page-1-0), belonging to the sturgeon family (40 are representatives of the species *Acipenser baerii*, 59 - *Acipenser Gueldenstaedtii*, 21 – *Huso huso,* and one hybrid – *Huso huso x Acipenser ruthenus*).

Phenotypic traits

The age of individuals in the studied groups varied between 6 and 9 years; the average live weight was 6.25±0.22 kg, and the body length was 112.19±1.35 cm. Each individual had a subcutaneous chip installed for further identification [\(Figure 1\)](#page-2-0). Samples were collected from 40 individuals of *Acipenser baerii,* and 40 individuals of Acipenser gueldenstaedtii kept in RAS conditions in the laboratory of ichthyology and aquaculture of NJSC "Zhangir Khan West Kazakhstan Agrarian and Technical University" (51°10′41″ N, 51°18′36″ E).

Figure 1: Installation of subcutaneous chips for identification of individuals.

Huso huso and *Huso huso x Acipenser*, as well as 19 individuals of *Acipenser gueldenstaedtii* representatives of the natural population caught in the Ural River delta and kept in the RSE conditions of the Ural-Atyrau Sturgeon Fish Hatchery, which is a complex of engineering structures designed for raising juveniles sturgeon species of fish in order to replenish sturgeon stocks in the Caspian Sea basin [\(Figure](#page-2-1) [2\)](#page-2-1).

Figure 2: Sampling location in Atyrau region.

DNA extraction

DNA extraction, genotyping, and results processing were conducted in the biotechnology laboratory, and diagnostics of infectious diseases of the Testing Center of the same university.

Ectodermal tissue fragments of the pectoral fin were selected as the starting material for DNA extraction and microsatellite analysis [\(Gnezdilova et al., 2024\)](#page-10-16). Two samples were taken from each individual; one of them was fixed in 96% ethyl alcohol at the site where the material was collected, and the other was placed in a plastic tube without fixation with alcohol. DNA was isolated from a sample not fixed with ethyl alcohol; samples with alcohol were placed in a low-temperature refrigerator to create a depository bank for biomaterial with which further research will be carried out. Each sample collected was assigned a laboratory identification number.

Genetic analysis

DNA was extracted from fin tissues using the commercial kit "DNA-Extran-2" (SINTOL LLC, Russian Federation).

For genotyping, we used the "GenExpert-Sturgeon" reagent kit (SINTOL LLC, Russian Federation) intended for genetic certification and determination of the relationship of sturgeon fish species. Genotyping was carried out using seven microsatellite loci (AoxD161, AfuG41, AfuG135, AfuG37, and AoxD165), the characteristics of which are indicated in [Table 2.](#page-3-0) We chose these loci since they are amplified in most individuals of sturgeon species; the genotyping results can be reproduced in other laboratories, and all listed loci are amplified in multiplex PCR.

The amplification reaction was carried out according to the manufacturer's instructions for the genotyping kit on a ProFlex PCR system thermal cycler (Аррllied Вуоsystems, USA).

Data analysis

Genetic diversity was analyzed using a specialized add-in for Microsoft Excel—GenAlEx 6.51. The following indicators were assessed: allele frequency, number of alleles per locus, adequate number of alleles (Na), observed (Ho) and expected (He) heterozygosity, fixation index (Fis), and genetic distance index by Nei [\(Nebesikhina et al.,](#page-10-20) 2017). Cluster analysis was performed using the STRUCTURE 2.3.4 program, which uses the Markov chain method (MCMC).

It implements a Bayesian algorithm for clustering genotypes into K clusters, considering a priori information about the geographical location of the populations under consideration. The Delta K coefficient was used to select the optimal K, where $1 \leq K \leq 8$. To visualize the results and their mathematical confirmation, the STRUCTURE Harvester web program is used, which uses Evanno's methods to calculate Delta K and determine the optimal number of clusters by sequentially enumerating them. The results of the STRUCTURE runs were summarized using the CLUMPP program. The resulting Q-matrices were presented in the form of histograms.

Results and discussion

Genetic diversity

Based on the analysis results, we found that in four samples initially identified as beluga (*Huso huso*), most of the seven genetic markers showed more than two alleles. This indicated that these samples did not belong to the species *H. huso*.

