Identification of Acipenseriformes species in trade

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Contents

Abbreviations

AFLP	Amplified Fragment Length Polymorphism
bp	base pairs (value for the length of a DNA fragment)
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
cytb	*cytochrome b* (gene of the mitochondrial genome)
d-loop	displacement-loop (part of the mitochondrial DNA)
DNA	Deoxyribonucleic acid
EMBL	European Molecular Biology Laboratory (DNA database)
IEF	Isoelectric Focusing
IUCN	The World Conservation Union
MDH	Malate Dehydrogenase
mt	mitochondrial
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SEM	Scanning Electron Microscope
ss	single-stranded
SSCP	Single Strand Confirmation Polymorphism
SSR	Simple Sequence Repeats
STR	Short Tandem Repeats
Taq	*Thermus aquaticus*
VNTR	Variable Number Tandem Repeats

Glossary

allele – Alternative states of a **gene**, generated by mutations.

autosomal DNA – All nuclear **DNA** which is located on an autosomal (non-sex determining) chromosome. In general, one allele is inherited from each of the parents.

***bp (*base pairs)** - Unit for the number of nucleotides currently used to characterize the length of **DNA** fragments or strands (pairs because **DNA** is a double stranded molecule and each nucleotide is base paired to its complementary nucleotide).

cytochrome b – Gene of the mitochondrial genome coding for cytochrome b and very commonly used for phylogenetic analyses of vertebrates.

diagnostic nucleotide or diagnostic substitution – Nucleotide or substitution which is only present in a single species (species-specific), **population** (population-specific) or stock (stock-specific). All specimens of this species, **population** or stock have this nucleotide or substitution and all specimens of other species, populations or stocks do not share it.

DNA – DNA (deoxyribonucleic acid) is a nucleic acid that contains the genetic instructions. In complex eukaryotic cells such as those from plants, animals, fungi and protists, most of the DNA is located in the cell nucleus (**nuclear DNA**). Additionally, cellular organelles known as chloroplasts (plants) and mitochondria (animals - **mitochondrial DNA**) also carry DNA. The mitochondrial (mt) genome of animals is a small genome containing about 16000 **bp** in length. In contrast, the human **nuclear DNA** contains 24 distinct chromosomes with a total of about 3 billion DNA **bp**. The number of chromosomes range between about 120 and >500 in sturgeons and paddlefishes. The number of **bp** is unknown for these species.

DNA sequencing – The process of reading the succession of nucleotides representing the genetic code by a machine called DNA sequencer.

EMBL - Nucleotide Sequence Database of the European Molecular Biology Laboratory in Germany.

endonuclease - An enzyme that breaks strands of DNA at internal positions; these enzymes are important tools in recombinant DNA technology.

gene – A region of the **DNA** which controls for a specific characteristic and is consequently regarded as the basic unit of heredity.

genetic drift - Genetic drift describes the statistical drift of the frequencies of neutral selected alleles over time.

genotype - The genotype will contain both member (diploid) or all member (tetraploid or higher ploidy levels) alleles of the nuclear **DNA**.

haplotype - A haplotype is the genetic constitution of an individual chromosome. In the case of diploid organisms, the haplotype will contain one member of the pair of alleles for each site. A

haplotype can refer to only one locus, to several loci or to an entire genome. A genome-wide haplotype would comprise half of a diploid genome, including one allele from each allelic gene pair. In this review, the term haplotypes refers to a set of nucleotide polymorphisms found to be in one or more genes. Furthermore, in the case of **mitochondrial DNA** haplotypes = **genotype**.

heterosomal DNA – Nuclear **DNA** which is located on an heterosomal (sex determining – X/Y or Z/W) chromosome. A small part of this **DNA** from the Y or W chromosome is inherited in a clonal (non-recombinant, non-**Mendelian**) fashion.

hybrid – A hybrid is a cross-breed between two (or sometimes more) species. In breeding biology, the term hybridization is also used for crosses between distantly (formerly separated) **populations** or hatchery **strain** of the same species.

interspecific variability – Genetic differences between species.

intraspecific variability – Genetic variability within one species.

introgression – An introgression is based on a hybridization event followed by back-crossing with one paternal species/**population**. This back-crossing with one paternal species results in the loss of nuclear **DNA** diagnostics for the other paternal species. The first hybrid generation (F1) has an allelic ratio of 50:50 from both species, the second generation resulting from a cross between a F1-hybrid and one paternal species has a ratio of 75:25, third 87.5:12.5 and so on. After a few more generations, no nuclear signs of the second paternal species are detectable. Based on their nuclear profiles and on their morphology this offspring is genetically identical to the first paternal species. However, **mitochondrial DNA** is passed on maternally and therefore, offspring can still have nuclear signs from one parent and at the same time their **mitochondrial DNA** can be from another paternal species.

lineage sorting - Lineage studies can be done based on the fact that the **mitochondrial DNA** only comes from the mother (maternal lineage), and the male Y chromosome only comes from the father (paternal lineage). Often these lineages are reflecting **population** (species) specific-selection resulting in **interspecific** or **intraspecific** variability.

Mendelian inheritance (fashion) – Inheritance following the rules discovered by Gregor Mendel (1822-1884).

microsatellite – A region of **DNA** that evolves very rapidly allowing the detection of recent evolutionary changes. Microsatellite regions form the basis of **DNA** profiling (genotyping) which is used in forensic applications.

mitochondrial DNA - **DNA** which is located in the mitochondrial genome. In vertebrates, mitochondrial **DNA** (mtDNA) is only inherited from the mother. This means that mutations of mtDNA can be passed from mother to all offspring independent of their sex.

morphological analysis - The form and structure of an organism or one of its parts (for example oocytes) is analysed.

mutation – A change in the **DNA** sequence that results in an alteration of this sequence. Reasons are non-correct replication or the presence of mutagenic factors, for example. Fixation of a mutation by selection or genetic drift can result in a diagnostic character state (**diagnostic substitution**).

nuclear DNA – DNA which is stored in the nuclear genome, unifying all **autosomal** and **heterosomal DNA**.

n-1 molecules – Normally **primers** are stored forzen. The successive thawing/freezing of **primers**, when they are used for **PCR,** cause their partial degeneration. This degeneration results in the cut of one (n-1) or more (n-x) nucleotides resulting in an shorting of **primers**.

oocyte - An oocyte or ovocyte is a female gametocyte or germ cell involved in reproduction. Unfertilised egg cell.

organoleptic analyses - Relating to perception by a sensory organ. In the case of caviar, colour and taste are proven mainly.

PCR – PCR stands for Polymerase Chain Reaction. This is a biochemical (enzymatic) reaction geneticists use to artificially synthesise millions of copies of a target region of **DNA**. It is the method by which microscopic quantities of tissue can be investigated in forensic and conservation genetic analysis.

population – In this paper the term is used for all specimens from the same river basin or water body (including tributaries).

phylogeny – The pattern of ancestral relationships between species or taxonomic groups.

primer - A short segment of **DNA** or RNA that is complementary to a given **DNA** sequence and that is needed to initiate replication by DNA polymerase (used for **PCR** amplification).

RFLP - Restriction Fragment Length Polymorphism is a fingerprinting technique based on sequence-specific cleavages of DNA by restriction enzymes. Because of the diagnostic differences between sequences (species), species-specific restriction patterns can be used for identification.

strain – Within this report this term is used for hatchery fish of the same genetic (breeding) origin.

sub-population – This term is used within this document for a separated stock of fish from a single river or tributary.

Summary

Sturgeons and paddlefishes (Acipenseridae) are highly endangered freshwater fishes. Their eggs (sold as caviar) are one of the most valuable wildlife products in international trade. Concerns of overharvesting and the conservation status of many of the 27 extant species of Acipenseriformes led to all species being included on the CITES Appendices in 1998. Since then international trade in all products and parts from sturgeon and paddlefish has been regulated. However, despite the controls on trade, unsustainable harvesting continues to threaten many populations. Illegal fishing and trade continues to be a threat to the management of these fish.

To enforce the regulation of legal trade and prevention of illegal trade, the development of a uniform identification system for parts and derivates of Acipenseriformes has been identified as an urgent requirement. Ideally this system should be suitable for (i) identification at the species-level of caviar and other products from Acipenseriformes, (ii) population identification, (iii) source identification (wild *versus* aquaculture), and (iv) determining the age of caviar because strict timeframes govern its international trade. This paper reviews the techniques currently available and their potential to be used in an identification system for Acipenseriformes species and their products in trade.

A review of all available identification techniques has shown that there is not a single method that can meet all requirements (see i-iv), and it does not appear to be feasible to develop such a method in the near future therefore the most appropriate methods need to be developed for each. Considering the advantages and disadvantages of all techniques reviewed in this document, the following conclusions can be drawn:

i) For the identification of species, approaches are recommended that target mitochondrial *cytochrome b* sequences (RFLP, nested PCR or direct sequencing). However, they show limitations for the detection of hybrids (although natural hybrids are rare, the number of artificially produced hybrids in aquaculture is increasing) and for the differentiation of the following closely related species complexes: *Acipenser*

gueldenstaedti - *A. baerii* - *A. persicus* - *A. naccarii*; *A. medirostris* - *A. mikadoi*; and *Scaphirhynchus albus* - *S. plathorhynchus* - *S. suttkusi*.

ii) The identification of different populations of the same species is currently not feasible because genetic data are incomplete for most populations, and stocking and release programmes, which have become more and more common, often result in a mixture of phenotypes and genotypes, thereby impeding the creation and application of such a population identification system.

iii) Source identification based on genetic approaches can be excluded at present because there are no genetic differences between wild and hatchery-raised fish. This is the result of the continuing restocking of natural populations with captive fish and *vice versa*. However, because rearing (i.e. environmental) conditions are different, methods focusing on differences in water quality or food seem to be more appropriate (for example differences in fatty acid composition). So far, very few studies have been conducted and therefore, source identification methods merit further exploration.

iv) The age of a product in trade cannot be detected by DNA-based methods and protein profiling is undoubtedly impractical due to hard-to-perform, labour-and cost-intensive methods, which are highly susceptible to protein degradation.

Arising from the limits discussed above, the next steps in the development of a uniform sturgeon identification system are proposed to be the following:

i) Designation of qualified reference laboratories at national levels in (re-) exporting and importing countries. These should be approved through a standardized testing procedure, for instance a ring test on blind samples. Registered laboratories should be published and disseminated and their accreditations should be subject to certain guarantees regarding quality, economic independence and scientific rigour. Operational procedures have to be determined and standardized among reference laboratories.

ii) Establishment of reference collections that are accessible to the reference laboratories containing DNA analyses results and information on the location and availability of tissue samples. This is highly recommended as an important step

towards a population identification system and indispensable for a general species identification system.

iii) Creation of a website access to the reference collections containing the reference database information about genetic samples, comparable to NCBI, which provides background data: sample location; population information; citation; available genetic data; location of archival storage; currently treated and distributed caviar and status of analysis. This website should also be a forum for the exchange of knowledge on and experiences with identification systems, species and population status information, relevant scientific research, etc.

iv) The outcome of the trade identification tests should be made available to the reference laboratories for future reference.

The universal caviar labelling system could incorporate an indication of the verification of the consignment.

In view of the lack of knowledge and the great need to develop a uniform identification system for Acipenseriformes with regard to the importance of the international caviar trade, further scientific guidance and appropriate research is strongly recommended. Progress should be assessed and exchanged on a regular basis.

1. Introduction

Sturgeon, the family of Acipenseriformes, are known as the producers of caviar, which today is one of the most highly valued animal products in trade (Pikitch *et al.*, 2005). One kilogram of Beluga caviar (*H. huso*), the most highly prized caviar, was sold for up to US$ 4,290 on Western markets in 2005 (Table 1). The following 27 species are included in the family of Acipenseriformes: 25 sturgeon species (***Acipenser baerii,*** *A. brevirostrum*, *A. dabryanus*, ***A. fulvescens***, ***A. gueldenstaedtii,*** ***A. medirostris***, *A. mikadoi*, *A. naccarii*, *A. nudivendris*, ***A. oxyrinchus***, ***A. persicus***, ***A. ruthenus***, ***A. schrenckii***, *A. sinensis*, ***A. stellatus***, *A. sturio*, ***A. transmontanus***, ***Huso huso***, ***H. dauricus***, *Pseudoscaphirhynchus kaufmanni*, *P. hermanni*, *P. fedtschenkoi*, *Scaphirhynchus albus*, ***S. platorynchus***, and *S. suttkusi*), and two paddlefishes (***Polyodon spathula***, *Psephurus gladius*). However only 14 of these species are commercially important (highlighted in bold). Sturgeon and paddlefish species are widespread, occurring in Eurasia and North America. The high economic value of sturgeons, mainly for the caviar they produce, and the failure to manage harvests at a sustainable level, in combination with the loss of spawning grounds, have resulted in population declines. Today, all but three species are considered to be threatened by IUCN, with some believed to be on the brink of extinction (Table 2).

