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AN OVERVIEW OF THE METHODS USED FOR GENETIC ANALYSIS OF STURGEON POPULATIONS

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Abstract

Sturgeons are a significant and ancient group of fish, often referred to as "living fossils" because their morphology has remained largely unchanged for millions of years. As some of the earliest extant vertebrates, their evolutionary history offers valuable insights into the development and diversification of vertebrates. However, human activities such as overfishing, habitat destruction and environmental pollution have severely impacted their populations. As a result, the majority of sturgeon species are now classified as endangered, vulnerable, or even extinct in the wild. Understanding the genetic diversity of sturgeons is critical for their conservation and for maintaining the long-term viability of their populations. Genetic studies using tools such as microsatellite markers and mitochondrial DNA (mtDNA) genotyping provide detailed information about the population structure, genetic variation and degree of inbreeding among sturgeon populations, enabling researchers to assess population health, understand phylogenetic relationships, support aquaculture programs and develop effective conservation strategies.The continued research into their genetic diversity and evolutionary history contributes to a broader understanding of biodiversity and the dynamics of ancient vertebrate lineages.

***Keywords**: Sturgeons; Phylogenetics; Biodiversity; Mitogenomics*

Introduction

Sturgeons are an important ancient group of biologically and economically important fishes that are threatened by extinction [1]. They have a long-life cycle, long migratory [1]movements, slow growth, large bodies, late sexual maturation and intermittent reproduction [2-6]. During the last decade, sturgeon populations are threatened by a drastic decline due to anthropogenic impacts such as dam construction, exploitation, ecosystem fragmentation, poaching, habitat destruction, pollution and water quality [6-8]. Therefore, due to reported anthropogenic factors that have affected populations, at the European scale, sturgeon species have been included on the International Union for Conservation of Nature's Red List of Threatened Species (IUCN, 2024), Appendix II of the Convention on International Trade in Endangered Species (CITES, 2020), the Natura 2000 list, the CMS - Bonn Convention (Convention on the Conservation of Migratory Species of Wild Animals), the Food and Agriculture Organization (FAO) and the Bern Convention (Convention on the Conservation of European Wildlife and Natural Habitats) [9].

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The importance of sturgeons goes beyond their economic value for meat and caviar. The study of sturgeon species is currently incomplete and still presents many unknowns regarding information about chromosomal evolution or the evolution of the species during the historical development of the living world. However, the decline in sturgeon species diversity is occurring at an accelerating rate and the time available for research on these individuals is becoming limited [10]. The unchanged morphology of sturgeons over many eras places this family of fish as the new biological model for understanding genomic stability.

Conservation genetics is a branch of genetics that is meant to help diminish the risk of population and species extinction. The levels established by the IUCN for biodiversity conservation are genetic diversity, species diversity and ecosystem diversity [11]. Understanding these levels gives insights into how anthropogenic activities and historical events shape the characteristics of a species [12]. As the main factors that are causing extinctions are inbreeding, loss of genetic diversity and mutational accumulation, conservation genetics become an important tool in overcoming these issues [11]. For better conservation and restoration of sturgeon species, programs should aim to include genetic studies and unravel more about population structure and status, species identity and hybridization as well as assessing the origins and relatedness between individuals and populations [13].

Sturgeon biology

The family Acipenseridae is thought to have originated 200 million years ago from the pre-Jurassic teleost lineage. The first records of sturgeons date back to the Upper Cretaceous period and their distribution is restricted to the Northern Hemisphere [5]. Biogeographical research suggested that Acipenseriformes originated in Europe and then started its diversification in Asia [14]. Sturgeons are known to be part of the phylum Chordata, superclass Osteichthyes, class Actinopterygii, order Acipenseriformes and family Acipenseridae [4]. Among vertebrates, they have two special characteristics: reduced molecular evolution rate and multiple levels of ploidy [15, 16]. For this reason, these species provide a unique background for evolutionary genetic research and genome duplication.