They were, therefore, excluded from further processing, as it is known that *H. huso* is a diploid species [\(Fazli et al.,](#page-10-8) 2020). Also, the hybrid (*Huso huso x Acipenser ruthenus*) is represented by a single sample, which needs to be revised for further analysis.

In general, tetrasomic inheritance of microsatellite loci was revealed in samples of Russian sturgeon and Siberian sturgeon, and disomic inheritance in the sample of beluga, which is consistent with most previously conducted sturgeon studies [\(Fazli et al.,](#page-10-8) 2020).

In all studied sturgeon samples, 127 alleles were identified at 7 STR loci, as shown in [Figure](#page-4-0) [3.](#page-4-0) This figure presents the allele frequencies for seven microsatellite loci (AoxD161, Afug41, LS19, Afug135, Afug37, Spl173, and AoxD165) across the studied sturgeon populations. Each bar in the figure represents the frequency of a specific allele within a locus.

Summarizing the data from the study of the genetic structure, we calculated the average values for all loci of the number of alleles per locus, the effective number of alleles, heterozygosity, Shannon fixation index, etc.; the data is presented in [Table 3.](#page-5-0)

As well as by loci, the Atyrau sample of *A. gueldenstaedtii* (AGn) was characterized by the most significant average number of alleles per locus (Na) and effective number of alleles per locus (Ne), which were 14,571 and 8,691, respectively.

Figure 3: Allele frequency by loci. X-axis: Represents the different alleles for a specific microsatellite locus. Each value on the X-axis corresponds to a specific allele size or repeat number. Y-axis: Represents the frequency of each allele within the population. The Y-axis values indicate how common or rare each allele is in the studied sturgeon samples.

Table 3: Average values of genetic diversity indices for the studied sturgeon samples.

Sample		Na	Ne		Ho	He	uHe	F
ABa	Mean	9,857	5,771	1,893	0,638	0,809	0,814	0,206
	SE	0,962	0,560	0,117	0,075	0,031	0,031	0,093
AGn	Mean	14,571	8,691	2,316	0,626	0,867	0,880	0,278
	SE	1,494	1,364	0,129	0.064	0,020	0,020	0,071
HHn	Mean	3,714	2,822	1,053	0,670	0,596	0,614	$-0,124$
	SE	0,522	0.462	0.144	0,111	0,052	0,054	0,176
AGa	Mean	10,429	5,980	1,980	0,660	0,829	0,834	0,208
	SE	0,782	0,367	0,052	0,057	0,010	0,010	0,060
Total	Mean	9,643	5,816	1,810	0,648	0,775	0,786	0,142
	SE	0,884	0,548	0,105	0,038	0,025	0,025	0,060

Na - number of alleles per locus; Ne - effective number of alleles per locus; I - Shannon information index; Ho - observed heterozygosity; He - expected heterozygosity; uHe - expected heterozygosity adjusted for sample size; F - Wright fixation level

Analysis of genetic distances

The Nei Genetic Distance indicator between the AGn and AGa samples was the smallest and amounted to 0.23, which shows the greatest genetic similarity.

[Figure 4](#page-5-1) shows the spatial distribution of the four sturgeon samples. Each point on the graph represents one sample, and its spatial position along the axes depends on the population genetic parameters of this sample.

Cluster analysis results

Analysis of the population structure showed that the Delta K indicator reaches its maximum value

(17.233784) when the number of clusters is 4 [\(Figure 5\)](#page-6-0). Thus, the most probable is the division of the study sample into K=4 groups, corresponding to 4 genetic populations.

Russian sturgeon – *Acipebser gueldenstaedtii*

According to the results of genotyping at the LS19 locus,16 specimens of Russian sturgeon (*A. gueldenstaedtii*) had six alleles; in other loci, the number of alleles did not exceed 4. 15 of the 16 fish specimens mentioned above were caught from natural conditions. Based on the information provided, we can assume the presence of hexaploid individuals in the studied population of Russian sturgeon.

Figure 4: Principal coordinates analysis (PCoA). X-axis (Principal Coordinate 1): Represents the primary axis of genetic variation, capturing the largest amount of genetic diversity among the sturgeon samples. Y-axis (Principal Coordinate 2): Represents the secondary axis of genetic variation, capturing the second-largest amount of genetic diversity among the samples. This figure serves as a standalone representation of the genetic structure of the sturgeon populations studied. The PCoA plot provides insights into the genetic relationships and diversity within the sturgeon samples, highlighting the genetic differentiation among populations and informing conservation strategies aimed at maintaining genetic diversity and reducing inbreeding.

