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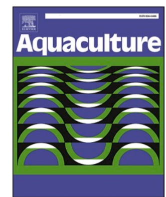

Review

# Identification and tracking of sturgeons and paddlefish products in trade: Implications for trade control and biodiversity management

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ABSTRACT

Despite strict protection of natural populations and CITES restrictions on international trade of caviar, sturgeons are increasingly at risk of extinction. The latest assessment released in July 2022 by the IUCN confirms sturgeons and paddlefish as the most endangered group of species on the planet. In this context, poaching and the illegal trade of caviar and meat have a significant weight and it is, therefore, necessary to support control activities by identifying diagnostic and reliable methods for the identification of species, interspecific hybrids, aquaculture or natural origin and geographical provenance of commercial samples. The technical approaches used for this purpose now include biochemical, isotopic, and genetic marker analyses, and are constantly evolving with remarkable developments proposed in recent years. Among these, particularly relevant are the numerous nuclear genetic markers, which allow the identification not only of pure sturgeon species but also of interspecific hybrids and the approaches based on the analysis of fatty acids which allow to distinguish feed-fed animals from fed with natural food. Additionally, the use of multi-isotopic fingerprint analyzes to determine the geographical area of origin is rapidly developing and is proving to be extremely useful for commercial controls and for the protection of natural sturgeon populations.This synthesis arises from the need of providing an exhaustive picture of the available technical approaches and to evaluate their potential and limits, focusing mainly on the innovations proposed in the last 10 years which have significantly improved our diagnostic power.

## 1. Introduction

Sturgeons and paddlefish (Acipenseriformes) are world's most endangered group of species - based upon IUCNs RED List Assessment (Congiu et al., 2023) – (https://www.iucn.org/content/sturgeon-more-critically-endangered-any-other-group-species). Besides habitat degradation through damming, channelization, gravel extraction etc. and pollution, a major factor contributing to this status is a long-lasting overharvest for production of one of the most prestigious and valuable food of wildlife origin, the caviar, obtained by processing their eggs.

In response to the dramatic decline of wild populations, listing under the Convention on International Trade in Endangered Species (CITES) was used to increase transparency and sustainability of trade in sturgeon products. This process started with two species (*Acipenser brevirostrum* and *A. sturio*) being listed on Appendix I in 1989, followed by the remaining 25 species in 1998 (https://cites.org/eng/app/appendices.php) listed under Appendix II. As such the control and supervision of international trade was mandatory for all sturgeon species.

In order to support national and international trade controls, in 2000 CITES introduced a labeling system for both wild and aquaculture caviar, which uses universal codes and non-removable labels (Resolution CITES Conf 12.7, Rev. CoP17). Although recommended also for domestic markets several states with extensive domestic markets, such as China, Japan, Russia, and the United States, have not adopted a

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mandatory labelling system. This renders the tracking of caviar in trade impossible and exposes domestic markets to an uncontrolled distribution of caviar from illegal sources (whitewashing).

In the same resolution, CITES also recommends range States with shared stocks to establish annual quotas for exporting caviar and meat from wild caught sturgeons. However, since 2011, no quota has been communicated, making any trade in meat or caviar taken from shared stocks illegal. This holds true for the Caspian Sea, the Danube basin, the Black and Azov seas, the Amur/Heilongjiang River. In addition, many states have independently enacted national or regional bans on fishing and trading in wild sturgeons.

In parallel with the dramatic decline of wild stocks, and driven by the significant market demand for caviar, a rapid development of commercial sturgeon aquaculture producing caviar took place profoundly changing global caviar production and trade dynamics, shifting the import ratio of farmed versus wild caviar internationally from 5% aquaculture source, in 2000, to 95% in 2015 (Bronzi et al., 2019). However, the 5% amount of caviar from wild fish seems to be significantly underestimated because the volume of illegal trade is unknown.

According to a report released by TRAFFIC in 2018 (30th meeting of the CITES Animal Committee, AC30 Inf. 33), there are different types of illegal trade. One comprises the "black" market in which wild products are sold "under the counter", through individual contacts or online offers, and the official market in which illegal caviar might be commercialized under 'false labelling' taking advantage of the absence of conformity checks between the actually sold product and what is declared on the label. Hence, wild caviar can be labeled as aquaculture product and thus enter the legal trade illegally. In some cases, paradoxically, farmed caviar is deliberately sold as "wild origin" on the black market to intentionally overprice the product. It is also possible that forged or counterfeit CITES documents, or genuine CITES documents obtained through corruption, are used for export.

Despite CITES restrictions and the high availability of aquaculture products, poaching and illegal trade in wild meat and caviar remain serious threats to the survival of wild populations, as documented in many regions (Birstein et al., 1998; Cohen, 1997; Daea, 2019; Doukakis et al., 2012; Fain et al., 2013; Hruby, 2019; Jahrl et al., 2021; Knight, 2017; Luca et al., 2020; Ludwig et al., 2015; Pappalardo et al., 2019; Savić and Gmizic, 2018; Van Uhm and Siegel, 2016; Zabyelina, 2014). A recent innovative study based on the combination of both genetic and isotopic analyses on 145 commercial products sampled in Bulgaria, Romania, Serbia and Ukraine, demonstrated that a substantial fraction (19% of the total) was of wild and thus illegal origin in these four countries (Jahrl et al., 2021). In addition, 17% of the total samples was sold without the mandatory CITES labels, with false codes or without the required CITES permits. This highlights the urgent need for a greater global effort to control whether sturgeon products in trade comply with all legality criteria.

Currently, most control efforts by law enforcement officials are limited to verifying the presence of proper CITES documentation and the integrity of the label. Conversely, less importance is given to compliance checks between the commercialized products and the declarations made on their labels. These checks should verify the species and the origin of the commercial product, making use of the numerous traceability markers already available and increasing constantly.

Since the publication of the most complete summary of sturgeon forensic markers (Ludwig, 2008), novel methods and approaches in genetic analysis have been developed which allow to improve the efficacy in species identification as well as in detection of hybrids. Additionally, the assessments of origin and provenance of samples through analysis of the isotope or chemical compositions have recently undergone rapid development.

In line with the requirements of CITES Resolution Conf. 12.7 (Rev. CoP17), this review examines available techniques for identification of Acipenseriformes species and their products in trade considering their advantages and disadvantages.

## 2. Identification of sturgeon species, hybrids, and their products

### 2.1. Morphological determination

Morphological and morphometric approaches, supported by dichotomous identification keys, are usually the first steps to identify intact specimens. One of the most comprehensive guides for the morphological identification of pure species is the CITES identification guide "Sturgeons and Paddlefish 2001" (Environment and Climate Change (ECC) Canada, 2001). However, correct identification based on dichotomous keys is often complicated by the fact that the geographical distribution area of the species is one of the criteria that must be taken into account. The various sturgeon species raised in aquaculture for production purposes have been globally redistributed and the information of geographical origin no longer makes any sense.