Table 1: Mean import prices (in US $/kg without tax) for caviar; n/i = no import (source Zuther-Grauerholz, head of Dieckmann & Hansen, Hamburg, Germany; pers. comm. 2006).

Origin / Species	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Russia								
H. huso (Beluga)	550	650	730	600	700	1400	1800	n/i
A. gueldenstaedtii (Osietra)	450	580	490	380	380	780	1300	n/i
A. stellatus (Sevruga)	400	545	470	350	350	570	980	n/i
Iran								
H. huso (Beluga)				863	1318	2306	4290	n/i
A. gueldenstaedtii (Osietra)				558	720	1203	2860	2406
A. persicus (Asetra)				558	720	1203	2860	2406
A. stellatus (Sevruga)				450	680	1180	2171	n/i
Romania								
H. huso (Beluga)	450	680	660	520	450	850	1600	n/i
A. gueldenstaedtii (Osietra)	360	470	500	450	380	680	n/i	n/i
A. stellatus (Sevruga)	330	440	380	380	350	n/i	n/i	n/i

Table 2: Overview of names, distribution, global threat category according to the IUCN Red List and CITES listing for all sturgeon and paddlefish species worldwide.

Species name	Common name	Caviar name	Distribution area	CITES App	IUCN Red List status*
Asian species					
Acipenser baerii	Siberian sturgeon	Baerioska (farmed)	Rivers of north coast of Russia	II	VU[a]
Acipenser dabryanus	Dabry's or Yangtze sturgeon		Yangtze River	II	CR[a]
Acipenser mikadoi	Sakhalin sturgeon		Siberia Pacific coast	II	EN[a]
Acipenser schrenckii	Amur sturgeon		Amur River (Russia, China)	II	EN[a]
Acipenser sinensis	Chinese sturgeon		Yangtze and Pearl rivers	II	EN[a]
Pseudoscaphirhynchus fedtschenkoi	Syr-Darya shovelnose sturgeon		Syr-Darya basin	II	CR[a]
Pseudoscaphirhynchus hermanni	Small Amu-Darya Shovelnose, Dwarf sturgeon		Amu-Darya basin	II	CR[a]
Pseudoscaphirhynchus kaufmanni	False or Large Amu-Darya Shovelnose sturgeon		Amu-Darya basin	II	EN[a]
Huso dauricus	Kaluga	Kaluga	Amur River drainage	II	EN[a]
Psephurus gladius	Chinese paddlefish, Sword sturgeon		Yangtze River	II	CR[a]
Eurasian species					
Acipenser gueldenstaedtii	Russian sturgeon	Osietra, Ossetra, Almas (white caviar)	Ponto-Caspian distribution	II	EN[a]
Acipenser nudiventris	Fringebarbel, Ship, Spiny or Thorn sturgeon	Ship	Ponto-Caspian distribution, Lake Balkhash, extinct in the Aral Lake	II	EN[a]
Acipenser persicus	Persian sturgeon	Asetra	Caspian Sea (mainly southern area), Black Sea (Rioni River)	II	EN[a]
Acipenser ruthenus	Sterlet		Ponto-Caspian distribution, Ob River (Siberia)	II	VU[a]
Acipenser stellatus	Star or Stellate sturgeon	Sevruga	Ponto-Caspian distribution	II	EN[a]

Huso huso	Beluga, Giant or Great sturgeon	Beluga, Almas (white caviar)	Ponto-Caspian distribution	II	EN[a]
European species					
Acipenser naccarii	Adriatic sturgeon		Northern tributaries of the Adriatic Sea	II	VU[a]
Acipenser sturio	Baltic, common, European, Sea sturgeon, German sturgeon		Gironde basin (France), formerly Black, Adriatic, Mediterranean seas, European Atlantic coast	I	CR[a]
North American species					
Acipenser brevirostrum	Shortnose sturgeon		North American Atlantic coast	I	VU[b]
Acipenser fulvescens	Lake sturgeon		Great Lakes, Central United States	II	LC[b]
Acipenser medirostris	Green sturgeon		North American Pacific coast	II	VU[c]
Acipenser oxyrinchus	Atlantic or Gulf sturgeon		North American Atlantic coast, Gulf of Mexico	II	LR/nt[c]
Acipenser transmontanus	White sturgeon		North American Pacific coast	II	LC[b]
Scaphirhynchus albus	Pallid sturgeon		Mississippi-Missouri basin, South-eastern United States	II	EN[b]
Scaphirhynchus suttkusi	Alabama sturgeon		Mississippi-Missouri basin	II	CR[b]
Scaphirhynchus platorynchus	Sand or Shovelnose sturgeon, Hackeback		Mississippi-Missouri basin, South-eastern United States	II	VU[b]
Polyodon spathula	Duckbill or Spoonbill Cat, Paddlefish, Spadefish		Mississippi-Missouri basin, South-eastern United States	II	VU[b]

** IUCN Red List global threat categories:*

LR/nt=Lower risk

VU=Vulnerable

EN=Endangered

CR=Critically endangered

a – assessed 1996 with 1994 Categories & Criteria (version 2.3) (http://www.iucnredlist.org/info/categories_criteria1994#categories). Species currently being reassessed. Species currently being reevaluated.

b – assessed 2004 with 2001 Categories & Criteria (version 3.1) (http://www.iucnredlist.org/info/categories_criteria2001#categories)

c – assessed 2005 with 2001 Categories & Criteria (version 3.1) (http://www.iucnredlist.org/info/categories_criteria2001#categories)

Biology and ecology

Acipenseriformes species are large slow-growing fish; the largest of these, *Huso huso* can reach over six metres. Although females produce large quantities of eggs, they are generally long-lived and mature slowly, with infrequent reproduction (Pikitch *et al*., 2005), characteristics which make them vulnerable to overharvesting. All species spawn in freshwater with most species migrating between marine and freshwater systems (diadromous), but some migrating within freshwater systems (potamodromous) (De Meulenaer and Raymakers, 1996) As well as overharvesting, habitat degradation and pollution have contributed to the population declines of the Acipenseriformes.

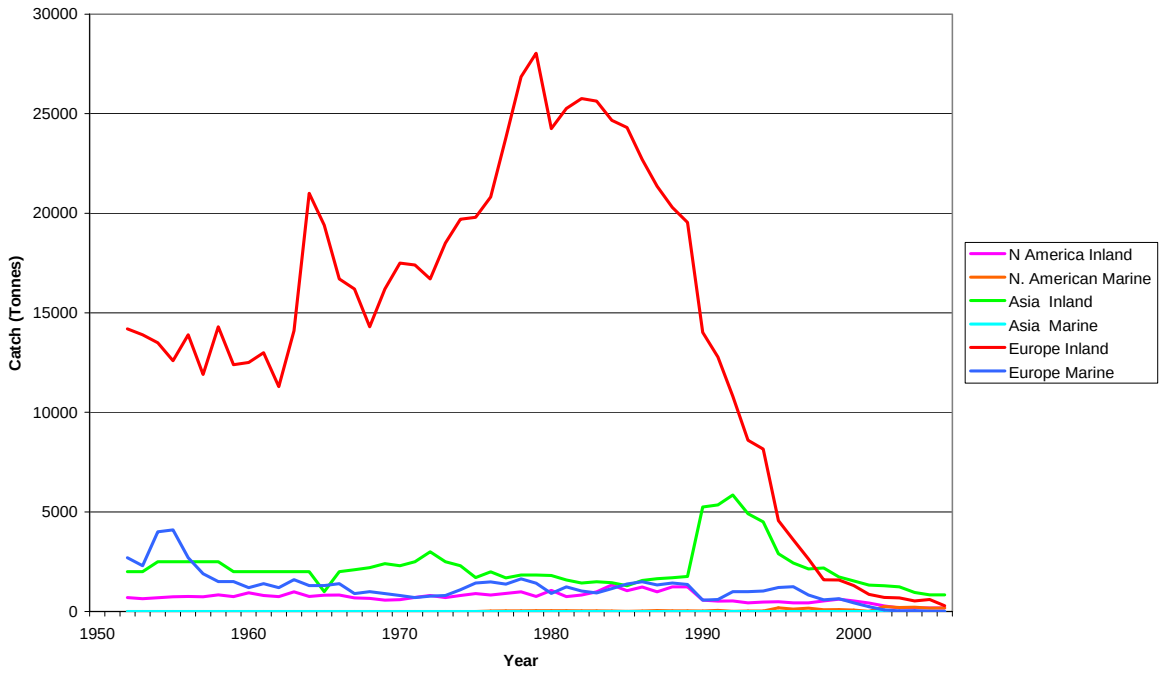
Trade in sturgeon and paddlefish

Sturgeon and paddlefish are predominantly harvested for their eggs, sold as caviar. Caviar has been traded for many centuries, but it was only in 1820 that the use of refrigeration allowed a dramatic increase in international trade and production. During this time, several, mainly West European, caviar companies traded caviar produced from Eurasian sturgeon species. Declines in catch led to a shift in trade with the United States becoming the top caviar producer, exporting caviar primarily from the Atlantic sturgeon (*A. oxyrinchus*) to Europe in the second half of the nineteenth century (De Meulenaer and Raymakers, 1996). However, by 1910, production from the USA had collapsed and the USSR again became the primary producer. Iran became a significant exporter following an agreement with the USSR in 1953 (Bourguignon, 1989) to share with them the Caspian Sea the stocks of the four most important caviar – producers; *A. gueldenstaedtii*, *A. persicus*, *A. stellatus*, *H. huso*, and two economically less relevant species *A. nudiventris* and *A. ruthenus*. Catch peaked at approximately 28,000 tonnes of fish in 1977 (Figure 1). After the break up of the USSR, fishing rights in the Caspian Sea were shared between Azerbaiijan, Kazakhstan, Russia, Turkmenistan and Iran. However, with the dissolution of the USSR came an increase in illegal harvest and trade (De Meulenaer and Raymakers, 1996; Pikitch *et al*., 2005).

Recent population and catch trends

Although population sizes of all Eurasian species declined, the situation has been most critical for *H. huso* (Beluga sturgeon). *H. huso* were harvested most intensively in the Volga basin, and the number of spawning adults has decreased dramatically since 1990 (De Meulenaer and Raymakers, 1996). Sturgeon reproduction decreased after the Volgograd Dam construction in 1960, when reproductive migration of adults was prevented by isolation of historic spawning grounds (Pikitch *et al.*, 2005). In order to stabilise caviar production of the Volga River, captive-raised fish were released from 1960 onwards. Nevertheless, despite these restocking efforts Caspian harvests had declined from the peak in 1977 to less than 15% of this level by the mid 1990s (see Figure 1). In contrast, trade in caviar from North America remained fairly constant until the late 1980s.

Figure 1: Volume of sturgeon and paddlefish catches landed 1950–2004 (based on FAO-FIGIS data downloaded 30th November 2006).



Concerns about overharvesting and the conservation status of many of the 27 extant species of Acipenseriformes led to all species being included in the CITES

Appendices in 1998, although some species had been listed on the Appendices previously (*Acipenser brevirostrum* (App I in 1975) *Acipenser fulvescens* (App II in 1975), *Acipenser oxyrinchus* (App I in 1975 transferred to App II in 1979) *Acipenser sturio* (App II in 1975 transferred to App I in 1983)) *Polyodon spathula* (App II in 1992). Since 1998 international trade in all specimens of Acipenseriformes, including live animals and all parts and derivatives such as caviar, meat, isinglass and fertilized eggs, has been regulated under the provisions of CITES. Two species, *A. sturio* (European sturgeon) and *A. brevirostrum* (Shortnose sturgeon), remain in CITES Appendix I, whereby commercial trade is prohibited. All other sturgeon and paddlefish species are listed in CITES Appendix II. Commercial trade is authorized pending the issuance of CITES documents. These are approved only when authorities are satisfied that the specimens in trade are of legal origin and, in the case of specimens from the wild, are harvested in a sustainable manner. Although trade is predominantly in caviar it also involves meat, isinglass, brood fish, including fertilized eggs, fingerlings and adult fish; the amount of hatchery fish trade increased rapidly from 2465 t in 1999 to 15,551 t in 2004 (*FAO Statistical Yearbook 2005-2006*).

Since all Acipenseriformes were listed in 1998 reported international trade has reduced. This is partly due the continuing decline in population numbers but also due to the introduction of quotas in 2002 (see Figure 2 and Table 3). However, large quantities of caviar are still produced for the domestic market and illegal catch and trade are said to exceed legal landings (Williot *et al*., 2002; Pikitch *et al*., 2005). Studies of international trade (DeSalle and Birstein, 1996; Birstein *et al.,* 1998; Wolf *et al*., 1999; Raymakers, 2002) give evidence for a high proportion (7–25%) of caviar with the wrong species origin assigned sold on the world market. Although the actual amounts of illegal trade are unknown, police and customs services of EU Member States seized over 12 tonnes of illegal caviar in the EU between 2000 and 2005 (EU-Commission Reference press release: IP/06/611, 15/05/2006).