Sturgeons typically inhabit temperate waters and can reach sexual maturity in marine and brackish environments [17]. However, certain species, like the sterlet (*Acipenser ruthenus*) and Siberian sturgeon (*A. baerii*), live solely in freshwater. Nevertheless, all sturgeon species reproduce exclusively in freshwater [10, 19]. Sturgeons have a specific morphology. Their bodies are elongated and fusiform with five rows of bone plates: a dorsal, two lateral and two ventral rows that form the external skeleton, specific to prehistoric animals. The snout (rostrum) is elongated with whiskers above the mouth that work as taste buds [19, 20]. Bony plates are present on their skin with a pectoral fin that is transformed in a spine. Adults have no teeth and their mouth has thick lips [17]. In order to feed, reproduce or overwinter, sturgeon migrate from one place to another. The movement can take place in several stages and, depending on the species can be accomplished at different times [21]. The spawning takes place either on the mainstream or the margins of the river or on hard bottom substrates [17]. In most cases, males are the first to arrive at the spawning site. Sex difference determines different times of migration and is an important characteristic to achieve the balance of sex ratio between males and females [21]. Sturgeons usually take 3 to more than 20 years to become sexually mature and spawn between long periods of time (2 to more than 5 year) [4]. A characteristic of sturgeon species is the high capacity of hybridization. In most cases, 2nx2n and 4nx4n hybrids produce fertile offsprings, while hybridization between 2nx4n results in sterile children [17].

Sturgeons are a divergent species of bony fishes considered to be among the earliest existent vertebrates [22, 23]. They are geographically distributed across Europe, North America and Asia [23, 24]. Establishing the phylogeny of the species helped to better understand the processes that influenced the variability between species. For example, phylogeny can show how opening of the Atlantic Ocean contributed to the divergence of two different lineages, today known as *A. sturio* (Europe) and *A. oxyrinchus* (North America) [25]. The largest freshwater fish, the Beluga sturgeon (*Huso huso*) is found in the Black, Caspian and Azov Seas. On the other side, the smallest sturgeon, called shovelnose (*P. kaufmanni*, *P. hermanni* and *P. fedtschenkoi*) can be found in the Aral Sea [4]. Currently, the species that spawn in the Lower Danube are *Huso huso, Acipenser stellatus, Acipenser ruthenus* and *Acipenser gueldenstaedtii* [26-28]. These are known to be part of the Atlantic clade of Acipenseridae family [29]. According to the International Union for Conservation of Nature (IUCN) (IUCN Red List), there are 25 species of sturgeon in the Acipenseridae family from which: 17 are Critically Endangered, 4 are Vulnerable, 3 are Endangered and Yangtze Sturgeon is listed as Extirpated in the Wild, the most severely affected species (Table 1).

Table 1. IUCN Red List conservation status where EX – extinct, EW – extinct in the wild, CR – critically endangered, EN – endangered, VU – vulnerable, NT - Near Threatened, LC – Least Concern.

	EX	EW	CR	EN	VU	NT	LC
Acipenser naccarii			x				
Acipenser schrenckii			x				
Acipenser sinensis			x				
Acipenser sturio			x				
Acipenser nudiventris			x				
Acipenser medirostris				x			
Acipenser oxyrinchus					x		
Acipenser fulvescens				x			
Acipenser persicus			x				
Acipenser gueldenstaedtii			x				
Acipenser mikadoi			x				
Acipenser brevirostrum					x		
Acipenser baerii			x				
Acipenser stellatus			x				
Acipenser ruthenus				x			
Acipenser transmontanus					x		
Acipenser dabryanus		x					
Huso huso			x				
Huso dauricus			x				
Scaphirhynchus suttkusi			x				
Scaphirhynchus albus			x				
Scaphirhynchus platorynchus					x		
Pseudoscaphirhynchus kaufmanni			x				
Pseudoscaphirhynchus hermanni			x				
Pseudoscaphirhynchus fedtschenkoi			x				

Until now, most of the sturgeon species have been karyotyped and it was observed that the number of chromosomes can vary as it was summarized in Table 2. Therefore, three ploidy levels were distinguished: 120 chromosomes, 250-270 chromosomes and 370 chromosomes [30]. Additionally, the ploidy of Acipenseriformes was divided in two scales: evolutionary, which includes tetraploid (4n), octaploid (8n), 12-ploid (12n) [9] and contemporary scale which includes diploid (2n), tetraploid (4n), hexaploid (6n) species [31]. Because of their large genome, mechanisms behind the sturgeon genetic material are not clear, but recent studies noted that the reduction of redundancy of the polyploid genome is mostly random [22].

The Ponto-Caspian region is the area with the highest diversity of endangered sturgeon species. Danube River plays an important role as a migration corridor because it provides access to the Black Sea habitat and is the richest European basin in freshwater fauna species like sturgeons [5]. Six species were present in the Lower Danube region in the past, of which only four species remain today [5, 42]. *A. nudiventris* and *A. sturio* are already extirpated [5, 42].