Another problem of morphological and meristic characteristics is the overlap of the ranges of values between species. Often the differences are valid only on a statistical basis, making the identifications meaningful only if carried out on many animals. Moreover, while this approach can be successfully used on live or dead animals if they are intact, this is not the case with carcasses where significant parts are often damaged or missing, or with sturgeons offered for sale in parts, often even without skin. In these cases, it is necessary to proceed with identification through a genetic approach.

Another source of ambiguity in using a morphological approach is the allometric growth. During ontogenetic development, which includes the larval, fry, juvenile and sub-adult phases, the proportions of the body undergo significant changes which are not systematically described in any sturgeon species identification key. In some species, also morphological characters can be modified, as for example in Beluga (*Huso huso*) which reduces its scutes during the sexual maturation. After attaining sexual maturity, which can be reached after 4–25 years depending on the species and environmental conditions, the morphological and meristic characteristics tend to be consistent.

The effectiveness of the morphological approach collapses when the animals to be identified are hybrids of unknown origin. Many interspecific hybrids between sturgeon species are in fact produced in aquaculture and possess intermediate morphological characteristics compared to the parental species. Furthermore, sturgeon hybrids are often fertile and can be crossed with third species, with other hybrids or backcrossed with parental species for multiple generations, potentially generating a multitude of morphologically indistinguishable intermediate forms.

This massive and growing presence of hybrids of various grades on the market makes the morphological/morphometric approach scarcely reliable, prompting the development of alternative markers, mainly genetic, which can identify the presence of hybridization events more efficiently.

Regarding both eggs and caviar (consisting of salted – traditionally unfertilized – eggs), the precise identification of species on a morphological basis is not reliable due to the similarity between the eggs of different species and the unpredictable intraspecific variability of size and color, which are affected by the degree of maturation, diet and individual genetic traits. The possibility to use the number of micropyles for identification (Debus et al., 2002) have not been followed up after the initial attempts. Again, the increasing number of species and interspecific hybrids used to produce caviar is decreasing the quality of diagnostic characters. If historically the eggs of the main three caviar producing species (*H. huso*, *A. gueldenstaedtii* and *A. stellatus*) were quite recognizable by experts, today, the contribution of genetic analyses is necessary.

All the by-products of sturgeon processing such as oils, isinglass, swim bladders, and other derived tissues or body parts are not morphologically identifiable, the only exception being the skin which, in some cases, can retain diagnostic characteristics. The morphometric approach can sometimes allow to distinguish animals of wild origin from

animals raised in aquaculture thanks to typical malformations that are observed in reared individuals such as deformed pectoral or anal fins, shortened opercula, missing nostril (Gessner and Friedrich, 2017) or eye abnormalities. However, these malformations do not constitute decisive evidence of the origin of the animals as they may not exist in farmed animals, or they may be observed in animals released within restocking programs after a period of intensive farming.

In any case, any type of morphological sturgeon species identification must be carried out by skilled experts. For a layman, the risk of misinterpreting the indications of the identification guides is high and can lead to incorrect results. This is very relevant for example in the case of animals accidentally caught by sport fishermen who should be able to distinguish native species of a given basin - to release them immediately - from alien species escaped from aquaculture facilities or released illegally - which should be removed. In these cases, it would be important to have the support of experienced personnel.

### 2.2. Genetic identification

Most limits concerning morphological and morphometric identification discussed above are overcome by DNA-based methods which, in recent decades, have become the approach of choice in the identification of animal and plant species of commercial interest. The analyses of different types of molecular markers as instruments to detect intra- and interspecific genetic variability became powerful approaches to elucidate species evolutionary and demographic histories, population differentiation as well as to search for diagnostic characters which allow to identify species, interspecific hybrids, populations, genetic lines, paternities or even individuals. In this chapter, the current status of the availability and application of DNA markers for sturgeon species is presented, highlighting their potential and limits.

#### 2.2.1. Mitochondrial markers

Mitochondrial markers are among the most frequently used genetic tools and often the prime standard to identify species (Ahmed et al., 2018; Cooper et al., 2009; Dalton and Kotze, 2011; Dawnay et al., 2007; Khedkar et al., 2014) and this is true also for sturgeons. This is mainly due to mitochondrial DNA (mtDNA) properties such as: i) number of copies hundreds or thousands of times greater than the nuclear genome, ii) small size and consequent high resistance to degradation, iii) presence of regions with different mutation rates and therefore with different levels of sequence heterogeneity, vi) ease of analyses, reliability and comparability with a rich reference database (e.g. Genbank, BOLD). Last but not least, this method is generally accepted as evidence during court cases. It is generally used by sequence analysis although several indirect approaches have been proposed in the past to limit costs and processing time (sequencing). For a review of these methods such as RFLP, SSCP nested or semi-nested PCR, see Ludwig (2008).

More recently, after sequencing costs dropped substantially, the mtDNA sequencing analysis became the most common technique for species identification through the comparison of results with sequences deposited in publicly available data banks. However, the use of the reference sequences present in the databases should be treated with caution as some individuals have been identified incorrectly and consequently their deposited sequences can create ambiguities. This is the case of a mitogenome (EU719645) deposited as *A. sinensis* but belonging to *A. gueldenstaedtii* (Dillman et al., 2014), highlighting the importance of vouchers and certified reference samples.

Mitochondrial DNA, for sturgeon in particular the sequencing of the control region, represents an excellent tool for the identification of pure species. Exceptions are sturgeon species that are not clearly differentiated or have encountered introgressions in the past. This is the case for the Persian sturgeon (*A. persicus*), morphologically very similar to the Russian sturgeon (*A. gueldenstaedtii*) and with overlapping distribution. The two species are not distinguishable based on mitochondrial DNA and in general no diagnostic genetic markers are available, raising doubts about the status of *A. persicus* as a valid species. Although separated geographically, the mitochondrial haplotypes of the Adriatic sturgeon (*A. naccarii*) do not form a separate cluster from haplotypes of *A. gueldenstaedtii* (Birstein et al., 2000). However, the species is distinguishable genetically because all existing individuals descend from a group of animals bred in captivity that are characterized genetically. For these species seven mtDNA control region haplotypes are known and these haplotypes are not shared with other species (Boscari et al., 2014a). Another typical ambiguity that often creates disputes in the case of analysis of commercial products is given by the introgression of the Siberian sturgeon (*A. baerii*) into Russian sturgeon (*A gueldenstaedtii)* gene pool, followed by backcrosses with the Russian sturgeon (Jenneckens et al., 2000; Mugue et al., 2008). Due to the non-recombinant inheritance of mtDNA along the matrilinear genealogy, over 30% of Russian sturgeons still has a *A. baerii* mitochondrial haplotypes that are defined as "*baerii-like*". These *baerii-like* haplotypes, based on a simple query of the databases are attributed to the Siberian sturgeon even if the animal, after several generations of backcrosses, has completely lost any morphological trace of the hybridization event. The only suitable marker for the identification of *baerii-like* haplotypes is a SNP proposed by Mugue et al. (2008).

