

## Four new polymorphisms of the mitochondrial gene cytochrome b in the beluga sturgeon (*Huso huso*) from the Black Sea

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### Abstract

The unique characteristics of sturgeons, as well as the difficulties in sampling determine the scarce knowledge about the genetics of this group of fish. The studies regarding taxonomic and phylogenetic relationships are limited, this group of fishes showing a great morphological variability due to their capacity of generating fertile hybrids with species belonging to different genera in their natural environment or in aquaculture conditions. Mitochondrial DNA represents a valuable marker in studies investigating the structure of the populations and phylogenetic relationships between sturgeon species. In our study we have sequenced a fragment of the cytochrome b mitochondrial gene from *Huso huso* individuals. By analyzing and comparing these sequences with those in the existing data bases we have identified new polymorphisms appearing in the *Huso huso* population from the Black Sea. The mutations involve four codons 234CTA, 280CTG, 390GGG and 405TGA of the cytochrome b mitochondrial gene. All these newly identified polymorphisms, detected in the beluga sturgeons from the Black Sea, do not alter the protein biological function and they might be employed as markers for the genetic characterization of this population.

Keywords: *Huso huso*, Black Sea, cytochrome b, polymorphism, mitochondrial DNA.

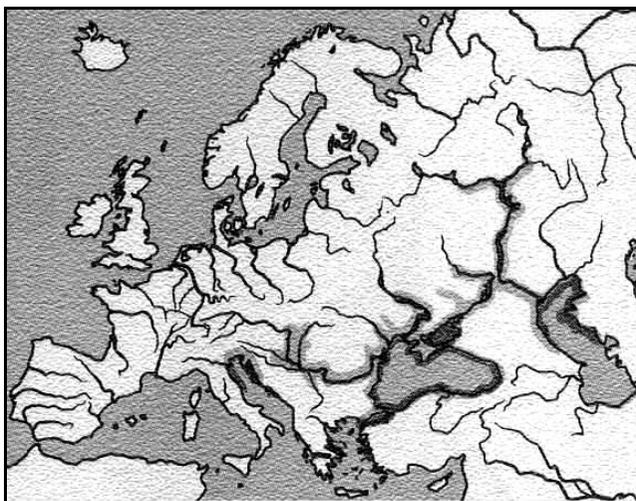
### Introduction

Sturgeons represent one of the most important marine natural resources. The interest for this ancient group of fish is both scientific and commercial as well. The molecular analysis of sturgeon species is extremely important since it provides valuable data which leads to an understanding of mechanisms underlying the evolution of vertebrates, thus they can be employed in the development of conservation programs for these species of fish.

Although the numbers of these populations have diminished for all sturgeon species, the beluga sturgeon (*Huso huso*) is presently in the most critical state, since the number of adult individuals has dramatically decreased in the last decades (T. De MEULENAER & al. [1]). Currently, the distribution habitat of this species has been confined to a few areas: the Black Sea, the Azov Sea, the Caspian Sea, the Adriatic Sea and the rivers that flow into them (Figure 1). In Romania, the beluga sturgeon can be encountered on the Black Sea coast and on the Danube, reaching the Iron Gates area (OȚEL [2]).

Being an anadromus species, the beluga sturgeon lives mainly in a sea environment and ascends rivers for spawning, usually to great distances from the inlet mouths. Among all sturgeons, the beluga sturgeon undertakes the longest up-stream migration. As a consequence, embankments and dams have had a strong impact on the natural breeding of this species. Based on the migration period, the beluga sturgeon is divided into two biological forms. One

of these forms migrates in the spring and the other in the autumn. The best spawning places are found in deep waters, on hard, sabulous ground, and are situated especially upstream the Danube River. Once they breed, adult individuals return to the sea, where they settle in waters as deep as 100 m (A. CIOLAC & al. [3]).



**Figure 1:** The natural habitat of *Huso huso* (www.ittiofauna.org).

Despite their scientific and commercial importance, the studies performed on this group of fish have been fairly limited. Due to the unique characteristics of sturgeons, as well as to the difficulties in sampling, few things are known about the genetics of this group of fish. Knowledge regarding taxonomic and phylogenetic relationships is fairly reduced due to the increased morphological variability and the capacity of these fish to generate fertile hybrids together with species belonging to different genera (*Acipenser*) in their natural environment.