Figure 2: Reported international trade in caviar from wild sources (tonnes = 1000 kg) (source: CITES trade data www.cites.org in Raymakers, 2005); 2a Caspian & Black Sea, and 2b Amur River.

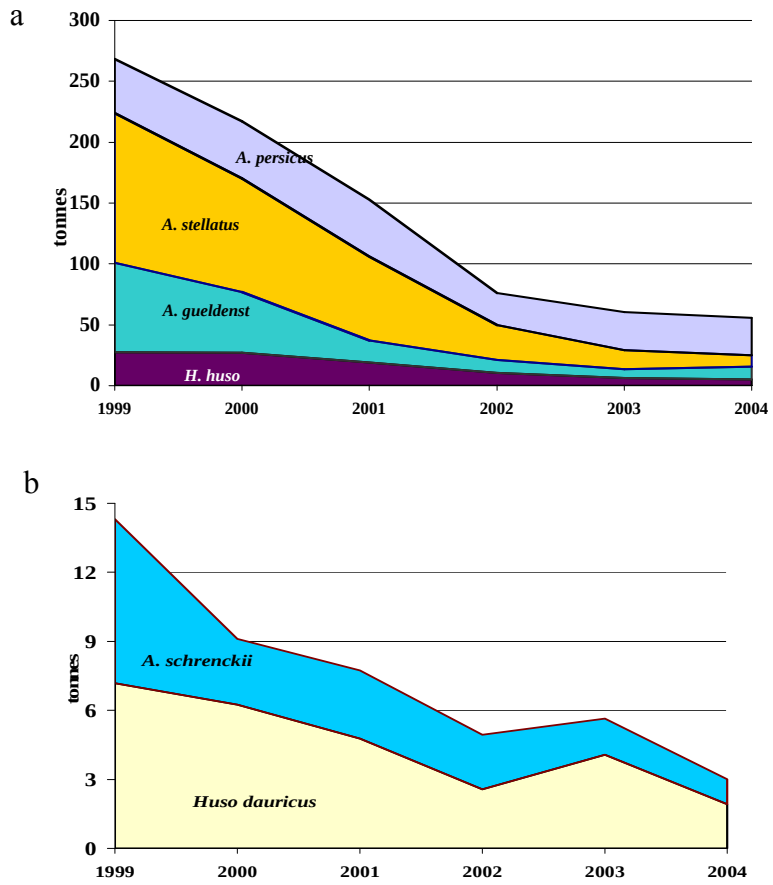


Table 3: Reported international trade in caviar from wild sources in metric tonnes (source: CITES trade data www.cites.org in Raymakers, 2005)

Origin/ Species	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**% change 1999–2004**
Caspian & Black Sea							
Huso huso	27.62	26.89	18.83	10.54	6.54	4.99	-81.9%
A. gueldenstaedtii	73.55	49.81	18.44	10.72	7.03	10.79	-85.3%
A. stellatus	122.62	93.61	68.71	28.79	15.71	9.18	-92.5%
A. persicus	44.38	46.93	47.01	25.86	31.02	30.78	-30.6%
A. nudiventris	0.00	3.34	4.89	0.71	0.08	0.08	-97.7%
Amur River							
Huso dauricus	7.18	6.25	4.77	2.57	4.07	1.92	-73.2%
Acipenser schrenckii	7.12	2.86	2.97	2.37	1.57	1.08	-84.8%
USA							
Polyodon spathula		3.39	1.95	2.17	4.85	4.62	43.0%
Total	**282.47**	**233.08**	**167.56**	**83.73**	**70.87**	**63.44**	

At the time of listing of sturgeon, a Resolution was adopted on the Conservation of Sturgeon (Res. Conf 10.12). This Resolution urged range States and Parties to encourage scientific research to promote the sustainability of sturgeon fisheries in the Eurasian region, to curtail illegal fishing and exports and to promote regional agreements between range States to bring about proper management and sustainable utilization of sturgeons. At COP 11 in Gigiri 2000, decision 11.58 was adopted which instructed range States to declare coordinated annual export and catch quotas and to inform the Secretariat of these before 31st December of the preceding year. Failure to do so would result in Parties having a zero quota for the following year. Resolution 10.12 also recommended that the Secretariat, in consultation with the Animals Committee, explore marking systems for sturgeon products, and that the Animals Committee consider sturgeons under the Review of Significant Trade. This Review concluded that for North American species, despite river damming and habitat deterioration, international trade is generally well managed under CITES provisions and was not a conservation concern. However, the Review showed continuing declines in harvested sturgeon stocks in the Caspian Basin and recommendations were made for actions to be taken by four of the Caspian Sea range States, no recommendations were made for Iran. As a result four range states of the Caspian Sea (Azerbaijan, Kazakhstan, the Russian Federation and Turkmenistan) committed themselves to a series of urgent measures in the "Paris Agreement". These included:

a) collaborative basin-level management system for sturgeon fisheries (including independent stock assessments for quota setting)
b) efforts to combat illegal harvesting and trade
c) regulation of domestic trade
d) establishment of caviar labelling system.

The establishment of a universal labelling system was considered a key instrument to assist law enforcers and consumers in distinguishing legal caviar from illegal caviar. At COP 12 Resolution 10.12 was repealed by Resolution 12.7 (revised CoP13). This described labelling requirements which include a standard species code; the source

code of the caviar; the ISO two-letter code for the country of origin; the year of harvest; the official registration code of the processing plant (e.g. xxxx); and the lot identification number for the caviar (e.g. yyyy). The formula should therefore read, for example, "Beluga/HUS/RU/2001/xxxx/yyyy". Currently the labelling is required for all caviar containers in trade, whether imported, exported, re-exported or in domestic markets.

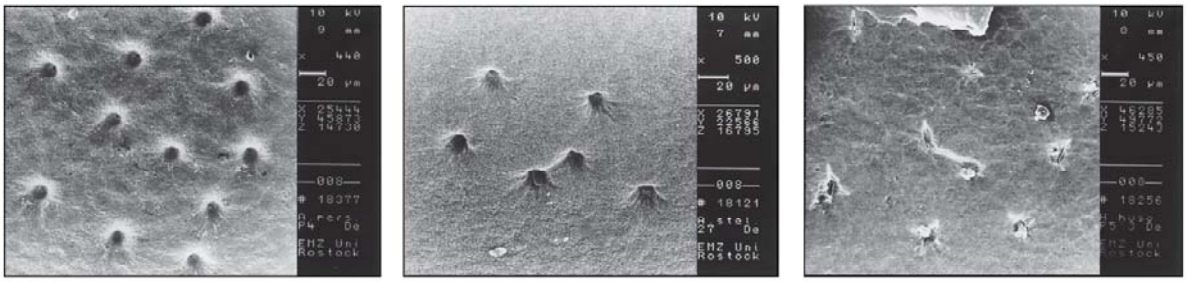
In addition this Resolution called upon "range States, importing countries and other appropriate experts and organizations such as the IUCN/SSC Sturgeon Specialist Group, in consultation with the Secretariat and the Animals Committee, to explore the development of a uniform DNA-based identification system for parts and derivatives and aquaculture stocks of Acipenseriformes species to assist in the subsequent identification of the origin of specimens in trade." Given that quotas are set for each range state sharing its sturgeon populations with neighbouring states, the sturgeon/caviar identification system ideally needs to be able to identify the species and the stock or local population where the product has come from. To this end, the IUCN/SSC Sturgeon Specialist Group was contracted through IUCN to write a paper discussing the techniques currently available for identification of Acipenseriformes species in trade, and further steps needed to develop these techniques as a tool for identification. This report discusses the techniques currently available for interspecific (species) and intraspecific (population) as well as source (wild *versus* aquaculture) identification. In order to use many of these techniques as a tool for identification, a reference collection for sturgeon samples would be necessary. This report also covers the requirements for establishing such a reference collection. The initial draft report was discussed and revised at a workshop held on this subject in Berlin from 29th September to 1st October 2006.

2. Species/caviar identification methods

2.1 Morphological and biochemical methods

Traditionally, morphological and organoleptic analyses have been carried out to identify species. These coarse methods are susceptible to several errors. For instance, egg size and egg coloration for Acipenseriformes, vary within species depending on age (size) of the specimens analysed as well as type of nutrition. In 2002, Debus *et al.* extended morphological studies to sturgeon oocytes (both caviar and unprocessed oocytes), which they compared for the following species: *A. gueldenstaedtii*, *A. stellatus*, *A. persicus*, *A. baerii*, *A. nudiventris*, and *H. huso,* using a Scanning Electron Microscope (SEM). The presence (*Acipenser* spp.) or absence (*Huso huso*) of a funnel on the micropyle (Figure 3) allowed the discrimination of the two genera. All species analysed differed in their oocyte diameter, the size of the micropylar fields (surface perforated by micropyles) and in the ultrastructure and size of the external micropylar opening. Nevertheless, the oocyte surface of all species was very similar and changes in the oocyte envelopes during caviar processing blur the diagnostic structural differences among species (Debus *et al.,* 2002).

Figure 3: Micropylar field of an egg from *A. persicus*, *A. stellatus*, and *H. huso* (from left to right); micropyles with funnels and clear grooves (x500 – figure modified after Debus *et al.*, 2002; Debus, pers. comm., 2006).



Biochemical methods were also used for species identification, especially isoelectric focusing (IEF), introduced in the 1980s. This technique is based on differences in the proteins of the different caviar producing species (Rehbein, 1985; Keyvanfar *et al.*,

1988; Chen *et al.*, 1996). But Rehbein (1997) concluded that the protein patterns produced by IEF are very similar for *H. huso* (Beluga), *A. stellatus* (Stellate sturgeon) and *A. gueldenstaedtii* (Russian sturgeon). All studies using IEF included the three commercial sturgeon species *A. gueldenstaedtii*, *A. stellatus*, *H. huso* and up to two additional species (*A. oxyrinchus*, *P. spathula*). In contrast to morphological and organoleptic analyses, IEF has the advantage that little training is needed to use this method for species identification. However, there are several disadvantages to using IEF. An examination of roe from most if not all sturgeon species must be carried out together in a single experiment to ensure species-specific and thus distinguishable patterns of protein banding, because species differ chiefly in the intensity of their bands rather than in the position of their bands (Rehbein, 1997). As sampling and their preparation are the main influence on the intensity of bands, standardization is necessary to make the results comparable.

In conclusion, morphological approaches to identify commercial caviar at species level are usually very time consuming, expensive and often unreliable because they are highly sensitive to environmental variation and processing techniques (Clayton, 1981). Moreover, the analyses of morphological traits are further limited by the absence of reference data for most species. The same limitations also exist for biochemical approaches (IEF produces only weak differences among species and is very sensitive to sampling and processing conditions).

2.2 Genetic methods

Morphological and biochemical analyses are not suitable when using processed caviar or meat and for this and the other reasons mentioned below, DNA-based techniques have become the most important for species identification during the last decade. This is due, in part, to the uniformity of the genetic code and that DNA can be extracted not only from all tissues of living specimens, but also from processed caviar and meat. Furthermore, only very small sample sizes are needed (in theory, one cell or oocyte would suffice).

Nearly all species comprise various degrees of intra- and interspecific genetic differentiation. The amount and pattern of this divergence is determined by the formation of new genetic lineages through DNA mutation, random lineage sorting through genetic drift, increased mixing of gene pools after geographic dispersal, and natural selection.