Primers suitable for all species and matching the proline (Pro1F: 5′-CACCCTTAACTCCCAAAGC-3′) and phenylalanine tRNA (Phe1R: 5′-CCCATCTTAACATCTTCAGT-3′), respectively can be used for the amplification of the entire control region, with the recommendation of using the using Phe1R and not Pro1F, for the sequencing reaction, to obtain unambiguous sequences, as justified by Congiu et al. (2011).

Due to its maternal inheritance, the main limit of mtDNA is the inability to track the paternal contribution, making it scarcely informative for relatedness analyses and detection of hybrids which is a critical limit for sturgeons. In fact, with the considerable increase in the production of interspecific hybrids for caviar or meat, the use of mitochondrial markers for commercial controls opens the way to a series of possible commercial frauds, the most common of which is to sell the hybrid caviar under the false label of the maternal species increasing its market value. In this case, mitochondrial markers would erroneously confirm the correctness of the label. Alternatively, pure-bred species wild caviar (illegal) is sold as being of captive hybrid origin (whitewashing). For this reason, the development of biparentally inherited nuclear markers has been particularly pursued in recent years.

#### 2.2.2. Nuclear markers

With the exception of the pioneering studies that had involved multilocus nuclear markers in the past (extensively reviewed in Ludwig, 2008 but scarcely used nowadays), the use of nuclear markers to detect species and hybrids has caught on only lately. In the last 10 years, the focus of the development has been upon two classes of nuclear markers, the microsatellites and the Single Nucleotide Polymorphisms (SNPs), used individually or in combination through different approaches (summarized in Table 1). For both classes of markers, loci can be informative if they have species-specific alleles, either fixed or with a frequency high enough to guarantee a good probability of detection. This condition is less common in microsatellites than in SNPs, probably because of their higher rate of variability and the high number of alleles resulting thereof. Furthermore, the published loci have generally been selected for high variability. Conversely, SNPs are usually biallelic and, especially those isolated with genotyping by sequencing approaches, can map in regions with different variability rates increasing the likelihood of having loci with fixed or high frequency species-specific alleles.

Nuclear molecular markers, being equally inherited by the parental individuals, are informative for all applications in which the genetic contribution of both parents is of relevance, such as relatedness analyses or identification of intra- and interspecific hybrids. The latter case is especially important for sturgeons for which artificial hybridization is recurrent in aquaculture and for the confirmed occurrence of various

**Table 1**
Summary of nuclear markers available for the sturgeon species ID and information addressing their efficiency and cross-species tests. Concerning the column "Efficiency" where the probability to detect the target species is estimated for each marker, the frequency of diagnostic alleles and the ploidy of the species have been considered for the calculation. References are numbered as follows: 1: Boscari et al., 2014a; 2: Havelka et al., 2019; 3: Barmintseva and Mugue, 2013; 4: Chassaing et al., 2011; 5: Havelka et al., 2017; 6: Boscari et al., 2017a; 7: Boscari et al., 2017b. The cross-species tested for each marker are reported by using the official CITES standard code for the identification of sturgeon species. Species codes: BAE = *Acipenser baerii*; FUL = *Acipenser fulvescens*; GUE = *Acipenser gueldenstaedtii*; MED = *Acipenser medirostris*; MIK = *Acipenser mikadoi*; NAC = *Acipenser naccarii*; NUD = *Acipenser nudiventris*; OXY = *Acipenser oxyrhynchus*; PER = *Acipenser persicus*; RUT = *Acipenser ruthenus*; SCH = *Acipenser schrenckii*; SIN = *Acipenser sinensis*; STE = *Acipenser stellatus*; STU = *Acipenser sturio*; TRA = *Acipenser transmontanus*; DAU = *Huso dauricus*; HUS = *Huso huso*. For all other species, no diagnostic markers are available. For primer sequences of the different loci please refer to the corresponding reference.

