New Perspectives for Monitoring Migratory Animals – Improving Knowledge for Conservation* –

by Vadim Birstein**

Molecular identification of sturgeon species: scientific and bureaucratic problems

Sturgeons are a group of ancient fishes which are usually called "living fossils" belonging to the order *Acipenseriformes*, family *Acipensendae*. There are about 25 sturgeon species worldwide and two paddlefish species, their close relatives (family *Polyodontidae*). Almost all sturgeons are very similar morphologically, but they differ in size. All of them have three main characters: a specific form of the tail, an unusual rostrum, and five rows of large scutes along the body. Paddlefishes have a specific long paddle-like rostrum and do not have scutes.

All sturgeon species live in the northern hemisphere. Paddlefishes inhabit two distant basins: the Yangtze River in China and the Mississippi-Missouri River system in the United States. Most sturgeons are anadromous fishes and can be considered migrating species: they live in the sea and migrate into the rivers for spawning. Some species live in the rivers only.

Because of morphological similarity, until recently almost nothing was known about the relationships between different species of sturgeons. All hypotheses were based on general morphology and the geographic range of particular species.

Five years ago, my colleague Dr. Rob DeSalle of the American Museum of Natural History (New York) and I started a research on the molecular phylogeny of sturgeons. We sequenced portions of the following three mitochondrial DNA regions: cytochrome *b* (650 bp), 12S (150 bp), and 16S (350 bp) genes. A parsimony analysis of the combined data resulted in one tree. This tree represents the first scheme of relationships of the sturgeon species based on their genetic relatedness.

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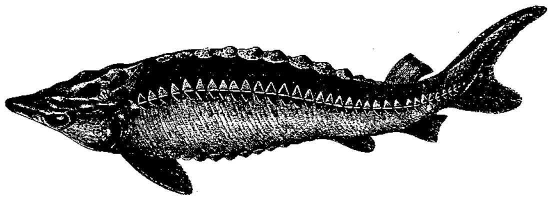
There are three main clades within the main sturgeon species: a cluster of the European and American Atlantic sturgeon species, a cluster of two American and two Asian species which live along both shores of the Pacific Ocean, and a cluster of Eurasian species. The last also consists of three clusters. One includes two species of the genus *Huso* which are embedded within the genus *Acipenser.* Apparently, *Huso* is not a separate taxonomic unit, as traditional systematists thought. One of these species, the beluga *Huso huso*, is important in terms of caviar production. Another caviar producing species, sevruga *A. stellatus*, is a sister-species of *Huso*. One more cluster includes three closely related species,



the Siberian sturgeon *A. baerii*, Persian sturgeon *A. persicus*, and Italian sturgeon *A. naccarii.*

This tree demonstrates a good topological similarity between patterns of ploidy and grouping and branching of sturgeon species. It seems that acipenseriforms originated from tetraploid ancestors and some of the species retained this ploidy. The octoploidization occurred independently in two lines: in the ancestral form of the Pacific species and in a group of four above-mentioned species, from *A. gueldenstaedtii* to *A. naccarii.* Also, it seems that even higher levels of ploidy appeared also in two lineages ancestral to the Asian Pacific species *A. mikadoi*, and to the species inhabiting rivers of the American Atlantic shore, *A. brevirostrum*.

Almost all sturgeon species are endangered. Three main factors caused the depletion of sturgeons: the destruction of the environment, especially construction of dams which prevent sturgeons from spawning, water pollution, and overfishing. At present, overfishing prevails and it threatens the survival of three Eurasian species traditionally exploited for the production of black caviar: the beluga *Huso huso*, sevruga *A. stellatus*, and Russian sturgeon *A. gueldenstaedtii*.

Overfishing is not a new problem. At the turn of this century the European Atlantic sturgeon, which historically inhabited all rivers of Central and Western Europe, was overharvested to the extent that it almost disappeared. Currently many European countries have restoration plans for this species, but at present there is no adult fish which could be used as a brood stock for these plans. Similarly, overfishing at the beginning of our century almost wiped out three sturgeon species commercially caught in the 19th century in the United States. Most Americans and Europeans do not know that 100 years ago Germany imported black caviar from the United States.