Figure 5: Graph of Delta K values. X-axis (Number of Clusters, K): Represents the number of potential genetic clusters considered in the analysis, ranging from 1 to 8. Y-axis (Delta K): Represents the rate of change in the log probability of data between successive K values. Higher Delta K values indicate a more significant change in the likelihood of the data fitting the model with that particular K value. The Delta K method is based on the rate of change in the log probability of data between successive K values. The graph provides clear evidence that K=4 is the most appropriate number of clusters, highlighting the genetic differentiation among the populations.

Siberian sturgeon *– Acipenser baerii*

In a population study of *A. baerii* (ABa), a moderate level of polymorphism was observed. Between 6 and 12 alleles were identified, with an average of 9,857 alleles per locus. The most polymorphic loci were Afug41, Afug37, and AoxD165. LS19 represents a lower polymorphism in this population. Alleles 145 and 149 at the Afug37 locus were found only in this species.

At the LS19 locus, allele 141 was the most common in both the studied Russian sturgeon (AGn, AGa) samples and the Siberian sturgeon (ABa) group [\(Fopp-Bayat et al., 2022\)](#page-10-1).

Beluga *- Huso – huso*

Among the studied samples, the lowest level of polymorphism was observed in a group from the natural Atyrau population of beluga sturgeon (*H*. *huso*, HHn), in which from 2 to 6 alleles were identified, and the average number of alleles per locus was only 3.714. The Afug41 locus, as in other samples studied in our work, was characterized by the highest level of polymorphism. AoxD161 loci, Afug135, Afug37,

and AoxD165, represent the lowest polymorphism, as they have 2-3 types of alleles, which makes them uninformative for studying the genetic structure of the beluga.

Genetic diversity within and between samples of sturgeon fish

Based on the data presented in [Table 4,](#page-7-0) some differences between the sturgeon samples can be identified.

HHn and AGa were characterized by the smallest number of effective alleles. Low Ne values and the possibility of loss of valuable genotypes due to genetic drift cause a decrease in the population's evolutionary potential. Thus, the most extraordinary allelic diversity at all loci was observed in the Atyrau population of Russian sturgeon *A. gueldenstaedtii* (AGn) despite the small sample size (n=19). In contrast, a sample of Russian sturgeon collected from 40 individuals from the WKATU laboratory (AGa) was characterized by lower allelic diversity. The natural origin of the Atyrau population and the artificial origin of the Ural population can explain this.

Na - number of alleles per locus; Ne - effective number of alleles per locus; I - Shannon information index; Ho - observed heterozygosity; He - expected heterozygosity; uHe - expected heterozygosity adjusted for sample size; F - Wright fixation level

The Shannon information index (I) indicates allelic diversity; its values can range from 0 to maximum, i.e. Imax value is not specific. However, [Galinskaya et al. \(2019\)](#page-10-21) noted that "Shannon index values for microsatellite markers are considered high if they are at least 1.5".

Low values of the I index in the HHn sample correlate with the data of a Belarusian group of scientists who studied belugas bred under artificial conditions [\(Slukvin et al.,](#page-10-22) 2021). The level of heterozygosity is also an indicator of the population's genetic diversity.

When comparing the distribution of heterozygosity indicators in Russian sturgeon with literature data, we found that the observed heterozygosity values are lower than in other populations. In the breeders of Russian sturgeon

of the Don region and fish of natural spawning, the value of Ho in different years was in the range of 0.988-1, according to the locus AoxD161-1, according to the locus AoxD165-0.840-0.936 [\(Nebesikhina et al.,](#page-10-20) 2017). The studied Atyrau and Ural samples of Russian sturgeon had values of observed heterozygosity below these ranges, which indicates a lower genetic diversity compared to them.

[Slukvin et al. \(2021\)](#page-10-22) studied the molecular genetic characteristics of the beluga aquaculture breeding stock of the Republic of Belarus for six microsatellite loci, three of which were the same as in our work: AoxD161, AoxD165, and AfuG41. The authors revealed that in belugas, the observed heterozygosity values at the AoxD161 and AfuG41 loci were higher than those observed [\(Slukvin et al.,](#page-10-22) 2021). Additionally, we obtained a

similar result for the sample from the Atyrau natural population *Huso huso* (HHn).