| Spp | Ref | Marker | Locus | Criteria | Efficiency | Cross-species tested | Comments |
|---|---|---|---|---|---|---|---|
| BAE | 1 | SNP | RPS7 (Intron RP1) | Diagnostic PCR product | 60.6% | FUL, GUE, NAC, PER, RUT, SCH, SIN, STE, TRA, DAU, HUS | Shared with RUT |
| | | | Vimentine | Diagnostic PCR product | 78% | GUE, PER | – |
| | 2 | SNP | Unknown | Diagnostic PCR product | 99% | FUL, GUE, NAC, PER, RUT, SCH, SIN, STE, TRA, DAU, HUS | Shared with GUE and highly present in PER |
| FUL | 1 | SNP | RPS7 (Intron RP1) | Diagnostic PCR product | 100% | BAE, GUE, NAC, PER, RUT, SCH, SIN, STE, TRA, DAU, HUS | – |
| GUE | 2 | SNP | Unknown | Diagnostic PCR product | 96% | BAE, FUL, NAC, PER, RUT, SCH, SIN, STE, TRA, DAU, HUS | Shared with BAE and highly present in PER |
| MED | 3 | Microsatellite | An20 | Species-specific range: 185–197 | >92% | BAE, GUE, MIK, NUD, PER, RUT, SCH, STE, DAU, HUS | – |
| | | | AfuG51 | Diagnostic allele: 296 | 100% | | |
| | | | AoxD161 | Diagnostic allele: 114 | 97% | | |
| | | | AoxD165 | Species-specific range: 230–254 | 68% | | |
| NAC | 1 | SNP | RPS7 (Intron RP1) | Diagnostic PCR product | 100% | BAE, FUL, GUE, PER, RUT, SCH, SIN, STE, TRA, DAU, HUS | – |
| | 4 | Microsatellite | LS54, Aox23 | specific allelic range | 100% | OXY, STU | Low number of species compared RS possibly related |
| | | | LS19, LS68 AoxD161 | SNPs on flanking region | 100% | | Need of sequencing; Low number of species compared |
| NUD | 3 | Microsatellite | An20 | Diagnostic allele: 153 | 100% | BAE, GUE, MED, MIK, PER, RUT, SCH, STE, DAU, HUS | – |
| | | | AfuG51 | Diagnostic allele: 272 | 100% | | |
| | | | AoxD165 | Diagnostic allele: 196 | 74% | | |
| OXY | 4 | Microsatellite | Aox23, AoxD161, LS68 | SNPs on flanking region | 100% | NAC, STU | Need of sequencing; Low number of species compared RS possibly related |
| RUT | 1 | SNP | RPS7 (Intron RP1) | Diagnostic PCR product | 96% | BAE, FUL, GUE, NAC, PER, SCH, SIN, STE, TRA, DAU, HUS | Shared with BAE |
| | 5 | SNP | Unknown | Diagnostic PCR product | 100% | BAE, GUE, MIK, NAC, PER, SCH, STE, TRA, DAU, HUS | – |
| | 3 | Microsatellite | An20 | Diagnostic allele: 177 | >80% | BAE, GUE, MED, MIK, NUD, PER, SCH, STE, DAU, HUS | |
| | | | AfuG51 | Diagnostic allele: 252 | >91% | | Partially shared with DAU |
| SCH | 1 | SNP | RPS7 (Intron RP1) | Diagnostic PCR product | 100% | BAE, FUL, GUE, NAC, PER, RUT, SIN, STE, TRA, DAU, HUS | Shared with DAU |
| | 3 | Microsatellite | An20 | Diagnostic allele: 137 | 100% | BAE, GUE, MED, MIK, NUD, PER, RUT, STE, DAU, HUS | – |
| | | | AfuG41 | Species-specific range: 185–197 | Moderate | | |
| | 6 | SNP | RPL8 (Intron RP5) | Diagnostic PCR product | 100% | DAU | Partially shared with DAU; To be used in combination with RPS7 (1) |
| SIN | 1 | SNP | RPS7 (Intron RP1) | Diagnostic PCR product | 100% | BAE, FUL, GUE, NAC, PER, RUT, SCH, STE, TRA, DAU, HUS | – |
| STE | 1 | SNP | RPS7 (Intron RP1) | Diagnostic PCR product | 100% | BAE, FUL, GUE, NAC, PER, RUT, SCH, SIN, TRA, DAU, HUS | – |
| | 3 | Microsatellite | An20 | Diagnostic allele: 141 | 85% | BAE, GUE, MED, MIK, NUD, PER, RUT, SCH, DAU, HUS | – |
| | | | AfuG51 | Diagnostic allele: 288 | 86% | | |
| | | | AoxD161 | Specific allelic range | 100% | | – |
| STU | 4 | Microsatellite | Aox23, AoxD161, LS68 | SNPs on flanking region | 100% | NAC, OXY | Need of sequencing; Low number of species compared. RS possibly related |
| TRA | 1 | SNP | RPS7 (Intron RP1) | Diagnostic PCR product | 100% | BAE, FUL, GUE, NAC, PER, RUT, SCH, SIN, STE, DAU, HUS | – |
| DAU | 1 | SNP | RPS7 (Intron RP1) | Diagnostic PCR product | 100% | BAE, FUL, GUE, NAC, PER, RUT, SCH, SIN, STE, TRA, HUS | Shared with SCH |
| | 3 | Microsatellite | AoxD165 | Private allele: 206 | 73% | BAE, GUE, MED, MIK, NUD, PER, RUT, SCH, STE, HUS | – |
| | | | AfuG41 | No amplification | 100% | | |
| | | | An20 | Diagnostic allele: 169 | 93% | | |
| | 6 | SNP | RPL8 (Intron RP4) | Diagnostic PCR product | 100% | SCH | Partially shared with SCH To be used in combination with RPS7 (1) |
| HUS | 7 | SNP | RPS6 (Intron RP2) | Diagnostic PCR product | 100% | BAE, FUL, GUE, NAC, PER, RUT, SCH, SIN, STE, TRA, DAU | – |
| | 5 | SNP | Unknown | Diagnostic PCR product | 100% | BAE, GUE, MIK, NAC, PER, RUT, SCH, STE, TRA, DAU | – |

(*continued on next page*)

**Table 1** (*continued*)

| Spp | Ref | Marker | Locus | Criteria | Efficiency | Cross-species tested | Comments |
|---|---|---|---|---|---|---|---|
| | 3 | Microsatellite | An20 | Diagnostic alleles: 145, 149 | 83%, 45% | BAE, GUE, MED, MIK, NUD, PER, RUT, SCH, STE, DAU | – |
| | | | AoxD161 | Diagnostic allele: 98 | 98% | | |
| | | | AoxD165 | Diagnostic allele: 178 | 96% | | |
| | | | AfuG41 | Species-specific range: 249–277 | Moderate | | |

events of natural introgression (Beridze et al., 2022; Ludwig et al., 2009). Since the fertility of many hybrids can generate genomes with various degrees of admixed contributions through backcross with parental species or further hybridizations with other species, the availability of one diagnostic marker for each species may not be sufficient. In fact, in first generation hybrids the entire chromosomal set of the two parental species is fully represented, and even a single locus marker can successfully detect their target species. From the second generation onward, only a subset of these chromosomes (or part of them, being everything further complicated by meiotic recombination) will pass the random bottleneck of segregation (Fig. 1). For this reason, the availability of several loci increases the possibility that at least one of them can be still detectable in the sample.

For some species, the occurrence of past events of natural introgression renders the identification of loci with 100% diagnostic power difficult if not impossible. For example, due to the past introgression documented for *A. baerii* and *A. gueldenstaedtii*, as mentioned above, no nuclear loci with alternative alleles in the two species are yet available. This is also the case of *A. schrenckii* and *Huso dauricus* hybrids (sympatric in the Amur River) (Boscari et al., 2017a).

*2.2.2.1. Microsatellites.* Microsatellites, given their high variability, the widespread occurrence and the easy analyses by PCR, have for decades been the most common markers for the study of genetic differentiation at different levels of diversity (relatedness and population analyses) in many eukaryotes including sturgeons and paddlefish (Ludwig, 2008).

The presence of a high number of alleles per locus (for example compared to SNPs) makes them very informative for the study of genetic