Mitochondrial DNA analysis is employed in order to investigate the structure of the populations and phylogenetic relationships between sturgeon species. The mitochondrial genome contains genetic information which permits scientists to determine the relationships between related species and the history of a species. The majority of studies performed based on the mitochondrial DNA polymorphism have indicated low levels of divergence between the sturgeon species confirmed by comparative analyses. As a consequence, it is possible to use certain mitochondrial sequences as markers for the identification of populations (P. DOUKAKIS & al. [4], [5], M. FERGUSON & al. [6]).

## **Materials and methods**

### **Sampling and DNA extraction**

Sturgeons tissue samples from 10 individuals of *Huso huso* collected in liquid nitrogen were used for DNA extraction. The genomic DNA was extracted from 50 mg fins by a specific method (J.B. TAGGART & al. [7]). The DNA concentration and quality was assessed spectrophotometrically at 260/280 nm.

### PCR amplification and sequencing

We used one set of primers Forward (F) 5'AAAACCACCGTTGTTATTCA3' and Reverse (R) 5'GCCCTCAGAATGATATTTGT3' which amplify a fragment of 462bp from the cytochrome b gene. Initially, the PCR conditions were optimized by varying the annealing temperature between 55-65°C on a gradient thermocycler IQCycler (BioRad) and the optimum annealing temperature we selected was 60°C. Amplification reactions were carried out in 25µL final volume and contained 1X PCR Buffer, 35nM of MgCl<sub>2</sub>, 200µM of each nucleotide, 10µM of each primer, 0.5 units of AmpliTaq Gold DNA polymerase, nuclease free water (AppliedBiosystems) and 50ng of DNA template. PCR amplifications were performed using a program with 40 cycles on GeneAmp 9700 PCR System (AppliedBiosystems) under the following conditions: denaturation was performed at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C (60 seconds). The first denaturation step was of 10 minutes at 95°C and the final extension was of 10 minutes at 72°C.

The amplified fragments were sequenced by ABI Prism 3130 Genetic Analyzer (AppliedBiosystems), using the ABI Prism ® BigDye v3.1 Terminator Cycle Sequencing Kit (AppliedBiosystems) after purification with the Wizard PCR Preps DNA Purification System Kit (Promega). The sequences were processed using BioEdit Software.

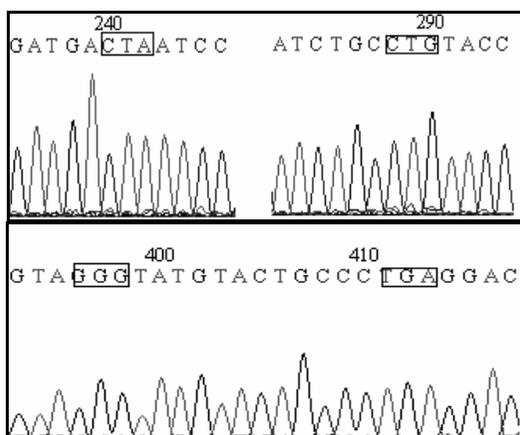
## Results and discussions

The recent development of molecular techniques is very useful for establishing new taxonomical and phylogenetic relationships among different populations with distinct geographical distribution. The molecular data, based on the mitochondrial genome to drop shed new light on intraspecific variability. Different regions of mitochondrial genome like cytochrome b gene may be employed to discriminate between closely related species or populations of the same species. The sequencing of a fragment of 462bp from the cytochrome b gene in *Huso huso* from the Black Sea reveals the existence of several polymorphisms in comparison with the same sequence from the *Huso huso* from the Caspian Sea. If confirmed in a higher number of individuals, this might be considered a characteristic of the Black Sea Beluga Sturgeons and it may become a molecular tool for differentiation between isolated reproductive populations.

In our study we have sequenced a fragment of the mitochondrial gene for cytochrome b. By analyzing and comparing these sequences with those in the existing data bases we have identified three polymorphisms characteristic to the *Huso huso* population in the Black Sea. The existence of the polymorphisms was confirmed for all the ten samples of *Huso huso* that we analyzed.