The three main Eurasian commercial species (beluga, sevruga, and Russian sturgeon or osetra) inhabit the Caspian, Black and Azov seas and rivers entering them. The Caspian Sea is in fact a large landlocked salt lake, not connected with the Black and Azov seas. There are numerous populations of each species within the Black and Caspian seas. It is known that during the geological history these seas were included in one water body and the three species of sturgeons are remnants of the fauna existing in the past in the united basin. The existence of numerous populations in a huge geographical area creates a technical problem in collecting tissue samples for molecular study.

The intensive overfishing of the three commercial species started in 1991 after the fall of the Soviet Union. Before that black caviar was produced in the Caspian Sea basin in two countries, the Soviet Union and Iran. After dissolution of the Soviet Union the number of countries harvesting sturgeons increased to five. Also, the Soviet centralized government control of the sturgeon catch disappeared and an uncontrolled wild poaching started in all parts of the former Soviet Union. In 1993–94, the European caviar market was flooded by a very low quality caviar made in Russia illegally. Later so-called "Turkish caviar" smuggled from Azerbaijan appeared in Turkey and Western Europe.

The International Conference on Sturgeon Biodiversity and Conservation held in 1994 in New York, was the first scientific forum which discussed the problem of the endangered status of sturgeons worldwide. After that I was appointed Chairman of the IUCN Sturgeon Specialist Group. I involved sturgeon scientists from many countries in the work of the group. Members of the group made a preliminary evaluation of the status of all sturgeon species, including that of different populations of the three commercial species.

Also, at the request of the IUCN Office in Cambridge, I prepared a draft proposal for the CITES listing of all sturgeon species with the hope that it would help control sturgeon overfishing. Soon it became clear that a scientific method for species identification of sturgeons was needed to support the proposal.

After the analysis of our database of DNA sequences, Rob DeSalle and I developed such a method. We compared partial sequences of the cytochrome *b* gene of all sturgeon species and selected nucleotide positions specific only to each of three commercial species. Using these data we created species-specific primers which served as boundaries for a PCR reaction. During this reaction a piece of DNA restricted by these boundaries is amplified. Our method was based on the specificity of the primers: if we were using primers for beluga, the reaction happened only for beluga caviar eggs and not for eggs of any other sturgeon species. The method is efficient for even a single caviar egg. We published the description of the method in *Nature and Conservation Biology.* Also, the method was patented in the United States and is presently being patented in the countries of the European Union. We assigned patent rights for the method in the United States to the American Museum of Natural History (New York), and in Europe, to the Karl-Schmitz-Scholl-Funds for Environmental Law and Policy (Bonn, Germany).

We used our method for a survey of the New York caviar market. During 1995–96, we purchased and tested about 100 lots of caviar. We found that more than 20 per cent of lots were mis-labeled, i.e. contained caviar not of the species mentioned on the label. For the identification of mis-labeled caviar, we sequenced regions of cytochrome b gene and compared these sequences with the sequences in our database. It appeared that we could not distinguish three species from each other: the Persian, *A. persicus*, Siberian *A. baerii*, and Italian, *A. naccarii*, sturgeons.

It took a long time to look into this puzzle. With the help of colleagues in different countries I collected tissue samples for the Russian and Persian sturgeon from the main populations of the Caspian, Black and Azov seas. The main expert on the Siberian sturgeon. Dr. Georgii Ruban (Moscow), provided us with samples from all three subspecies of this species (*A. b. baerii*, *A. b. stenorrhynchus*, and *A. b. baicalensis*). Besides the cytochrome *b* gene (850 bp), we collected sequence data for two more mitochondrial genes, the ND 5 gene (645 bp) and d-loop (725 bp). These regions theoretically evolve faster than the cytochrome *b* gene. The combined results of the whole research were quite unexpected.

Phylogenetic analysis of sequences of these three regions indicated that the Russian sturgeon is actually a polyphyletic assemblage consisting of two lineages: one is phylogenetically interspersed with *A. persicus* and *A. naccarii*, while another includes *A. baerii*. In the first clade *A. persicus* and *A. gueldenstaedtii* cannot be distinguished from one another. Apparently, *A. persicus* should not be considered as a separate species.

Acipenser naccarii can be identified by one position in the d-loop (G in 164), but this conclusion needs confirmation because we had tissue samples from only two individuals.

As for the second clade, *A. gueldenstaedtii* can be discriminated from *A. baerii* by C-T transition at position 432 in the d-loop. We suggest that this second genetic form of *A. gueldenstaedtii* in fact might be a cryptic taxon (a species?) known as a winter race of the Russian sturgeon (these sturgeon migrate into the river in the fall, stay in the river during the winter and spawn the next spring).