Overall, analysis of the sturgeon genetic structure data presented in [Table 5](#page-8-0) allows us to conclude differences in genetic diversity, heterozygosity, and sampling structure of sturgeons that may have important implications for the understanding and conservation of these species.

The Fis index values indicate the absence of excessive homozygosity within the samples. This may mean the preservation of genetic balance and the absence of a negative effect on genotype diversity. Fst is a measure of the degree to which the process of fixation of alleles in subpopulations is complete (demographic

differentiation) [\(Jost et al.,](#page-10-23) 2018). Fst rarely exceeds 0.5 and is often much less. In our case, Fst values indicate the presence of some genetic differentiation between populations. And Nm values may indicate some level of gene exchange between sturgeon populations. The genetic distance determined by M. Ney's method is a universal indicator of genetic distance. The values in [Table 6](#page-8-1) indicate low interpopulation genetic differentiation between the studied groups of sturgeon fish.

The highest genetic distance values were observed between Siberian sturgeon (ABa) and beluga sturgeon (HHn), while Russian sturgeon populations (AGa and AGn) were intermediate between them [\(Table 6\)](#page-8-1).

Fis, Fit, Fst - inbreeding coefficients at different levels of the population hierarchy; Nm is an indicator of gene flow intensity

Aga 0,048 0,023 0,117 0,000

	Aba	AGn	HHn	Aga
Aba	0,000			
Agn	0,032	0,000		
HHn	0,136	0,106	0,000	

Table 6: Nei genetic distance.

Based on the results, we see a clear separation of the four sturgeon samples. Thus, the gene pools of the Siberian sturgeon are different from the gene pools of the Russian sturgeon and the gene pools of the beluga; the gene pools of the two studied samples of the Russian sturgeon are the closest. Similar findings were reported by [Kjartanson et al.](#page-10-24) [\(2023\),](#page-10-24) who observed substantial genetic differentiation among sturgeon species, attributed to their varied geographic distributions and historical separation. The analysis results performed in the STRUCTURE program showed that all samples are differentiated [\(Figure 6\)](#page-9-4).

Analysis of the Neighbor-Net graph shows that some Russian sturgeon individuals stand out from the general group. It is worth noting that all *A. gueldenstaedtii* individuals forming separate clusters were caught from natural conditions. This clustering of the Russian sturgeon populations is consistent with the findings of [Nazari and Pourkazemi,](#page-10-25) who reported low genetic distances among Russian sturgeon populations in the Caspian Sea basin, suggesting historical gene flow and ongoing genetic exchange [\(Nazari](#page-10-25)

Figure 6: Results of sturgeon clustering in the STRUCTURE program at $K = 4$. This illustrates the clustering results of the sturgeon samples as determined by the STRUCTURE software for $K = 4$, which represents the most likely number of genetic clusters identified. Each bar in the figure represents an individual sturgeon, and the colors within each bar indicate the proportion of the individual's genome that belongs to each of the four genetic clusters.

[and Pourkazemi, 2023\)](#page-10-25). Because interspecific hybridization often occurs in sturgeons and there are problems with morphological identification, it can be assumed that there is an error in determining species identity.

Conclusions

As a result of this work, both wild sturgeon populations and RAS broodstocks were studied. The population structure of samples from two populations of Russian sturgeon, one artificial population of Siberian sturgeon, and one natural population of beluga was identified. Based on the findings obtained, the following recommendations can be made related to the breeding of groups of sturgeon fish in aquaculture conditions: (i) to increase the genetic diversity and sustainability of populations, increase and replenish the stock of breeding stock with individuals caught from natural conditions and other similar aquaculture populations are necessary; (ii) regularly analyze newly arrived individuals and resulting young animals in order to monitor and study the dynamics of significant indicators of genetic diversity is required.

Despite the comprehensive nature of this study, several limitations need to be addressed. The study provides a snapshot of genetic diversity at a single point in time. Longitudinal studies tracking genetic changes over multiple generations would be beneficial to understand trends and the impact of conservation efforts. Future studies could include sturgeon populations from different geographic regions, and environmental conditions will provide a

more comprehensive picture of genetic diversity and help identify region-specific conservation needs.

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