Table 2. Taxonomic classification of sturgeon species

Species	Number of chromosomes	References
Acipenser nudiventris	116 ± 4	[32]
	118 ± 2	[33]
Huso huso	116 ± 4	[34]
	118 ± 2	[35]
Acipenser sturio	116 ± 4	[34]
	121 ± 3	[36]
Acipenser ruthenus	118 ± 2	[37]
	118 ± 4	[38]
	118 ± 2	[39]
Acipenser stellatus	118 ± 2	[39]
Acipenser gueldenstaedtii	249 ± 2	[40]
	250 ± 8	[39]
	258 ± 4	[41]

Importance of molecular techniques in genetic diversity studies on sturgeon species

Studying genetic diversity in sturgeon species is paramount for effective conservation and restoration efforts. It provides a foundation for understanding population dynamics, health and adaptability, ultimately contributing to the long-term survival of these ancient fish in the face of numerous environmental challenges. Thus, in the context of determining their genetic diversity structure, multiple techniques have been developed and used including mitochondrial DNA sequencing, microsatellite studies, AFLP (Amplified Fragment Length Polymorphism), RFLP (Restriction Fragment Length Polymorphism) and RAPD (Random Amplified Polymorphic DNA) [43-45]. The restoration and conservation of sturgeon populations demand precise information about their population genetic structure and status. Accurate information on the population's genetic structure has an essential role in aquaculture and stock enhancement activities, as well as for the development of conservation strategies to ensure the persistence of species [46]. Genetic diversity is crucial for the survival and adaptation of a species to various environmental changes and challenges. Higher genetic variation within populations can improve the chances of populations to endure environmental challenges, like climate change, disease, or contamination [47]. Genetic diversity in sturgeon species has been studied over the past decades. An understanding of the current pattern of within- and among-population genetic variation can provide insight into the history of a species. Mitogenome study is used in investigations of phylogeny relationships between organisms and phylogeography as well as in population structures. The sequencing of mitochondrial DNA, including the Cyt B gene and the D-Loop control region, has been used as a method to identify species and to establish their phylogenetic relationship [48]. Mitochondrial DNA has several characteristics that make the method simpler and more efficient, such as its small size of about 15-20 Kb. Its circular molecule is inherited on the maternal line, having a conserved content and organization. MtDNA has 37 genes of which 13 are encoding proteins, 2 encoding ribosomes, 22 encoding tRNA and 2 control regions [49, 50].

Antognazza et al. [51] conducted a comprehensive genetic analysis on four broodstocks of the beluga sturgeon (*Huso huso*) using thirteen SSR loci, mtDNA cytochrome b gene sequencing (Cyt b) and the entire mtDNA control region (D-Loop). The analysis revealed significant genetic variability within the broodstocks, similar to that found in wild populations. These results indicated a healthy genetic diversity among the broodstock sturgeons. Additionally, through Baesyan evaluation it was shown that these broodstocks have a low potential for inbreeding. The genetic diversity and low inbreeding levels indicate that these broodstocks have good potential for sustaining future breeding programs, ensuring the continuation of genetically healthy beluga sturgeon populations [51]. Another study, examined the genetic structure of beluga sturgeon populations in the Caspian Sea using the mtDNA D-Loop region and nine microsatellite markers. Mitochondrial DNA showed that certain areas of the Caspian Sea host distinct genetic stocks of beluga sturgeon. Moreover, microsatellite markers revealed at least two distinct populations: one in the northern Caspian Sea and one in the southern Caspian Sea [52]. Both of these studies highlight the importance of genetic analysis in understanding and managing beluga sturgeon populations by treating distinct populations as independent management units to preserve their genetic integrity and sustainability [51, 52].

For the species *Acipenser stellatus, Huso huso* and *Acipenser gueldenstaendtii*, sequences from the mitochondrial control region D-Loop were downloaded from Genbank and aligned to assess genetic diversity (Table 3). Alignment was performed with the program Jalview, after which they were exported to iTol to perform the rectangular phylogenetic tree (Fig. 1).