The discovery that traditionally recognized *A. gueldenstaedtii* consists of two separate genetic forms (or species?) points to the necessity of a revision of this species in conjunction with the closely related species, *A. persicus*, *A. naccarii*, and *A. baerii*. This revision should be done as a joint effort of specialists on morphology and genetics of sturgeons. Unfortunately, currently it is not possible to organize such a research, basically because of bureaucratic problems.

The listing of all sturgeon species in the CITES Appendix II (controlled trade) was accepted at the CITES (COP 10) meeting in Harare in June 1997. The listing came into force on April 1, 1998. No general structure or mechanism for implementation of the listing was recommended or created and now each country introduces its own practice. Almost all of them simply restrict their efforts by paperwork. The CITES authorities of the exporting side provide caviar shipments with the CITES permits, and the authorities of the importing country check these permits without any scientific testing. The law enforcement branch of the United States Fish and Wildlife Service, which is in charge of the CITES implementation, declared that its Forensics Laboratory had developed its own DNA method of sturgeon species identification which would be used for random tests of caviar shipments to the United States.

The method is based on sequencing and analysis of a short fragment (280 bp) of the cytochrome *b* gene. It is unclear how it was developed. At the time of the agency's announcement that they had a DNA method the Forensics Laboratory did not have tissue samples from Eurasian sturgeons. Also, at that time there were no sturgeon DNA sequences available in the international GenBank. However, the Laboratory had in its possession our unpublished materials, including sequences, which it promised to treat confidentially.

The Forensics Laboratory's method has not been published or reviewed by any independent molecular geneticist. It is clear that it has not been proven scientifically: the Laboratory is using a gene region that is too short and does not have the number of samples necessary to provide certainty (it has only a few samples from one population of each species). Many results of tests given by the Laboratory are simply impossible: the Laboratory claims that it identifies caviar of Siberian sturgeon instead of the Russian sturgeon. Taking into consideration our result about two forms of the Russian sturgeon, one of which cannot be distinguished from Siberian sturgeon if sequencing cytochrome *b* gene only, these claims are not scientifically supported. Despite all, beginning from April 1, 1997, the agency stopped and destroyed a few tons of caviar using its Laboratory results.

This activity has no impact on the wild stocks of sturgeons: the fish had already been caught and killed, caviar produced and sold. The producer of caviar is not hurt and the whole system leads to the financial destruction of small American businessmen whose shipments were tested by a scientifically inadequate and unreviewed method. If the caviar producers lose their unfortunate American partners, they can easily sell the next shipments to any country whose authorities check papers only. American businessmen have no alternative for an independent testing and checking of the Laboratory's species identification.

The second consequence of the sturgeon CITES listing is that international projects and research have become almost impossible. The CITES listing made an exemption for 250 g of caviar which can be bought by anybody without special permission. But it did not make any exemption for scientific samples and now a scientist cannot send even one egg fixed in alcohol to a colleague in another country for a genetic study. My appeal to the CITES Secretariat with the description of this absurd situation was in vain: the response was that the Secretariat could not do anything about that. This means the end of working relationships between scores of scientists located in different countries because of bureaucratic reasons and is absolutely destructive to the long term survival of sturgeons. It is unbelievable that such an Orwellian situation is allowed to continue. This does not give me much hope for the future of CITES.

A good example of this situation is a recent expedition to Turkmenistan in which I took part two months ago. The organization of the expedition from the United States to Turkmenistan took several years. We paid several hundred dollars per each sturgeon for official permission to catch it in the Amu-Darya River. But we had no opportunity to bring tissue samples to the United States for genetic work and several fish for the museum collection. Supposedly, we should receive CITES permits from two countries, Turkmenistan and Russia, which is technically unrealistic. In the meantime, the sturgeon species of that area are the most endangered. Taking into consideration the state of economy and science in Central Asia it is evident that only international conservation efforts based on research free of bureaucratic rules can save these endangered species.

Recently most of the sturgeon species, migrating and non-migrating, were proposed for the listing in Appendix II of the Bonn Convention. I hope that this will not create even more restrictions for the international scientific research, without which the implementation of environmental laws is senseless. It is more than obvious that conservation measures and control can be effective only if the government structures in charge of environmental law implementation are under the oversight of independent scientific organizations. The scientifically unsupported and unreviewed methods should not be used for legal implementation. ❐