2.2.1 DNA sequencing

Traditionally, divergence analyses are based on nuclear genotypes (for example autosomal sequences, microsatellites, RFLP patterns) or on haplotypes (when considering haploid genomes such as mitochondrial DNA or hetero (sex) chromosome sequences). The degree of DNA sequence variation allows separation of sturgeon species, sub-species, populations (stock, river system) and lineages (e.g. hatchery strains) when reference data sets are available for evaluation. Sequence analysis is the analysis of the order of the four bases (cytosine, guanine, adenine, and thymine) of both types of DNA (mitochondrial and nuclear). The comparison of the sequences from different specimens results in the detection of differences (substitutions). Sequencing analysis is necessary for the validation of each genetic difference observed through the different methods discussed below. As this sequencing is expensive and time consuming it is impossible for each laboratory to sequence all species and/or populations so most scientists store their sequencing results in public gene banks (e.g. EMBL, GenBank). The data contained in these libraries may be used for sequence comparison. **Sequencing analysis has the largest diagnostic power of all DNA-based methods but it is time consuming and expensive, and thus, it is not a suitable technique for the screening of large sample sets.**

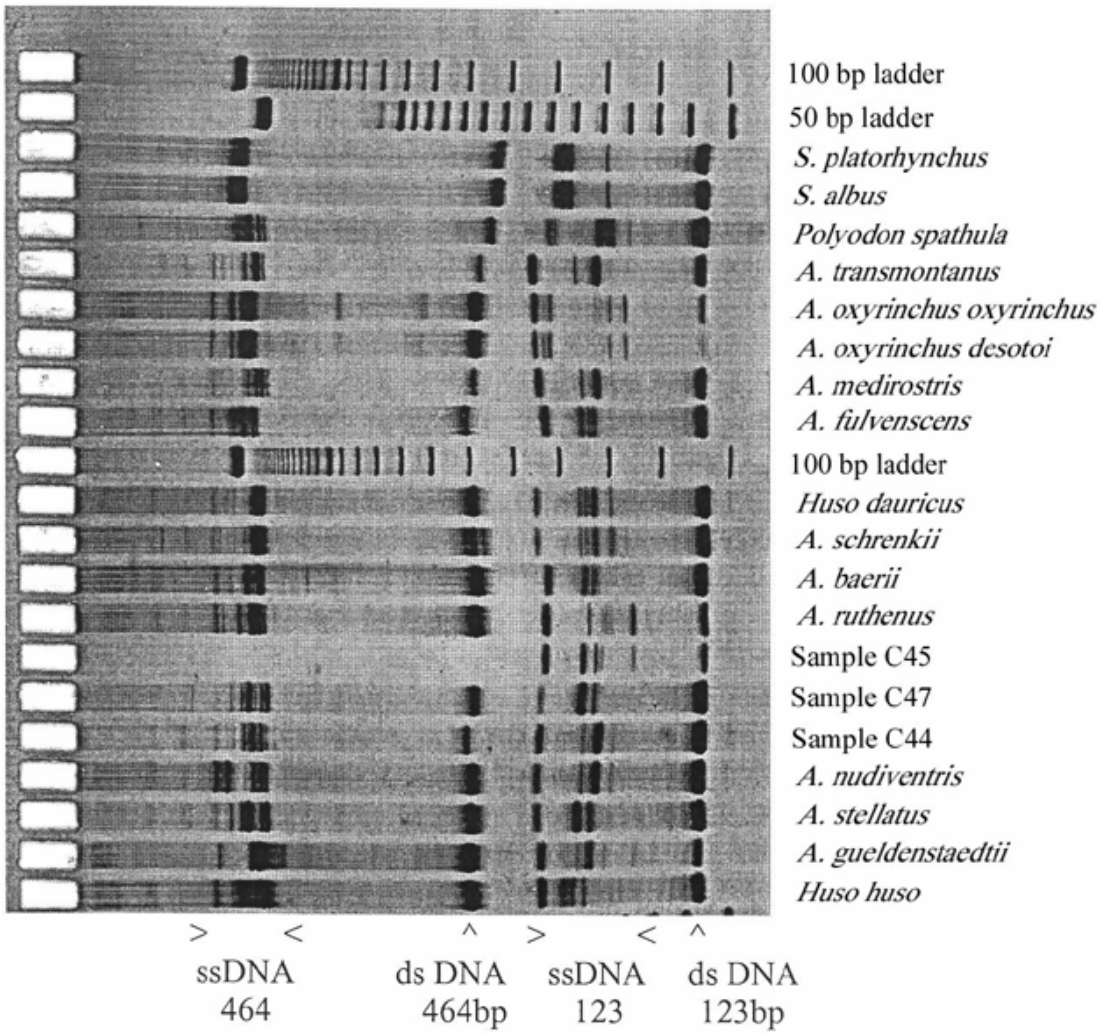
2.2.2 Genetic identification techniques based on mitochondrial DNA (mtDNA)

2.2.2.1 PCR-Single Strand Confirmation Polymorphism (SSCP)

SSCP is a method based on the electrophoresis of single-stranded (ss) DNA fragments of suitable size through a non-denaturating polyacrylamide gel, followed by visualization (Sunnucks *et al*., 2000). This method has been used for sturgeon species identification based on a region of the mitochondrial *cytochrome b* gene[1] (Figure 4, Rehbein, 1997). An advantage of this technique is that it easily distinguishes single nucleotide substitutions and shows intraspecific variability through variable band patterns representing different individuals. This technique allows identification of complex diagnostic profiles, and therefore has advantages for detecting intraspecific variability in large sample sets. However, this method is only valuable for short PCR-fragments (normally <250 bp) and thus a complex system of several PCR reactions is necessary to distinguish between all different sturgeon species. Despite this disadvantage, the method has been successfully used for maternal lineage detection of the Russian sturgeon complex, covering *A. baerii*, *A. gueldenstaedtii*, *A. naccarii*, and *A. persicus* (Ludwig *et al*., 2000). In future, SSCP could be a **valuable tool for the differentiation of (sub) populations**, tracing back population origin on the basis of one specimen or egg. SSCP requires reference data from wild populations which are sometimes available from public gene banks (see also Appendix Table I).

[1] Rehbein (1997) amplified a 358 bp fragment of the *cyt-b* gene. The entire *cyt-b* is 1141 bp long in sturgeons.

Figure 4: Discrimination of sturgeon species based on Single Strand Confirmation Polymorphism of *cytb*-fragments. This figure was presented by Rehbein (pers. comm., 2006).



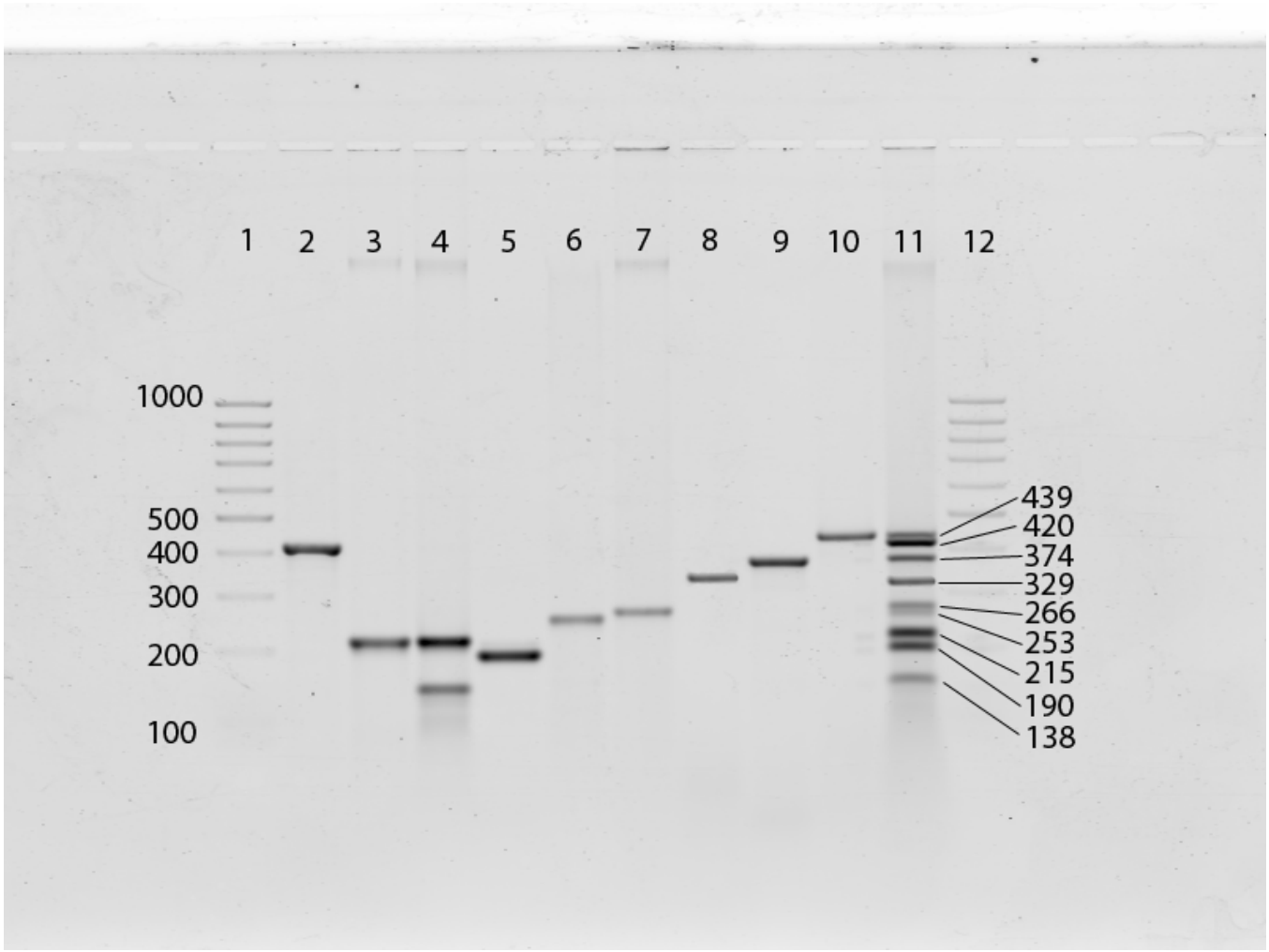
2.2.2.2 Species-specific PCR

This method is based on the presence of diagnostic nucleotide differences observed among species' mitochondrial DNA sequences and is used for the identification of *H. huso*, *A. stellatus* and *A. gueldenstaedtii* (DeSalle and Birstein, 1996; Birstein *et al*., 1998). For each of these species, at least one set of mitochondria, consisting of two specific primers, matches the diagnostic nucleotides. In the above mentioned studies, the occurrence of an amplification product, obtained with a species-specific primer pair, was assumed to allow a correct identification for these species. In the case that species-specific PCR is inconclusive, sequencing of the control fragment is required. Later work from the same group of scientists indicated that the **species-specific PCR did not allow accurate identification of *A. gueldenstaedtii* and its differentiation from closely related species** (***A. baerii***, ***A. naccarii*** **and** ***A. persicus***) due to overlapping mitochondrial DNA profiles (Birstein 1999; Birstein *et al*., 2000; Ludwig *et al*., 2002; and see 2.2.3). Although this method has advantages in that it is usually **easy, inexpensive and fast** for identification of *H. huso* and *A. stellatus*, two very important caviar producing species, it has failed in the separation of *A. gueldenstaedtii* and *A. persicus.*

Although the technique is based on unique interspecific nucleotide differences, the following aspect may lead to false positive results. The last nucleotide of each primer molecule is solely responsible for the species identification. Theoretically, this principle is vulnerable to i) primer solutions containing n-1 molecules, ii) an effective amplification of primer-template mismatches by *Taq*-DNA polymerases, even if they occur at the ultimate nucleotide position (Kwok *et al*., 1990), and iii) partial primer degradation resulting from successive thawing/freezing of primer solutions thereby again leading to n-x molecules. Reliable exclusion of false positive results requires the inclusion of reference specimens in each analysis.

Mugue *et al*. (2006) has conducted the most recent efforts to improve this method for species identification (Figure 5), which show potential, but so far their results have not yet been published.

Figure 5: PCR using species-specific primers presented by N. Mugue during the 2nd Status Workshop in Berlin at the IZW from 29th Sep. to 1st Oct. 2006 (Mugue *et al*., 2006). Lane 1/12 are length standards, lane 2/3 are *A. gueldenstaedtii*, lane 4 is *A. baerii*, lane 5 is *A. ruthenus*, lane 6 is *A. schrenckii*, lane 7 is *A. stellatus*, lane 8 is *A. nudiventris*, lane 9 is *H. huso* and lane 10 is *H. dauricus*.



2.2.2.3 PCR-Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) is a technique involving analysis of patterns derived from cleavage of DNA. This technique requires restriction enzymes, which normally detect and cut specific DNA sequences of 2- 6 bases. If two organisms differ in the distance between cleavage sites of a particular restriction endonuclease, the length of the digestion fragments produced will differ. **The patterns generated can be used to differentiate species (and even populations and sub-populations) from one another**. PCR-RFLP was first introduced for sturgeon species identification by Wolf *et al*. (1999) and has many advantages. Unlike with other techniques, a single universal primer pair is used without the need of reference specimens. After establishing the species-specific restriction patterns, this approach has been extended to 22 acipenseriform species (Ludwig *et al*., 2002). In addition, data set restriction sites of *A. dabryanus*, *Psephurus gladius*, *Pseudoscaphirhynchus hermanni*, and *P. kaufmanni* were analysed based on archived sequences (Table 4). Due to its extreme rarity, no data could be obtained for *Pseudoscaphirhynchus fetschenkoi*. Ludwig *et al.* (2002) investigated the restriction pattern of five enzymes for all *cytochrome b* sequences of 861 specimens of 22 Acipenseriformes. Species-specific nucleotide differences proved suitable for the differentiation of 17 species using the PCR-RFLP approach (see also Figures 6 and 7). Due to their unresolved phylogenetic relationship as well as their close genetic similarity, no diagnostic substitutions could be found for the species pair *A. gueldenstaedtii*/*A. persicus* and the three species of the genus *Scaphirhynchus*.