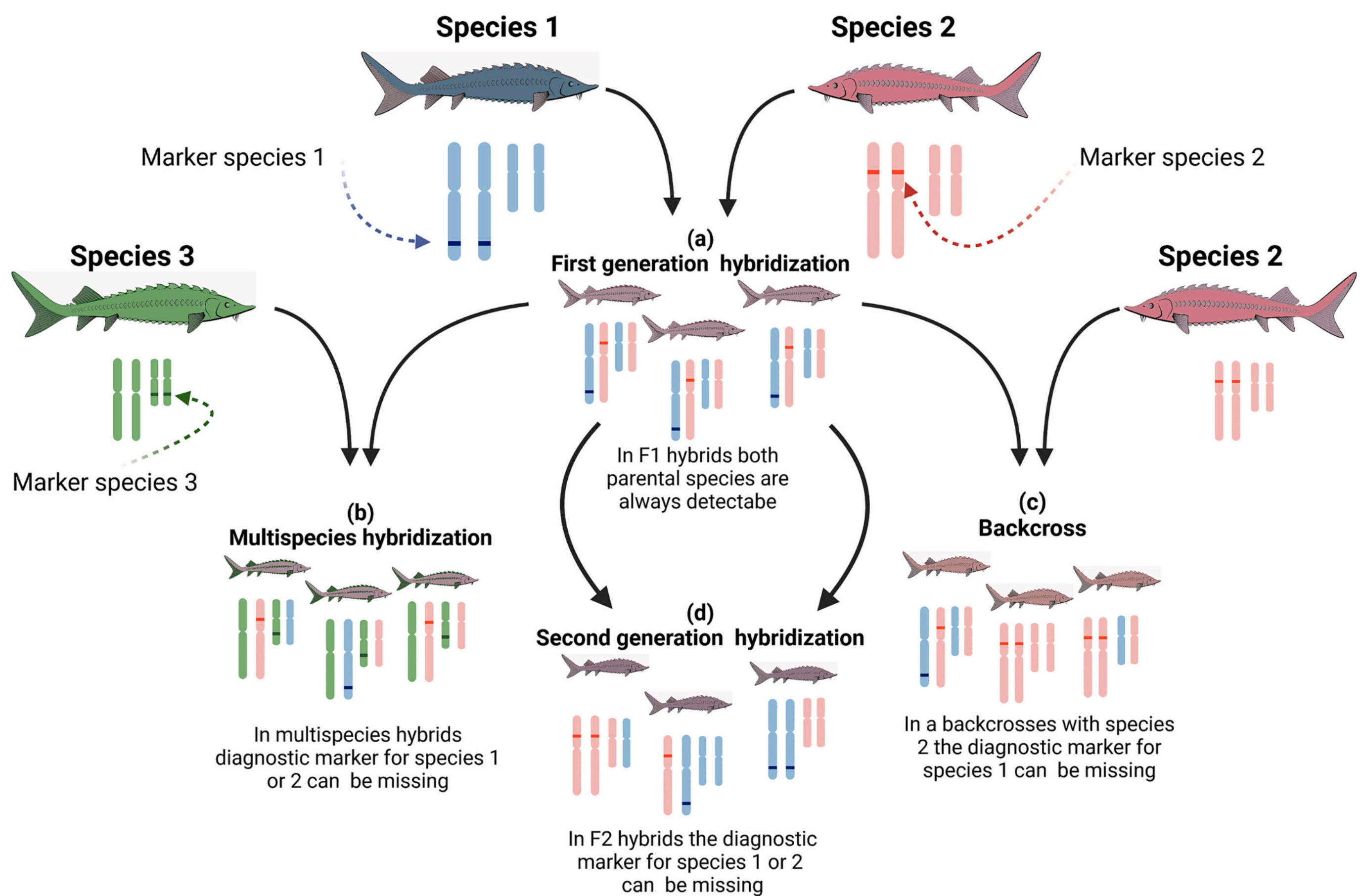


**Fig. 1.** Schematic representation of different hybridization events: a) hybridization between two pure species (species 1 and 2) generating first generation hybrids (F1); b) hybridization between a first generation hybrid (F1) with a third pure species (Species 3) producing multispecies hybrids; c) hybridization between a first generation hybrid (F1) with one parental species (Species 2) producing a backcrossed hybrid, d) hybridization between two fist generation hybrids (F1) generating second generation hybrids (F2). Genomes of the three species involved in the different hybridization events are schematized by chromosomes of the corresponding colors and bands on chromosomes highlight the presence of diagnostic markers for different species. Examples of chromosome inheritance and different possibilities to inherit the diagnostic markers are represented below each type of hybridization. The use of a single nuclear marker per species guarantees the detection of hybrids only in first generation. In the case of second (or more) generation hybrids, backcrosses or multispecies hybrids, the traceability of original contributing species can fail due to the loss of the corresponding marker. (Created with BioRender.com).

diversity, potentially including species identification. However, these markers also have some limitations for species identification, such as the limited comparability of results among laboratories due to different analytical conditions. Small allele shifting may lead to discordant genotyping of the same individual analyzed in different laboratories. To have a reliable allele size, all laboratories applying this approach should include some reference samples with known genotypes in their sample set for the opportune inter-calibration. Other problems such as misidentification due to allelic dropout, null alleles or wrong allele dosage estimation can also occur but they are locus-dependent and require preliminary validation on large sample sets.

For the application of microsatellites in species identification, the presence of alleles fixed in a given species and absent from all the others (private) is a very rare condition, also due to the high intrinsic variability rate of microsatellites. However, in some cases, it was possible to identify efficient markers, such as for the stellate sturgeon (*A. stellatus*) in which at the locus Afu-39 (formerly known as LS -39) (Jenneckens et al., 2001) the allele with 111 base pairs is private and monomorphic for this species. This locus can be used in combination with mitochondrial DNA for the identification of both the pure species and stellate sturgeon hybrids. In this case, the discordance with the mitochondrial haplotype will allow to detect the hybrid.

Loci in which no fully diagnostic alleles are present can also be used if some alleles are present at a high frequency in a given species. In these cases, the identification is performed by estimating the probability of belonging to a given species, following allocation procedures. However, the use of these markers for forensic purposes is much more critical as they cannot provide a certainty, but only an allocation probability. It is worth noting that the probability of observing a given allele in a genome depends not only on the allele frequency in the species but also on its ploidy level (See paragraph 2.2.5 for details). Based on these criteria, several microsatellite loci have been proposed as diagnostic markers for the detection of different sturgeon species (Barmintseva and Mugue, 2013; Boscari et al., 2017a; Chassaing et al., 2011).

*2.2.2.2. Single nucleotide polymorphisms (SNPs).* Single Nucleotide Polymorphisms (SNPs) are substitutions of a single nucleotide and represent the most frequent case of genetic polymorphism. Such substitutions can be localized both on coding and non-coding regions and their characterization can be carried out through different approaches. Species identification based on SNP analysis has become increasingly important in recent years, especially thanks to the advent of massive genotyping by sequencing methods (Elshire et al., 2011). The advantage of these approaches is that they allow the simultaneous genotyping of several SNPs in hundreds of individuals without the need to preliminarily identify a panel of polymorphic loci. However, these approaches, being time and lab consuming, are useful for the detection of polymorphism but are not convenient for applications on a limited number of samples. In these cases, it is necessary to use diagnostic SNPs (or combinations of SNPs) that can be genotyped quickly and reliably. Once the diagnostic SNPs have been identified using the above-mentioned whole genome approaches (Havelka et al., 2017; Ogden et al., 2013) or focusing on highly polymorphic regions such as intronic sequences (Boscari et al., 2014b, 2017a, 2017b), a locus-specific test can be developed, for example by developing PCR-based tests based on the presence/absence of diagnostic alleles. The good transferability and replicability of this approach among laboratories represent important advantages of SNPs compared to microsatellites.