Table 3. Sturgeon subspecies investigated in this study

Species	**Subspecies**	**Location**	**Access number GenBank**
Acipenser stellatus	*Acipenser stellatus donensis*	Azov Sea	AF168546 AF168547 AF168548 AF168549 AF168550
	Acipenser stellatus ponticus	Black Sea	AF168514 AF168515 AF168516 AF168517 AF168518
	Acipenser stellatus stellatus	Caspic Sea	AF168504 AF168505 AF168506 AF168508 AF168509
Huso huso	*Huso huso maeoticus*	Azov Sea	AY846666 AY846667
	Huso huso caspicus	Caspic Sea	AY846640 AY846641 AY846642 AY846643 AY846644
	Huso huso ponticus	Black Sea	AY846668 AY846669 AY846670 AY846671 AY846672
Acipenser gueldenstaedtii	*Acipenser gueldenstaedtii colchicus*	Azov Sea	AF238751
	Acipenser gueldenstaedtii colchicus	Black Sea	AF238739
	Acipenser gueldenstaedtii gueldenstaedtii	Caspic Sea	AF238719

One approach to studying the molecular diversity of sturgeons is by using microsatellites to identify the genetic variation between species. Microsatellite loci, also known as simple sequence repeats (SSRs), compose stretches of DNA consisting of single to numerous repeating units of 2–6 base pairs that are used as markers for studies in genetic diversity [53, 54]. To date, very few microsatellite loci have been characterized, developedand used in research studies of sturgeons.

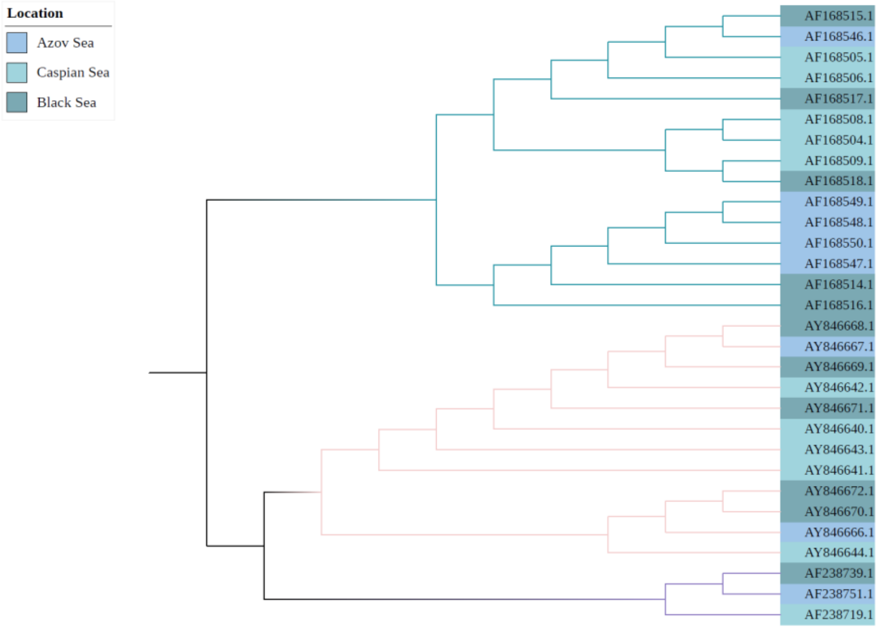

Fig. 1. Phylogenetic analysis of Neighbor-Joining based on sequences from mitochondrial control region (D-loop) using iTol [57]

Because of the characteristic traits of somatic microsatellites from one cross-species of the sturgeon family and the absence of mitochondrial DNA mutations during sturgeon interspecific hybridization, these loci could be used in genetic diversity and relationship studies of the sturgeon family, evaluations of different cultured sturgeon germplasm pool compositions and studies of the genetic effects of using interspecific hybrids as the germplasm of the sturgeon family [55, 56].

Conclusions

The natural, biological and conservation importance of sturgeons requires an understanding of their genetic structure. Therefore, protecting sturgeon diversity is essential for the survival of wild populations. To this end, it is necessary to establish several conservation strategies, such as: establishing genetic databases, monitoring population structures, studying reproduction and growth and estimating the genetic diversity of sturgeon species.

Habitat restoration plays an important role in conserving biodiversity and increasing the population size of a species. However, predicting its outcome remains challenging as restoration success is evaluated through indices of species abundance and diversity which require extensive resources. Another relevant component in developing conservation strategies is monitoring sturgeon biodiversity in aquatic ecosystems, a vital aspect that contributes to maintaining ecological balance and sustainable fisheries. For example, the presence of multiple genetic forms within *A. gueldenstaedtii* indicates a complex evolutionary history shaped by geographic isolation and independent adaptation. Studying the biodiversity of sturgeons can contribute to more efficient broodstock management and a successful conservation strategy for sturgeons. Additionally, through controlled breeding practices and rigorous monitoring, we can ensure that broodstocks contribute positively to both conservation and aquaculture goals. Therefore, adaptive management research strategies will continue to play a vital role in supporting the health and sustainability of sturgeon populations worldwide.

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