Figure 6: Restriction pattern of several sturgeon species using *Mse*I based on Ludwig *et al.* (2002). Lanes 1/12/22 are size standards, lane 2 is *A. fulvescens*, lane 3 is *A. transmontanus*, lane 4 is *A. nudiventris*, lane 5 is *A. naccarii*, lane 6 is *A. persicus*, lane 7 is *A. baerii*, lane 8 is *A. gueldenstaedtii*, lane 9 is *A. sinensis*, lane 10 is *A. schrenckii*, lane 11 is ***H. huso***, lane 13 is *H. dauricus*, lane 14 is *A. mikadoi*, lane 15 is *A. medirostris*, lane 16 is ***A. ruthenus***, lane 17 is ***A. stellatus***, lane 18 is ***A. oxyrinchus***, lane 19 is ***A. sturio***, lane 20 is ***S. albus*** and lane 21 is ***P. spathula*** – bold species are showing species-specific band patterns.

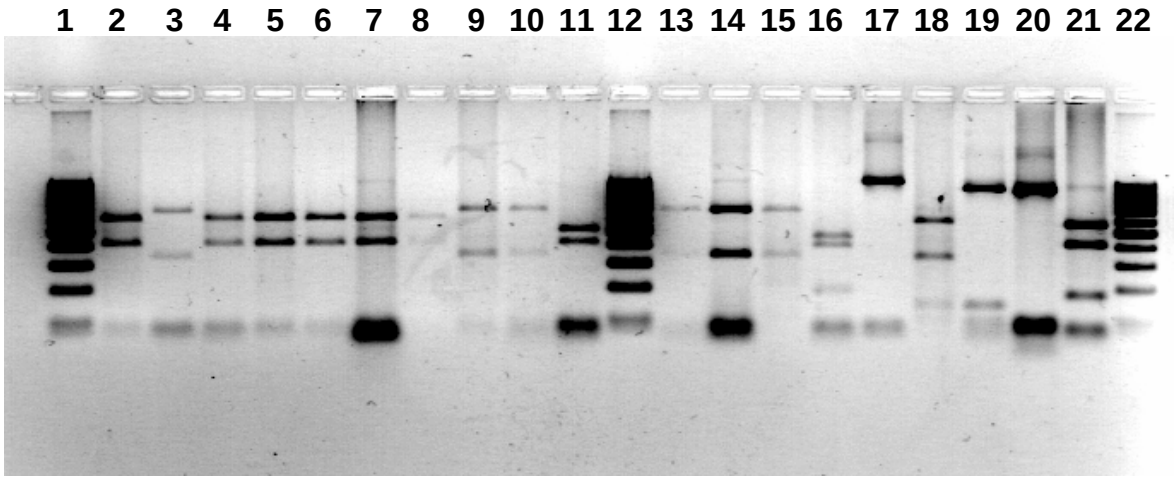


Table 4: Diagnostic restriction sites of amplification products* containing the entire *cytochrome b* gene and additional 80/81 bp of related tRNA genes grouped for each restriction enzyme among all acipenseriformes (modified after Ludwig *et al*., 2002).

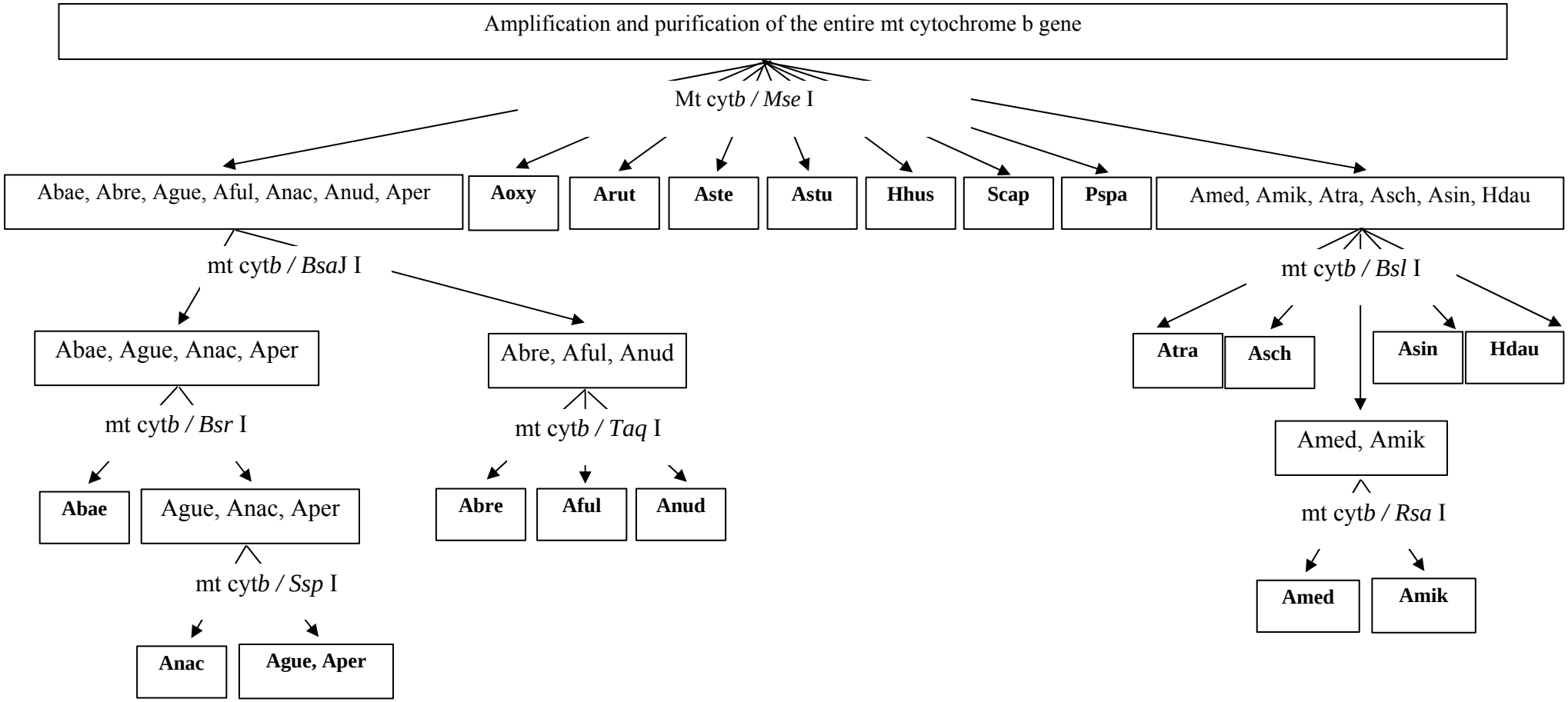
Genus and Species	**Restriction enzyme**						
	***Mse* I**	***Bsl* I**	***Rsa* I**	***BsaJ* I**	***Bsr* I**	***Taq* I**	***Ssp* I**
Polydontidae:							
Polyodon spathula	9, 66, 177, 413, 556						
*Psephurus gladius***	66, 85, 89, 981						
Acipenseridae:							
A. baerii	9, 58, 66, 84, 414, 590			142, 207, 233, 639	81, 111, 112, 951		
A. brevirostrum	9, 58, 66, 84, 414, 590			142, 440, 639		no RS	
*A. dabryanus***	9, 58, 66, 84, 345, 659						
A. fulvescens	9, 58, 66, 84, 414, 590			142, 440, 639		213, 1008	
A. gueldenstaedtii	9, 58, 66, 84, 414, 590			142, 207, 233, 639	81, 112, 1062		275, 284, 662
A. persicus	9, 58, 66, 84, 414, 590			142, 207, 233, 639	81, 112, 1062		275, 284, 662
A. medirostris	9, 58, 67, 84, 345, 659	69, 81, 130, 175, 327, 440	39, 55, 90, 112, 185, 319, 422				
A. mikadoi	9, 58, 67, 84, 345, 659	69, 81, 130, 175, 327, 440	39, 90, 112, 185, 319, 477				
A. naccarii	9, 58, 66, 84, 414, 590			142, 207, 233, 639	81, 112, 1062		99, 275, 284, 563
A. nudiventris	9, 58, 67, 84, 414, 590			143, 440, 639		158, 1064	
A. oxyrinchus	66, 104, 222, 339, 570						
A. persicus	9, 58, 66, 84, 414, 590			142, 207, 233, 639	289, 932		275, 284, 662
A. ruthenus	9, 42, 58, 66, 191, 399, 456						
A. schrenckii	9, 58, 66, 84, 345, 659	68, 88, 130, 441, 494					
A. sinensis	9, 58, 66, 84, 345, 659	88, 109, 430, 494					
A. stellatus	9, 58, 66, 84, 1004						
A. sturio	95, 222, 909						
A. transmontanus	9, 58, 66, 84, 345, 659	88, 130, 494, 509					
Genus *Huso*							

H. huso	9, 58, 66, 84, 95, 414, 495						
H. dauricus	9, 58, 66, 84, 345, 659	88, 130, 175, 319, 509					
Genus *Scaphirhynchus*							
S. albus	9, 58, 66, 84, 95, 909						
S. plathorhynchus	9, 58, 66, 84, 95, 909						
*S. suttkusi***	9, 58, 66, 84, 95, 909						
Genus *Pseudoscaphirhynchus*							
*P. kaufmanni***	9, 58, 66, 84, 414, 590						
*P. hermanni***	no sequence						
*P. fetschenkoi***	no sequence						

* Amplification products are 1222 bp in *A. medirostris*, *A. mikadoi* and *A. nudiventris* or 1221 bp (all other species) including entire *cytochrome b* 1141 bp and additional 80/81 bp of related tRNA genes

** Sequences of blue species were taken from GenBank. Only restriction sites situated within the *cytochrome b* gene were taken into consideration. For this reason, total fragment lengths may differ slightly depending on primer binding sites.

Figure 7: Protocol of species-identification using PCR-RFLP analyses with (in bold) the names of identified species in relation to identification point (Key to species names: *Acipenser baerii* - Abae, *A. brevirostrum* - Abre, *A. fulvescens* - Aful, *A. gueldenstaedtii* - Ague, *A. medirostris* - Amed, *A. mikadoi* - Amik, *A. naccarii* - Anac, *A. nudiventris* - Anud, *A. oxyrinchus* - Aoxy, *A. persicus* - Aper, *A. ruthenus* - Arut, *A. schrenckii* - Asch, *A. sinensis* - Asin, *A. stellatus* - Aste, *A. sturio* - Astu, *A. transmontanus* - Atra, *Huso huso* - Hhus, *H. dauricus* - Hdau, genus *Scaphirhynchus* - Scap and *Polyodon spathula* – Pspa – Figure was taken from Ludwig *et al*., 2002).



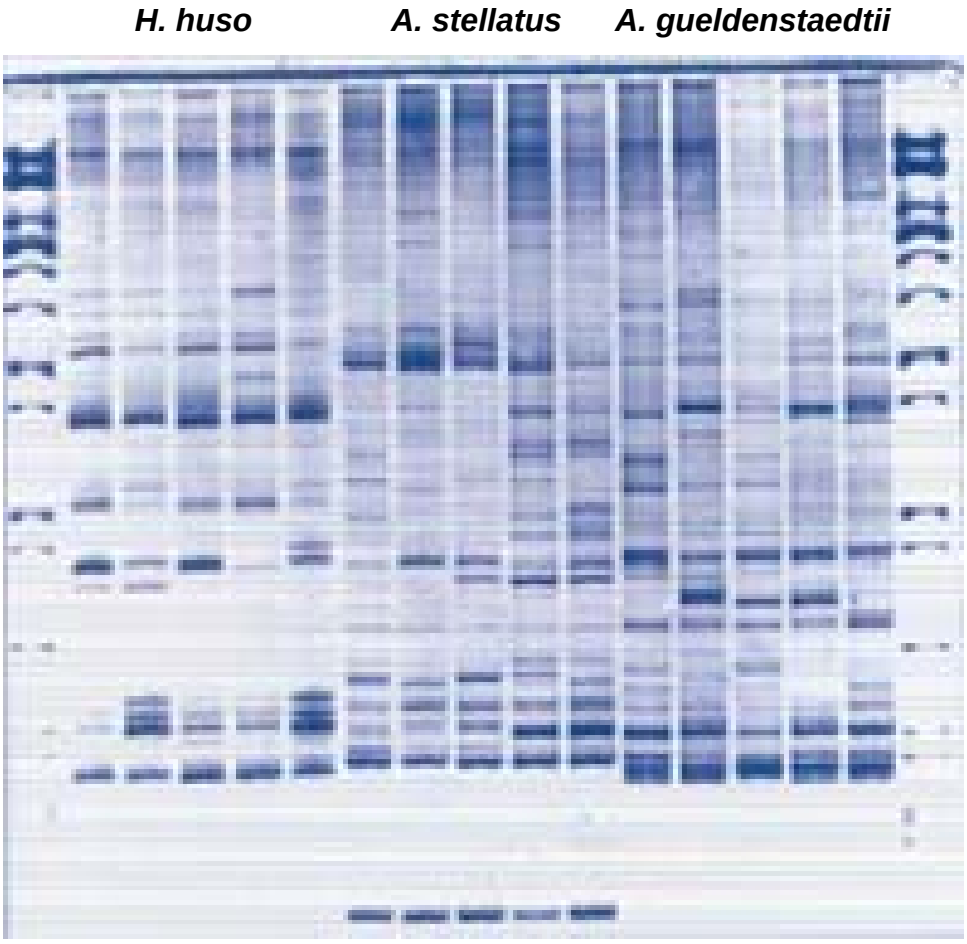
2.2.3 Nuclear DNA techniques

Mitochondrial DNA markers/polymorphisms are commonly used in species identification, because they are not influenced by recombination, which makes mathematic modelling easier, and evolve several times faster than nuclear DNA, resulting in a faster fixation of diagnostic substitutions. Importantly, being maternally inherited in all vertebrates, they only record the genetic variability of the maternal lineages. This could lead to misinterpretations, for instance in the identification of hybrids and/or ancient introgression events. Several hybridizations and introgression events have been described in sturgeons (Holcik, 1989; Arefyev, 1997; Ludwig *et al*., 2003; Tranah *et al*., 2004). For example, genetic evidence for hybridization events of *S. albus* and *S. plathorhynchus* were recently reported (Tranah *et al.*, 2004) and historical introgression events of *A. gueldenstaedtii* were observed in the Po River population of *A. naccarii* (Ludwig *et al*., 2003).