The application of SNPs to sturgeon identification was first proposed as a single locus approach by Boscari et al. (2014a). The authors set up a PCR-based identification tool based on a panel of diagnostic SNPs within an intronic region of the RPS7 gene. The approach, combined with mtDNA, allows the unambiguous detection of five species (*A. naccarii, A. fulvescens, A. stellatus, A. sinensis* and *A. transmontanus*) and two hybrids, the Bester (*H. huso* ♀x *A. ruthenus* ♂) and the AL (*A. naccarii* ♀x *A. baerii* ♂), with an 80% and 100% success, respectively. The use of intronic SNPs has been later extended to another intronic region, of the gene RPS6, again with a single locus approach, allowing for the identification of *H. huso* (Boscari et al., 2017a; Fig. 2A). Introns of RPL4 and RPL5 genes, combined with a microsatellites panel, allowed to setup a multi-locus approach for the differentiation between *A. schrenckii* and *H. dauricus* with discrete overall diagnostic results (Boscari et al., 2017a) even if no fixed markers were found that differentially segregate between the two species (see chapter 2.2 on the importance of reliable reference samples). Havelka et al. (2017) have identified other diagnostic SNPs for *A. ruthenus* and *H. huso* after a massive screening of loci obtained through Next Generation Sequencing. More recently, through a ddRAD approach, Havelka et al. (2019) isolated new SNPs able to differentiate *A. baerii* and *A. gueldenstaedtii* from all the other species, thus allowing to detect the presence of one of them as paternal species in hybrids. A similar whole genome rarefaction approach (2bRAD) enabled Boscari et al. (2021) to differentiate geographical origins in the Beluga, but this aspect can be relevant mainly in a conservation perspective, for a reliable identification of conservation units.

Multiple, in linkage, SNPs (microhaplotypes) are the focus of more recent studies that demonstrated how their use, instead of stand-alone SNPs, can effectively increase the informative potential for diversity analysis (Baetscher et al., 2018; Pakstis et al., 2021). The University of Padova is currently developing the multilocus SNPs approach focusing on intronic regions, which had showed interesting potentials for sturgeon species identification also in a single locus approach (Boscari et al., 2014a). This new approach is based on the simultaneous amplification and sequencing of several intronic loci through illumina sequencing with the aim of identifying species-specific haplotypes to be used for the subsequent development of diagnostic primers. In a preliminary application, individuals of fourteen species were simultaneously genotyped at 18 loci and the results, based on allele sharing among individuals, show a promising detection power as all species are clearly differentiated (Fig. 2B). The only exceptions are *A. schrenckii* and *H. dauricus*, supporting the hypothesis of hybridization events between the two species, at least in the individuals used in this analysis, consistently with what observed by Boscari et al. (2017a).

### 2.2.3. Effects of ploidy on the efficiency of nuclear markers

Whatever nuclear marker used, the probability of identifying a given species depends on the frequency of the diagnostic allele and the degree of ploidy of the organism.

The 27 species of sturgeon and paddlefish can be separated in different groups based on their ploidy level. The first group with about 120 chromosomes, the second with some 240 and the third, represented only by *A. brevirostrum* with 360 chromosomes. The ploidy level to be ascribed to each group depends on the criteria of classification (evolutionary of functional) as synthesized by Fontana et al. (2008). Focussing on the evolutionary perspective, the first group, having experienced two rounds of whole genome duplications, can be considered as tetraploid, with consequent status of ottaploids and dodecaploids for the other two groups. On contrary, when focussing on functional aspects, due to the significant functional genome re-diploidization, the three groups can be considered as di-, tetra- and hexaploidy (Fontana et al., 2008). The functional reduplication, however, is a dynamic and still ongoing process and the ploidy level at different loci cannot be taken for granted and should be viewed with caution (Ludwig et al., 2001). These aspects should be considered in developing species identification markers, especially in the case of Unfixed Diagnostic Alleles (UDA). These are alleles which are present only in one target species with a frequency lower than one. The target species can be unambiguously identified by the corresponding UDA but cannot be excluded if the UDA is not detected. Being an UDA not fixed by definition, a certain percentage of genomes in the population have alternative alleles; consequently, a genetic test (for example an allele-specific PCR) developed to specifically detect the UDA will yield negative results if, for chance, all genome