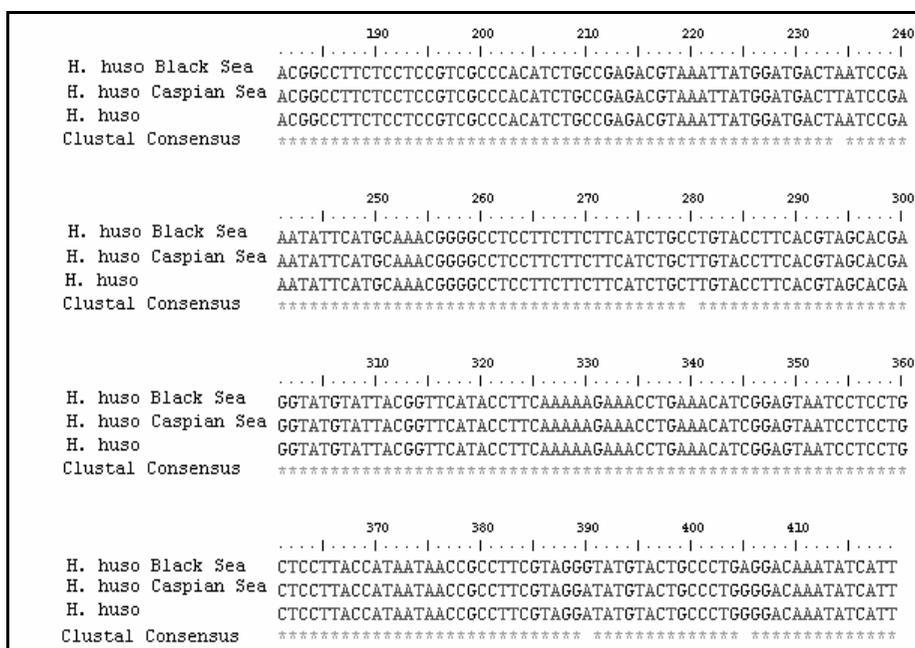
The data obtained by sequencing the 462bp fragment from the cytochrome b mitochondrial gene reveal the existence of several new polymorphisms in comparison with the sequences for the cyt b gene from GenBank Database. The mutations affect four codons 234CTA, 280CTG, 390GGG and 405TGA in the open reading frame (Figure 2).

The sequences were aligned and compared with similar ones from the data base which have the following accession numbers AJ245840 and AY442351 respectively (A. LUDWIG & al. [8]). By comparing the sequences from the Black Sea *Huso huso* individuals with cyt b gene sequences from the GenBank (AY442351), we identified three new polymorphisms in positions: 280T→C, 390A→G and 405G→A.



**Figure 2.** Polymorphisms of cyt b gene in *Huso huso* from Black Sea.

The mutations affected 234CTT→CTA, 280TTG→CTG, 390GGA→GGG and 405TGG→TGA codons.



**Figure 3.** Alignment between the cytochrome b gene fragments from *Huso huso* (GenBank Accession Number AY442351), *Huso huso* from the Caspian Sea (GenBank Accession Number AJ245840) and our PCR products of 462bp (*Huso huso* Black Sea).

All polymorphisms represent conservative missense mutations and do not alter the cytochrome b protein biological function. The modified codons 280CTG, 390GGG and 405TGA codify the same amino acids Leu, Gly and Trp respectively, similarly to the codons in the sequence of cyt b AY442351 (Figure 3).

The sequence of cyt b gene with accession number AJ245840 originates from a Caspian Sea *Huso huso* individual. By aligning and comparing the Black Sea *Huso huso* sequence with the Caspian Sea *Huso huso* sequence we have identified four polymorphisms. In addition to the three polymorphisms previously specified, we have identified the T>A polymorphism in position 234 of the cyt b gene. The codon 234 CTA in the Black Sea *Huso huso* sequence specifies for Leu, similar to 234 CTT in the Caspian Sea *Huso huso* sequence (Figure 3).

## Conclusions

The recent development of molecular techniques is very useful for establishing new taxonomical and phylogenetic relationships among different populations with distinct geographical distribution. The molecular data, based on the mitochondrial genome shed new light on intraspecific variability. The characterization of populations based on morphometric features is no longer sufficient and the molecular characterization is very useful and seems more adequate.

All these newly identified polymorphisms may be found to be characteristic to the *Huso huso* in the Black Sea and they might represent specific markers for this population. They are characteristic for the indigenous *Huso huso* individuals and may be employed for differentiating between populations from different geographical regions.

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