2.2.3.1 Random Amplified Polymorphic DNA (RAPD)

In RAPD analysis, the target sequences to be amplified are unknown. Random primers (only one short primer is used in each PCR reaction) are used for a non-specific, multilocus PCR reaction using nuclear DNA as template. Multiple-band RAPD profiles, usually run on agarose gels or sometimes on polyacrylamide gels, are scored as presence/absence for each generated fragment in the different individuals. The many amplification products are simultaneously synthesised from different genomic regions, with different degrees of variability: some of the fragments (= bands) are very well conserved amongst different individuals while some others show lower frequencies. Accordingly, species-specific, population-specific, and individual-specific bands can be identified.

Figure 8: RAPD band pattern for *H. huso*, *A. stellatus* and *A. gueldenstaedtii* [modified after Barmintsev *et al*., (2001)]



RAPD was first introduced by Comincini *et al*. (1998) for the discrimination of six sturgeon species. Some of the bands obtained by using eight different primers were found to be species-specific. In addition, a molecular marker was discovered in the process to differentiate caviar of *H. huso*, *A. nudiventris* and *A. stellatus* from caviar of *A. persicus* and *A. gueldenstaedtii* (Rezvani Gilkolaei, 1997). Gharaei *et al*. (2005) studied the genetic make up of *A. persicus* and *A. gueldenstaedtii* using RAPD techniques and observed only a few differences, but only a limited number of stocks/populations were included in this analysis. The RAPD technique was also used for population genetic studies of *H. huso* from two regions, the western and eastern shores of the South Caspian Sea. The population study showed a significant difference

at RAPD loci between the two regions. The RAPD approach was further developed by Barmintsev *et al.* (2001, Figure 8) who concluded "that RAPD-technique is a helpful method to identify not only species of sturgeon fish, but populations, hybrids and such derivatives as caviar." **The main limitation of the RAPD-method is the low reproducibility due to its high sensitivity to experimental conditions.** In fact, all samples to be compared, including reference specimens, have to be run in the same experiment.

2.2.3.2 Amplified Fragment Length Polymorphism (AFLP)

AFLP is a combination of RAPD and RFLP. The AFLP procedure typically detects more polymorphisms per reaction than RFLP or RAPD analysis, and is especially useful in screening backcross (crosses between a hybrid and one of its parental species) individuals or hybrids. Congiu *et al*. (2001) discussed the identification of sturgeon hybrids by AFLP. The increase of commercial hybrids in aquaculture (for example: *A. gueldenstaedtii* x *A. baerii*; *H. huso* x *A. ruthenus*; *A. transmontanus* x *A. naccarii*) and the rising number of escaped fish, including sturgeon hybrids, during the last decades renders this method particularly valuable (Kirschbaum *et al*., 2004). Congiu *et al.* (2001) used cluster analyses and assignment tests based on observed and simulated AFLP profiles to separate hybrids from their parental species. The advantage of AFLP techniques in discriminating species with a low level of genetic divergence is the possibility of estimating a probability of assignment based on multilocus information without the need of species-specific monomorphic bands (alleles). This method is very valuable for the identification of hybrids, but the inclusion of reference specimens of the parental species is strongly recommended.

2.2.3.3 Microsatellites

Microsatellites are tandemly repeated motifs of 1-6 nucleotides found in all eukaryotic and many prokaryotic genomes. These usually non-coding motifs are inherited in a Mendelian fashion. They are also called simple sequence repeats (SSR), short tandem repeats (STR), or variable number tandem repeats (VNTR). Alleles (fragment lengths)

at a specific locus can differ in the number of repeats. These length variations are used for allele definition. Because microsatellites are widely dispersed in eukaryotic genomes and highly variable, and their detection is PCR-based (requiring minute amounts of template), they have been used in many different areas of research. Because microsatellite profiles (fragment lengths of many loci) may vary among specimens, they are used in several ways: i) to determine paternity – PCR-fingerprinting, ii) for population studies – microsatellite variation is used for population structure analyses; and iii) for conservation biology – detection of population management units, introgression events and hybridizations.

Jenneckens *et al*. (2001) demonstrated that the microsatellite locus *Afu-39* (May *et al*., 1997) is a useful nuclear marker to identify caviar and other products of *A. stellatus*, because it contains a unique allele which is missing in all other sturgeon species. Besides the conventional application of microsatellites to investigate the diversity within and between populations or even species, *Afu-39* was the first genomic marker described for sturgeon with the potential to identify one of the main caviar-producing species and its hybrids. However, this method is not applicable for other species which have null alleles (*A. baerii*, *A. gueldenstaedtii*). A null allele is an allele which is not amplified/detectable by a specific set of primers. Null alleles often exist if primers developed for one species are used for another species.

2.2.3.4 Future developments – DNA barcoding

Over the past years, DNA barcoding using chip technology has become more and more prevalent. Barcoding is a technique used to characterize species using short, standardized DNA sequences (for a detailed description see http://barcoding.si.edu/DNABarCoding.htm). Currently *cytochrome c oxidase subunit 1* mitochondrial region (*COI*) is used for barcoding with the plan to create a system that can readily identify higher animals (identification of plants will require an additional gene). The process of barcoding requires generation of a reference collection, DNA sequences, a sequence database, and an analysis framework. This is

being applied on a large scale through The Consortium for the Barcode of Life (CBOL) (http://www.barcodinglife.org). Once a database is generated the short species specific oligonucleotides (synthetically synthesised sequences) can be used with a chip-based method. For this approach, diagnostic oligonucleotides are stored on the chip (like a microarray) which can be used for DNA-DNA hybridization analysis which produces a signal if similar (i.e. assumed homologous) sequences are stored on the chip. Chips can now have multiple levels of redundancy to yield positive identification to family, genus, species-complex, species, and population level. A positive identification involves four to five levels of matching and the system alerts the researcher to the possibility of a new species if higher level probes give positive results but species probes do not. Species mixtures could also be detected, for instance different DNA samples from a single tin of caviar could be extracted and the chips then used to detect species mixtures, as opposed to traditional methods analysing individual eggs.

The advantages of this technology are that i) a nearly unlimited (several thousands) number of diagnostic sequences, including both nuclear and mitochondrial DNA, can be stored on one chip; and ii) only one reaction is necessary to identify the species/population of origin. Unfortunately, this method also has some important disadvantages, especially as only known sequences can be stored on the chip, and the number of diagnostic nuclear sturgeon sequences is very low at the moment. By contrast, other data matrixes such as sequences, microsatellites or RFLP can be used for both species/population identification and phylogenetic reconstructions. In the case where no identical sequence is known, these phylogenetic reconstructions can be used for the identification of the phylogenetically most closely related species/population. Statistical tests (bootstrap analysis) are needed to either support or reject this grouping. Another disadvantage is that the development of a genetic chip is still very expensive. **Barcoding and chip technology may be important in the future but it should be taken into consideration that there is no approach available using these techniques for sturgeon identification at this time.**

BOX 1: Further complications in sturgeon identification: the *Acipenser gueldenstaedtii*-problem

There are four main caviar producing species: *H. huso*, *A. stellatus*, *A. persicus* and *A. gueldenstaedtii*. *H. huso* and *A. stellatus* can be easily identified using molecular techniques. The situation is much more difficult for the other most important caviar producers: *A. persicus* and *A. gueldenstaedtii*. Caviar from *A. gueldenstaedtii* has been the topic of several controversial discussions during recent years because most of the caviar mislabelled with respect to species identification is actually the product of *A. gueldenstaedtii* (Birstein *et al*., 1998; Jenneckens, *et al*., 2001).

Three major mitochondrial genotypes within what is presently known as *A. gueldenstaedtii* have been detected (Birstein *et al.,* 1998; 2005; Jenneckens *et al.,* 2001). These major groups of genotypes include the pure *A. gueldenstaedtii* and *A. baerii* plus a third rare genotype whose mtDNA is similar to the mtDNA of *A. naccarii*. Additionally, *A. persicus* genotypes are very similar to the *A. gueldenstaedtii* too. No diagnostic substitution has been observed between *A. persicus* and *A. gueldenstaedtii* until now. Taken together, all four species of this complex display a high genetic variability (Pourkazemi *et al.,* 1999; Birstein *et al.*, 2005; Doukakis *et al*., 2005), which suggests further sub-division. Uncontrolled release programs and artificial river connections (canals) have caused further mixture of genotypes of these closely related species. For these reasons, it is impossible to identify *A. persicus* or *A. gueldenstaedtii* on mitochondrial DNA exclusively. Further research on the phylogeny of this group is strongly recommended to discover and evaluate diagnostic substitutions.

3. Population (stock) identification

3.1 Genetic methods

Nuclear DNA-based methods are mainly used to define population because nuclear DNA represents both maternal and paternal origins of an individual. Indirect methods such as RAPD, AFLP and microsatellites (see 2.2.3.1, 2.2.3.2 and 2.2.3.3) are mainly used for the screening of nuclear DNA variability and were discussed in section 2.2.3. The use of the RAPD technique for population genetic inference has been questioned because of concerns about reproducibility, dominance, and homology. Given these concerns and the ready availability of alternative markers, **studies based primarily on RAPD are not considered a valuable tool for the discrimination of (sub-) populations**. **Alternatively, AFLP and microsatellite analyses are characterized by a high level of reproducibility. These higher levels of reproducibility result from the inclusion of specific primers (microsatellites) or restriction enzymes (specific cutting sites – RFLP)**.

3.1.1 Microsatellites

This technique, described under section 2.2.3.3, can be used for population determination studies in combination with assignment tests. The procedure of an assignment test requires microsatellite data from the source populations. Although details on many microsatellite loci have been published during the last years, only a few populations have been screened so far for these markers (Appendix Table I). Box 2 describes two studies which **demonstrated the value of this technique and of population genetic structure for forensic and conservation approaches. Despite these examples, studies focusing on population genetics have been lacking for many sturgeon species until now (**see Appendix Table I).

BOX 2: Two studies detecting hybridization and introgression events

Tranah *et al*. screened specimens captured from three sampling sites of *Scaphirhynchus albus* and *S. platorynchus* and detected evidence for large-scale hybridization events. The authors combined mitochondrial and nuclear data.

(Tranah, G., Campton D.E. and May, B. (2004): Genetic evidence for hybridization of pallid and shovelnose sturgeon. *Journal of Heredity* 95: 474-480.)

Ludwig *et al.* studied the population structure of the last remaining populations (Po River (Italy), Buna River, (Albania/Montenegro)) of *Acipenser naccarii* based on mitochondrial (d-loop), microsatellite and nuclear DNA (AFLP). A previously reported diagnostic substitution in the mitochondrial D-loop separating two *A. naccarii* specimens (Po River) from *A. gueldenstaedtii* (Birstein *et al*., 2000) could not be verified in later tests (Ludwig *et al*., 2003). Nevertheless, Albanian (Buna River) specimens of *A. naccarii* can easily be identified, as their mitochondrial D-loop is characterized by a minimum of 13 diagnostic substitutions. They also carry one diagnostic substitution in the mitochondrial cytochrome b gene and several diagnostic microsatellite alleles as well as private AFLP bands (Ludwig *et al*., 2002; Ludwig *et al*., 2003). Allelic distribution of the different loci (microsatellites) was used for an assignment test. All individuals were correctly assigned to their populations (Po, Buna or Volga).

(Ludwig, A., Congiu L., Pitra C., Fickel J. *et al.* (2003) Nonconcordant evolutionary history of maternal and paternal lineages in Adriatic sturgeon. *Molecular Ecology* 12: 3253-3264.)