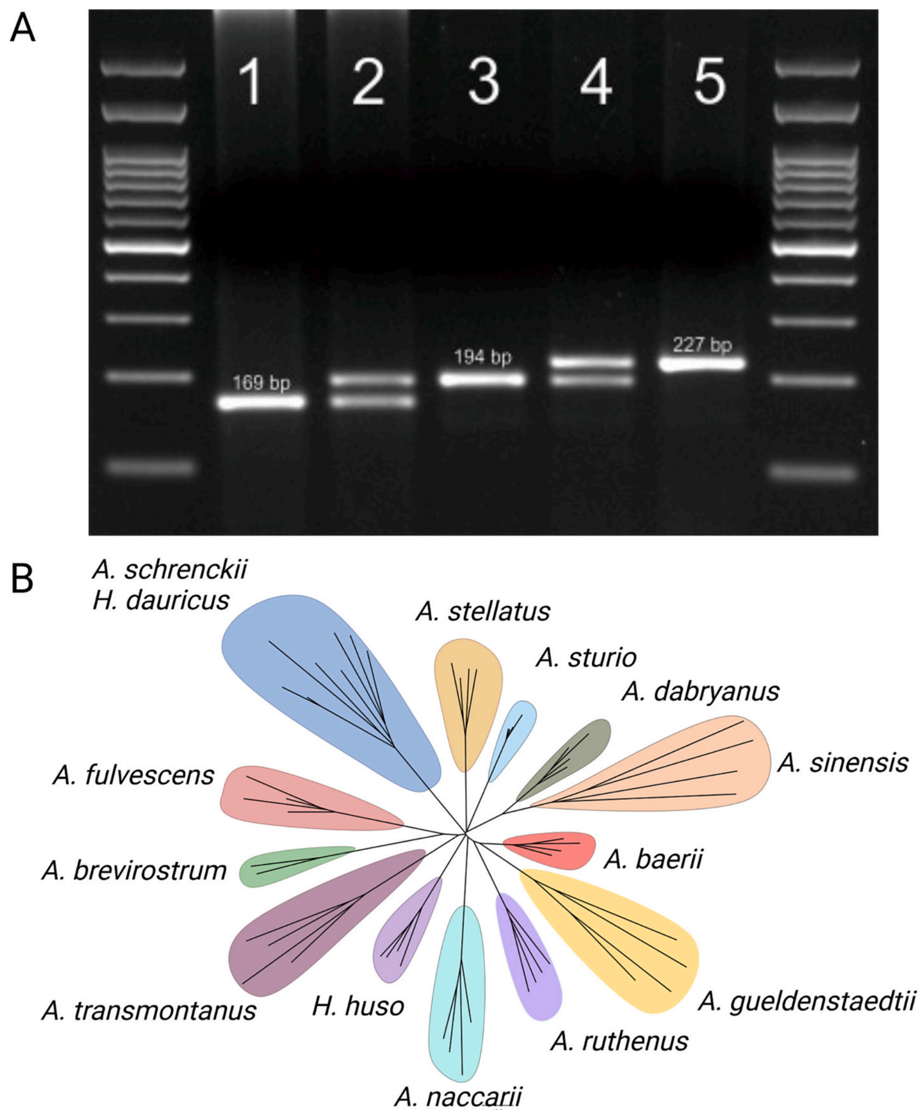


**Fig. 2.** Different genetic approaches to species and hybrids identification. A. Identification of two sturgeon hybrids (*H. huso x A. ruthenus* and *A. naccarii x H. huso*) in lanes 2 and 4 and their pure parental species (*A. ruthenus*, *H huso* and *A. naccarii*) in lanes 1, 3 and 5, based on specific PCR targeted to diagnostic SNPs in introns of RP2S6 and RP1S7 genes (Boscari et al., 2017b). B. Preliminary results of multilocus intron analyses under investigation at the University of Padova showing a good potential as species identification tool. (Created with BioRender.com).

copies of an individual carry these alternative alleles. The probability that a given individual does not have any copy of the UDA but carries only alternative alleles is counter proportional to his ploidy as schematized in the Fig. 3. To better explain this concept, let consider a hypothetical UDA with frequency of 0.33 on which an allele-specific diagnostic PCR is set up. The probability that a diploid animal has only alternative alleles being negative to the PCR, is $0.66^2 = 0.44$, that means that only 54% of the individuals are detectable. If an UDA with the same frequency of 0.33 is observed in a tetraploid species, the probability to carry only alternative alleles is $0.66^4 = 0.19$, and as a result 81% of individuals will be positive to the diagnostic PCR.

For this reason, even relatively low frequency diagnostic alleles can provide valuable detection power, especially in polyploids.

### 2.3. The importance of reference samples (RS)

Irrespective of the methods or the genetic markers used, the selection of Reference Samples (RS) in the developmental phase of forensic tools is crucial. RS must be certified with regard to their purity and should be representative of the genetic variability of the species or population of origin to avoid the following biases.

Firstly, difficulty in identifying diagnostic markers due to not pure individuals used as RS. Often, for the development of diagnostic markers, sturgeons from aquaculture facilities or from captive stocks are used as RS. The history of these animals is sometime unclear. In many cases, following translocations among plants or mixing of individuals of different origins, traces of their individual histories are lost. This generally does not represent a big problem since the species are morphologically recognizable. However, in aquaculture, pure individuals often coexist with different degrees of hybrids showing intermediate features which render the morphological-based identification a problem. This is especially true when the hybrid does not represent a first-generation specimen, but the hybridization event occurred in their earlier genealogy, followed by backcrosses. In this case, the contribution of the introgressed species is retained in the population or in the captive stock for several generations being progressively diluted. Consequently, some animals that phenotypically are classifiable as pure species might have a certain fraction of genome inherited from other species. Their inclusion in the reference dataset dramatically decreases the possibility to identify diagnostic markers. This situation was hypothesized in the case of the previously mentioned study conducted by Boscari et al. (2017a) on *H. dauricus* and *A. schrenckii* (sympatric in the Amur River). The authors have failed in identifying diagnostic markers to distinguish the two species, despite belonging to different genera and the fact that they are morphologically very dissimilar. The production of hybrids between the two species is in fact very common in aquaculture in China