Although nuclear DNA techniques predominate for population identification, mtDNA polymorphisms may also be helpful. Sequence analysis and/or SSCP are valuable tools for the detection of mtDNA variability, because mitochondrial DNA accumulates more substitutions than nuclear DNA over time. Under the assumption that a population was not influenced by introgression events or release programmes, the polymorphisms of mitochondrial genes are suitable for population identification. Unfortunately, many sturgeon and paddlefish populations were, or still are, the subject of unscientifically managed, haphazard release programs.

3.2 Biochemical methods

3.2.1 Allozyme electrophoresis

Several proteins have been investigated electrophoretically using polymorphisms for determination of stocks or populations. For example, Chikhachev (1983) found differences between *A. stellatus* populations from the Sea of Azov and from the North Caspian Sea on the basis of transferrin and albumin phenotype frequencies. Albumin fractions were tested as genetic markers for population studies in *A. gueldenstaedtii* and *H. huso* (Ivanenkov and Kamshilin 1991). However, several other studies suggest that albumin is not a reliable marker for population discrimination, since the variability of albumin depends largely on the functional status of the organism (Luk'yanenko *et a*l., 1975; Chikhachev and Tsvetnenko, 1979; Kamshilin, 1983).

The population structure of *Acipenser baerii* from the river Ob as well as *Acipenser ruthenus* from the rivers Don and Kama were investigated using the muscle malate dehydrogenase isoenzymes (MDH) (Kuz'min, 1991). Three different phenotypes of MDH were detected. A great similarity in MDH allelic mobility was found between *A. baerii* and *A. ruthenus*. Luk'yanenko *et al.* (1975) used double immunodiffusion and immunoelectrophoresis methods for a comparative study of the antigenic composition of serum proteins of *A. stellatus* from the rivers Volga and Ural. Additionally, Nikonorov *et al*. (1986) and Ryabova and Kutergina (1990) also performed biochemical investigations on *A. stellatus* from the North Caspian Sea. Both studies identified a high level of variability. This was in agreement with research results for *A. stellatus* from the South Caspian Sea (Pourkazemi, 1996).

Different **allele frequencies** were also observed between males and females and also between the summer and winter races of *A. gueldenstaedtii* (Chikhachev, 1983). Although **allozyme** electrophoresis is a reliable method for population genetics, there are several limitations in using this technique. The main disadvantage of protein electrophoresis is that a very high quality, undegenerated tissue is required and therefore, **it can not be used for the identification of caviar or sturgeon**

products in trade. Samples have to be taken from live specimens or specimens stored in dry ice or liquid nitrogen. But most caviar in international trade is preserved in salt or boric acid, which may prevent enzyme activities. Another limitation is that only non-silent substitutions (mutations which result in an amino acid exchange) can be detected. However, most substitutions are silent mutations and/or situated in non-coding regions. Therefore, genetic variability will be underestimated by allozyme electrophoresis, which can result in false positive results. In conclusion, this method is not suitable for caviar or any other processed sturgeon product. It can only be used for fresh (eggs or meat samples taken immediately after the catch of the fish) tissue. **Since microsatellites and AFLP analyses are available, most researchers focus on those methods for which any kind of tissue (including processed tissue) can be used. In addition, they are easier to use and less expensive than allozyme electrophoresis and the results are comparable between different laboratories.**

4. Source identification (wild vs. aquaculture)

Most hatchery strains were created only a few decades ago as commercial aquaculture in Western Europe and North America started in the 1970s. As yet, there are no genetic differences between wild populations and aquaculture strains. In addition, ongoing poorly managed restocking with captive fish prevents the manifestation of genetic differences. For these reasons, genetic methods are inappropriate for discriminating hatchery fish from wild specimens. Because rearing (i.e. environmental) conditions are different, methods that focus on differences in food or water quality seem to be most appropriate. Unfortunately, to date only very few studies have focused on such methods. They are discussed below.

4.1 Gas-liquid chromatography

Gas-liquid chromatography was used for the analysis of the fatty acid composition of caviar from wild and domestic (i.e. farmed) fish (Wirth *et al*., 2002). The fatty acid composition in neutral lipids of muscle and adipose tissue is mainly influenced by the fatty acid pattern of the food. During the gonadogenesis, the fatty acids of muscles and adipose tissue are incorporated into eggs. Additionally, the contents of long-chain poly-unsaturated fatty acids are also influenced by water temperature (Steffens *et al*., 1995; Wirth and Steffens, 1998). Wirth *et al*. (2002) analysed the fatty acid compositions of both lipid classes (triglycerides and phospholipids) in caviar from 34 wild specimens (*H. huso*, *A. gueldenstaedtii*) and 12 farmed specimens (*A. baerii*, *A. stellatus*, *P. spathula*). The authors observed differences between wild and farmed caviar, and between the different sample sites of the wild caviar. A significantly higher content of linoleic acid and a lower amount of arachidonic acid was found in farmed caviar. These differences are likely based on the use of commercial rainbow trout food, a source which contains a high content of Omega-3-fatty acids. These Omega-3-fatty acids are not present in natural sturgeon food, **which renders this approach valuable for discrimination between domestic and wild caviar**. Nevertheless, its value for species/population discrimination may be limited because the technique is time-consuming and expensive, while limited data from only a few

species/populations/hatchery strains are currently available. Changes in the feeding strategy (use of other food without Omega-3-fatty acids) are likely to result in the loss of the diagnostic differences observed between wild and domestic fish.

5. Age determination of caviar

Aging of caviar is not possible with molecular or genetic methods; microbiological approaches are more appropriate. Chemical changes in caviar during refrigerated storage depend on the composition of caviar, the temperature and storage time, and the microbiological state. Lipid and salt content, addition of borate, as well as pasteurization, may influence the speed of deterioration of caviar.

Several (bio) chemical reactions can be used to follow the loss of quality or degree of spoilage of caviar:

- Determination of the content of free fatty acids

  The lipid content of sturgeon caviar ranges from 10 to 20% wet weight. Free fatty acids are formed by lipolysis of triglycerides and can be determined titrimetrically.

- Measurement of lipid oxidation

  Caviar contains considerable amounts of polyunsaturated fatty acids, which are susceptible to oxidation. For measurement of lipid oxidation the thiobarbituric acid value is suitable.

- Determination of the content of "free" tyrosine

  During storage of caviar, proteins are degraded into peptides and free amino acids. The formation of tyrosine can be determined by a simple colorimetric assay.

Details of these methods are described in Lehmann *et al*. (2003) and Gussoni *et al*. (2006). These three methods could be used for determination of the storage time of caviar under the precondition that the temperature during storage is known. If this is not the case, the degree of lipid and protein degradation can deliver some information on the quality of the caviar, but it cannot be decided whether the chemical changes result from high temperatures or long storage time. In reality, reliable information on

storage temperature is unlikely to be available, making these aging techniques unreliable.

6. Overview of identification techniques

Table 5 lists the main features and suitability of the alternative identification methods described within chapters 2, 3 and 4, as well as their relative ease of handling, cost and availability of reference samples. Based on this overview, sequencing, species-specific PCR and PCR-RFLP analyses based on mitochondrial DNA are found to be the most valuable for species identification. AFLP and microsatellite analyses are the most suitable techniques for population identification. Gas-chromatography has the greatest diagnostic power for source identification. However, sequencing and gas-chromatography analyses are expensive and time consuming, which make both methods unsuitable for the screening of large sample sets.

Although identification techniques are available, their application for sturgeon identification and trade control is still in its initial, sometimes experimental, stage and three to five more years of research seem necessary to fully develop methods for population and source identification. As noted in the previous chapters, for most of the techniques discussed a comprehensive reference collection is a prerequisite for their development as an identification system for sturgeon products and parts. This reference collection should provide an open source of data including information about the location of reference samples. For example, it is not possible to use microsatellite data from different laboratories without verification between them because differences in allelic length detection are depending on laboratory specific conditions and/or equipment.

All methods differ widely in their running costs. The costs of an analysis per sample can only be estimated given the high differences between countries and laboratories in the cost of labour, chemicals, and technical equipment, etc.

Table 5: Overview of the different methods and their relative suitability for species, population and source identification (+++++ = most valuable, + = less valuable), the necessity to include reference samples (yes or no) or the availability of data in public data sources (e.g. GenBank), and their estimated costs (low ≤ 10 €; medium ≤ 20 €; high > 20 € per sample).

Method	Species ID	Population ID	Source ID	Handling	Reference samples	Costs (only chemicals)	Time required
SSCP	+++	++++	+	+++	Yes	low	1 day
Nested-PCR	++++	+	+	+++++	No	low	4 hours
Sequencing	+++++	++++	+	+++++	Yes (GenBank)	high	1 day
PCR-RFLP	+++++	+	+	+++++	Yes (GenBank)	medium	5 hours
AFLP	+++	+++++	+	+++	yes	medium	1 day
RAPD	+	+	+	+	yes	low	4 hours
Microsatellites	++	+++++	+	+++++	Yes (publications)	medium	5 hours
Gas-chromatography	?	++++	++++	+	no	high	1 day

7. Development of a reference collection

In order to develop many of the techniques discussed in the previous sections into a standard procedure for sturgeon identification, it would be necessary to have access to two sources of information; 1) data of previously published and unpublished genetic analyses, and 2) information on availability of stored reference samples (including laboratory and contact information).

Although GenBank already provides a virtual databank containing some of the necessary published sequences, in most cases, there is no information archived about populations, for example, allelic frequencies or distribution of haplotypes. GenBank archives only the first descriptions of a haplotype or sequence, but no information about how many individuals/river systems are sharing these haplotypes. A specific sturgeon and paddlefish reference collection should provide all this information (see Table 6). The barcoding initiative can provide guidance on establishing such a reference collection of tissues and sequences. The barcoding system reference library links DNA barcodes to reference specimens and details of their particular collections. Currently, there are two main barcode databases: The International Nucleotide Sequence Database Collaborative (http://www.insdc.org/ run by GenBank, EMBL and the DNA Data Bank of Japan) and the Barcode of Life Database (BOLD – http://www.boldsystems.org/views/login.php created and maintained by the University of Guelph in Ontario). Management of these databases could provide guidance for a specific reference collection for Acipenseriformes.

Table 6: Suggested information to include in reference database.

Species	Sample local/wild or hatchery/collecting date	River basin (for hatchery fish the place of origin)	mtDNA-haplotype (GenBank Accession number)	Nuclear data (type/locus/allele)
Huso huso	Astrakhan/wild/05/26/2006	Volga	d-loop H4 (XY123456)	microsatellites Afu-19 (132/146); Afu-39 (122/125)

Secondly information on availability of stored reference samples is necessary as identification analyses require the inclusion of reference material (tissue). Therefore, reference specimens (DNA or tissue) for genetic analysis must be available. The potential for creating an international single reference sample collection was discussed during the workshop on Identification of Acipenseriformes Species in Trade held at the Institute for Zoo and Wildlife Research in Berlin (Germany) from 29th September to 1st October 2006. However, it was concluded that genetic scientists would not be supportive of this as reference samples are considered to be their capital, preferring that reference samples should instead be stored at different registered laboratories. Reference samples could then be requested when necessary for analysis. Therefore, information on locations of reference material, contact persons and storing conditions should also be made available as part of the reference collection.

Reference samples from all sturgeon populations worldwide would need to be available with a representative number of specimens from each population (e.g. for natural populations a minimum of 30 non-related, randomly caught individuals would be needed and should be sampled for as much of the distribution range as possible). Samples must be collected by qualified scientists with knowledge of sturgeon systematics; wrongly assigned reference specimens would result in mis-identification of sturgeon products. Species assignment of reference samples must be verified by both morphological and genetic methods. Finally, official identification of trade samples should be restricted to a limited number of evaluated and registered laboratories worldwide, which would facilitate the exchange of samples between different countries. The exchange of tissue samples from CITES-listed species is subject to CITES simplified procedure for issuing permits and certificates for biological samples. The creation of this reference specimen database would take up to three years, because data have to be collected, reference specimens verified, and a data base structure developed.

As an existing network of scientists working on sturgeon, a reference collection could be developed and managed by the IUCN/SSC Sturgeon Specialist Group. The information could be made available on request for members of the Sturgeon Specialist Group and law enforcement personnel including scientists from the registered reference laboratories. Genetic data is in the ownership of the scientists carrying out the analyses; use of these data for public presentations and publications requires the permission of the scientist therefore the reference collection would need to have limited access. Password protected access to the reference collection could be facilitated through the Sturgeon Specialist Group web-pages. Data should be accessible for trade control by the members of the IUCN/SSC Sturgeon Specialist Group. The development of such a reference collection is a priority for the IUCN/ SSC Sturgeon Specialist Group over the next three years and the participants of the 2nd Status Workshop recommend the next steps:

1) Creation of an internet databank under the supervision of IUCN within the next 12 months
2) Collecting the data about reference specimens and previously published genetic analyses
3) Storage of these data, password protection

It is anticipated that a reference collection could become fully operational within the next three years.

8. Concluding remarks and next steps

Considering the present state of knowledge, there is no single tool that meets all the requirements of an identification system (species/caviar identification, population identification and source identification). There are no fully developed techniques for population or source identification available at this time. However, genetic methods are valuable for both species and population identification. Sequencing, nested-PCR and PCR-RFLP based on mitochondrial DNA sequences seem to be the most suitable techniques for species identification. Microsatellites or AFLP have shown to be effective for population identification.

Ongoing poorly managed, random restocking programmes result in an ongoing mixture of genotypes preventing any manifestation of differences between wild and hatchery specimens; fish still share the same genetic polymorphisms as their wild ancestors. Therefore genetic methods are not suitable for the differentiation between wild and hatchery fish nor the determination of the age of caviar. However, biochemical approaches (e.g. gas-chromatography) can be applied for source identification because environmental conditions influence the biochemical composition of individual fish, and rearing conditions and feeding regimes produce readily identifiable differences between wild and hatchery-raised fish. Microbiological approaches would be suitable to identify the age of caviar; however, these require reliable information on storage temperature which is unlikely to be available making these techniques unreliable.

Impediments to identification

Stocking and release programmes, partly of non-native fish, are a barrier to identification, reducing the genetic differences among populations. These stocking and release programmes, if not well managed, also present other risks for wild populations such as; the introduction of parasites, loss of adaptive genetic variability, inbreeding and outbreeding depressions. The release of non-native fish which affect

the genetic differences among populations should thus be avoided in the future and restocking programmes should be strictly managed.

Development of an identification system

For most genetic analyses discussed there is a need to develop a reference data collection. A large body of mitochondrial sequences is currently available in public data sources (GenBank, EMBL) but further Acipenseriform information is necessary and a specific database would be a useful tool. Moreover, some methods (e.g. SSCP, PCR-AFLP, microsatellites, nested-PCR) require the inclusion of reference samples. It would not be possible to create a reference sample collection, however a reference collection could include information on locating reference samples archived in different institutes and laboratories. This reference collection would be vital to using genetic methods for the identification of parts and products in trade.

Until now genetic methods have been inappropriate for discriminating hatchery fish from wild specimens; if the introduction of wild fish into hatchery stocks is stopped in the future and these stocks are managed consistently (lineage breeding) then genetic fingerprinting could also be a valuable tool. Biochemical approaches are most appropriate currently and it has been suggested that specific characteristics of a farmed fish may be introduced through a specific feed design in aquaculture (Rosenthal *pers. comm.,* 2006). Studies into the subject of "tagging" fish through specific feed composition should be encouraged as there may be an option to design feeds that allow site and area specific identification of origin of the cultured product. However, until today, gas-liquid chromatography is limited to a small number of referenced specimens and species, and it needs further evaluation. Taken together, further research is necessary to overcome these limitations.

Although there is no “one for all method” addressing all goals of an identification system it would be possible to develop the existing methods within the next three years. The next steps towards this would be:

1) Collection of reference specimens and nomination of reference laboratories by the participating countries;
2) Blind test of species identification for the evaluation of the reference laboratories;
3) Registration of the reference laboratories;
4) Introduction of the genetic species identification system in international trade;
5) Creation of a reference specimen database including also all genetic data available.
In time, a special letter code containing the laboratory and test method used could be included within this labelling system.

Given the importance of the international trade in caviar, enforcement of catch quotas and trade controls is an essential part of ensuring sustainable harvest of these species. Although further work is necessary to develop the methods into a uniform identification system for Acipenseriformes, some techniques look promising and it is anticipated that an identification system could be developed within the next three years

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Appendix

Table I: CITES trade in caviar from wild sources (tonnes = 1000 kg) (sources: Anonymous 2006a - CITES Annual Reports, www.unep-wcmc.org, Raymakers, 2005).

Scientific name **(Common name)**	**Basin**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	Total Exports Reported 1999-2003	Total Export Quotas 1999-2003
TOTAL:		**288.95**	**233.10**	**167.66**	**83.73**	**74.20**	**63.37**	**847.65**	1028.70
Sub-TOTAL per basin:	Caspian	267.32	209.68	152.59	69.01	59.03	52.30	**757.63**	927.88
	Danube R. / Black Sea	7.33	10.90	5.29	7.61	4.86	3.45	**36.00**	40.48
	Amur River	14.30	9.10	7.74	4.93	5.37	3.00	**41.46**	55.84
	North America	0.00	3.41	2.05	2.17	4.93	4.62	**12.57**	
Species									
A. gueldenstaedtii	Caspian	72.25	48.18	17.50	10.05	6.67	10.71		
(Russian sturgeon)	Danube R. / Black Sea	1.30	1.63	0.95	0.67	0.37	0.08		
	Total	**73.55**	**49.81**	**18.44**	**10.72**	**7.03**	**10.79**	159.56	222.20
A. nudiventris	(Caspian origin only: KZ & IR)			57.342.00	44.381.00				
(Ship sturgeon)	**Total**	**0.00**	**3.34**	**4.89**	**0.71**	**0.08**	**0.00**	9.02	11.01
A. persicus	Exporter's (IR) Reports	44.38	46.53	48.80	15.74	12.58	18.66		
(Persian sturgeon)	Importers' Reports	57.34	47.33	45.22	35.97	49.45	42.91		
	Average (Caspian Sea)	**50.86**	**46.93**	**47.01**	**25.86**	**31.02**	**30.78**	201.67	274.89

A. schrenckii	China	4.15	1.08	2.69	1.78	1.32	1.07		
(Amur sturgeon)	Russia	2.98	1.77	0.28	0.58	0.25	0.01		
	Total (Amur River)	**7.12**	**2.86**	**2.97**	**2.37**	**1.57**	**1.08**	16.89	18.89
A. stellatus	Caspian	120.75	90.88	67.32	27.28	18.82	9.05		
(Stellate sturgeon)	Danube R. / Black Sea	1.87	2.73	1.39	1.51	0.40	0.14		
	Total	**122.6**	**93.6**	**68.7**	**28.8**	**19.2**	**9.18**	332.94	377.71
H. dauricus	China	3.55	0.38	4.24	2.43	1.48	1.45		
(Kaluga)	Russia	3.63	5.87	0.52	0.13	2.32	0.47		
	Total (Amur River)	**7.18**	**6.25**	**4.77**	**2.57**	**3.80**	**1.92**	24.57	36.95
H. huso	Caspian	23.45	20.35	15.87	5.11	2.45	1.76		
(Beluga)	Danube R. / Black Sea	4.17	6.54	2.95	5.43	4.09	3.23		
	Total	**27.6**	**26.9**	**18.8**	**10.5**	**6.5**	**5.0**	90.43	80.92
Polyodon spathula	**Total (North America; USA)**		3.39	1.95	2.17	4.85	4.62	12.36	
Others*:		0.00	0.02	0.10	0.00	0.08	0.00	0.20	

***** *Acipenser baerii* (Siberian sturgeon): only caviar from farmed sources appeared in CITES trade from 1999 to 2004;

A. fulvescens (Lake sturgeon): only one shipment of 4.9 kg caviar exported from Canada to France in 2000;

A. oxyrinchus (Atlantic sturgeon): no international trade in caviar was reported by CITES Parties from 1999 to 2004

A. ruthenus (Sterlet): only 5 kg and 9.8 kg caviar exported in 2000 from Bulgaria to Switzerland and the USA respectively.

Table II: Reference sequences and population genetic studies previously published and available in public data sources.

Species	Common Name	mitochondrial DNA	Population genetics
A. baerii	Siberian sturgeon	*cytb*, *d-loop* (Lena, Lake Baikal, Ob, Yenisei – AF238625-31, AF168496-502, AF238655-8, AF168480-3, AF238644-54, AF168469-79, AF238625-63, AF168496-502	mtDNA - reviewed in Birstein et al. 2005
A. brevirostrum	shortnose sturgeon	*cytb*, *d-loop* (populations from the St. John River, NB, to the St. Johns River, FL - AJ275194-5, AF402848, AJ245828)	mtDNA - Grunwald et al. 2002
A. dabryanus	Dabry's sturgeon	entire mt genome, d-loop (Yangtze - AY510085, AF362129)	mtDNA - Zhang et al. 2001
A. fulvescens	lake sturgeon	*cytb*, *d-loop* (Great Lake basin - AJ245829, AY947813-34)	
A. gueldenstadtii	Russian sturgeon	*cytb*, *d-loop* (North CS, South CS, Dnieper, Donau, Black Sea, Sea of Azov – AF238662-9, AF238720-7, AF238673-6, AF238678-9, AF238731-4, AF238736-7, AF238681, AF238739, AF238682-90, AF238740-48, AF238691, AF238749, AF238692, Af238750, AF238693-9, AF238651-754, AJ563385-96, AJ245825-7	mtDNA - reviewed in Birstein et al. 2005
A. medirostris	green sturgeon	*d-loop*, *cytb* (Asian Far East, unknown - AF362130, AF184106, L01509, AJ245830)	mtDNA - Zhang et al. 2001 microsatellites – Israel et al. 2004
A. mikadoi	Sakhalin sturgeon	*cytb*, *d-loop* (Tumnin River - AJ245831, AJ275189,	mtDNA - Zhang et al. 2001

A. naccarii	Adriatic sturgeon	*cytb*, *d-loop* (Po, Buna - AF238659-60, AF238717-8, AJ275199, AJ245833, AF283730, Ludwig et al. 2003)	mtDNA, microsatellites, AFLP – Ludwig et al. 2003
A. nudivendris	ship sturgeon	*cytb*, *d-loop* (AF402853, AJ275202-3, AJ275193, AJ249668)	
A. oxyrinchus	Atlantic sturgeon	*cytb*, *d-loop* (all populations – see Ong et al. and Wirgin et al. , AJ245838)	mtDNA, microsatellites – King et al. 2001, Ong et al. 1996, Waldman et al. 2002, Wirgin et al. 2000, 2002
A. persicus	Persian sturgeon	see Russian sturgeon	mt-DNA - Pourkazemi et al. 1999
A. ruthenus	sterlet	*cytb*, *d-loop* (AJ249694, AF308926-7, AF402846, AJ249697, AJ249671)	
A. sinensis	Chinese sturgeon	*cytb*, *d-loop* (Yangtze - AJ252186)	mtDNA - Zhang et al. 2003 microsatellites - Zhao et al. 2005, Zhu et al. 2005
A. stellatus	stellate sturgeon	complete mt genome (AJ585050) *cytb*, *d-loop* (CS, Sea of Azov, Donau - AY846680-701, AJ249693, AY847790-1)	mtDNA - Doukakis et al. 1999, 2005
A. sturio	European sturgeon	*cytb*, *d-loop* (Gironde - AJ245839, AJ249673)	mtDNA, microsatellites - Ludwig et al. 2004
A. transmontanus	white sturgeon	complete mt genome (NC_004743, AB042837)	microsatellite (Fraser River) – Smith et al. 2002
H. huso	beluga	complete mt genome (NC_005252, AY442351) *cytb*, *d-loop* (Sea of Azov, CS, Black Sea – AY846640-79, AJ245840)	mtDNA - Doukakis et al. 2005
H. dauricus	kaluga	*cytb* (Amur – AJ252187, AF402858)	

Ps. kaufmanni	large Amu-Dar shovelnose sturgeon	*cytb*, *d-loop* (Amu-Dar – AF404788, AF404800, AF404812, AF402861)	mtDNA - Birstein et al. 2002
Ps. hermanni	Amu-Dar shovelnose sturgeon	*cytb*, *d-loop* (Amu-Dar - AF404787, AF404789, AF404811, AF402860)	mtDNA - Birstein et al. 2002
Ps. fedtschenkoi	Syr-Dar shovelnose sturgeon		
S. albus	pallid sturgeon	*cytb*, *d-loop* (U56987, U56983, AF224875-9, AF236144-8, U43740-1)	mtDNA - Szalanski et al. 2001a, Campton et al. 2000 microsatellites - Tranah et al. 2004
S. platorynchus	Shovelnose sturgeon	*cytb*, *d-loop* (U56984-6, U56988, AF176341, AF224880, AF224873-74, AF236131-43, U43742-4, U43897)	mtDNA - Szalanski et al. 2001a, Campton et al. 2000 microsatellites - Tranah et al. 2004
S. suttkusi	Alabama sturgeon	*cytb*, *d-loop* (AF402862, AF236149, U43679, U55994)	mtDNA - Campton et al. 2000
Polyodon spathula	North American paddlefish	complete mt genome (AY510086, NC_004419, AY442349, AP004353) cytb, d-loop (AJ245841, AF176331-40)	mtDNA - Szalanski et al. 2001b
Psephurus gladius	Chinese paddlefish	complete mt genome (NC_005834, AY571339)	