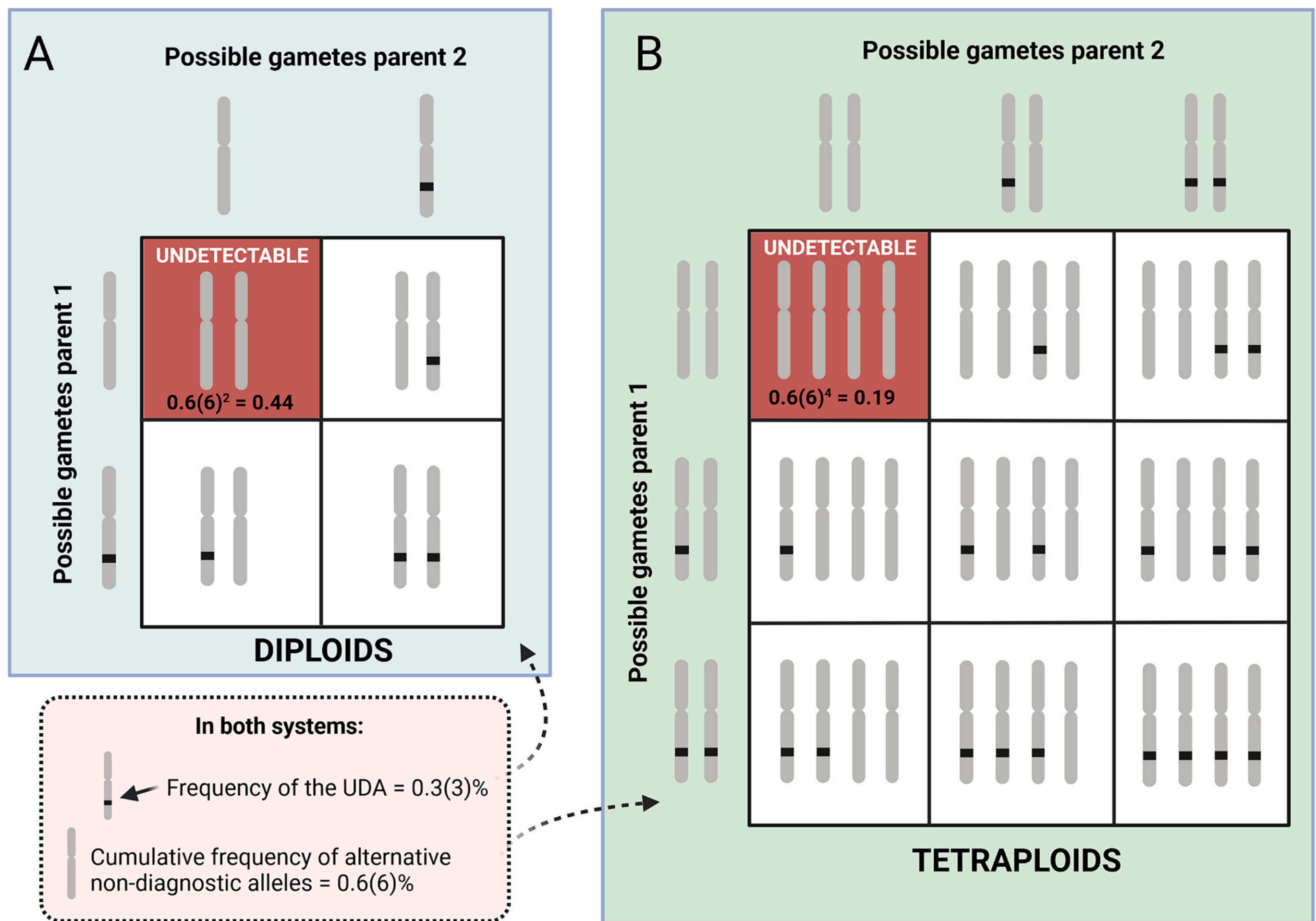


**Fig. 3.** The effect of ploidy on markers' effectiveness. Schematic representation of possible genotypes resulting in diploids (A) and in tetraploids (B) from the inheritance of chromosomes carrying either an Unfixed Diagnostic Allele (UDA) or alternative non-diagnostic alleles. For both ploidies the UDA, represented by a black square on the chromosomes, is arbitrarily supposed to have a frequency of 0.33 to match the example reported in the text. All genotypes are detectable by a selective amplification of the UDA, with the only exception of those in the red squares. The undetectable genotype has a lower probability to occur in tetraploids, regardless of UDA frequency. (Created with BioRender.com). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

but the two sympatric species are also known to occasionally hybridize in nature. In this case, a potential alternative is developing markers that identify the two species as a whole (Boscari et al., 2014a) and limiting any testing to the compatibility of a sample with these two species, without attributing them with certainty to one of them. This approach, however, does not meet the identification criteria required by CITES regulations.

Secondly, incomplete representation of a species in the RS. Due to a deficit in sampling effort, it is possible that the individuals used as a reference for a species are not completely representative of its genetic diversity. Often, animals originated from one or few crosses are reared in aquaculture resulting in a high degree of relatedness. In these cases, a genetic variant can be wrongly identified as always present or always absent from a given species and its application to forensic analysis could lead to false interpretations. For this reason, it is important to verify that the validation process of the identified markers has been carried out on the largest number of animals possible, with different origins, possibly analyzed by different laboratories. An example of this type of bias is given by Zhang et al. (2013) in which the authors used microsatellites to differentiate several reciprocal hybrids generated by switching the sire's and dam's species (*A. baerii*♀ × *A. gueldenstaedtii*♂ versus *A. gueldenstaedtii*♀ × *A. baerii*♂; *A. baerii*♀ × *A. schrenckii*♂ versus *A. schrenckii* ♀ × *A. baerii* ♂ and *H. dauricus*♀ × *A. schrenckii* ♂ versus *A. schrenckii* ♀ × *H. dauricus* ♂) as genetically distinct. This is not genetically expected because first-generation hybrids between two species should group together regardless of the maternal or paternal species. It is conceivable that the group of hybrids analyzed in this study comprised related animals and were not representative of the potential variability.

Thirdly, limited number of reference species. A suitable species identification marker is expected to show one state in the target species and alternative states in all other ones. This condition can be theoretically verified only if all other species are included in the validation test, but this is unfortunately often not the case as in most studies only a limited number of species is included. The set of species used to validate the efficiency of a marker should be as broad as possible, otherwise the risk is that a marker that we believe to be specific for a target species is actually also present in other species that we have not analyzed. An example is given by a *A. sinensis* marker that was proposed by Boscari et al. (2014b) which, following further validation tests, proved to be partially shared with a part of the individuals of *A. dabryanus*, a species that was not included in the original dataset.

## 3. Determining and tracking the source and geographical origins of sturgeons and their products

Along with the problem of setting up reliable methods for species and hybrid identification, a second challenging issue is providing tools able

to identify the origin of sturgeons and to trace the derived products. Particularly relevant to defeat the illegal trade and the whitewashing of wild sturgeon sold as captive-bred is the possibility of distinguishing wild from aquaculture products. Different approaches for these purposes were proposed on fish (Arechavala-Lopez et al., 2013), among which two have proven to be effective in addressing this question: fatty acid profiling and stable isotope analysis.

### 3.1. Specific chemical compound profiling (fatty acid)

Fatty acids are both important dietary sources and important structural components of cells in all biological organisms. Their composition is largely determined by the animal's diet (Zhang et al., 2020).

The analyses of fatty acid composition can be used to distinguish wild from farmed origin, as shown in salmonids (Molkentin et al., 2015) as well as in sturgeon caviar (DePeters et al., 2013; Gessner et al., 2002). While Czesny et al. (2000) pointed out that focusing on stearic and oelic acids would provide suitable tools for this purpose, basing the investigation on the entire fatty acid profile instead of relying on only one or few discriminating acids provides more reliable results (Vasconi et al., 2019). In fact, since the possibility to distinguish wild from farmed caviar depends on the food ingredients which are variable across farms, the more informative fatty acid for discrimination might change as well.

Unfortunately, fatty acid profiling is a labour-intensive and time-consuming procedure (approximately 5 to 10 days). The proteins need to be hydrolyzed, and the fatty acids are derivatized breaking them into the original esters (triglycerides, waxes, phospholipids etc.) before converting them to methyl esters that can be finally analyzed by gas chromatography. New, upcoming technologies, like Direct Analysis in Real Time (DART), coupled with a time-of-flight (TOF) mass spectrometer (MS) (e.g. DART-TOF-MS), may improve routine analysis of fatty acids by massively cutting down on costs and shortening the time needed to less than an hour including data analyses (Black et al., 2016; Cajka et al., 2013). The biggest advantage of this technology is that almost no sample preparation is required, and the chemical profile of a suitable sample can be obtained within seconds.

Therefore, DART is already used in various fields of authenticity verification. Thus, the field of work ranges from the verification of the authenticity of foods such as olive oil (Vaclavik et al., 2009) to forensics (Sisco and Forbes, 2021) and the routine application of species verification in wood (Price et al., 2022). However, the application of this approach to the analyses of caviar is still at its infancy but the results later described of a preliminary analysis performed by Agroisolab (Berlin) show a remarkable potential of the approach in distinguishing the different origins (Fig. 4). In combination, DART-TOF-MS provides a typical mass fingerprinting of those reference samples. The differentiation of caviar from wild and farmed origin using Kernel Discriminant Analysis (KDA) of the fatty acid chemical fingerprint from the DART-TOF analysis was performed on caviar obtained from farmed and wild origin (Fig. 4). The analyses of 58 test samples with both approaches yielded concordant results in 88% of cases (51 samples). In three samples the DART-TOF-MS gave no significant assessment, so the result could not be compared, and in four cases (7%) results were discordant. In these cases, further background information (i.e. on the feed used in aquaculture) might be helpful to draw the right conclusions. Regarding the costs, the method with a price of about 40 Euros per sample is quite inexpensive.

### 3.2. Stable isotopes

Stable Isotope Ratio Analysis (SIRA) is the analysis of variations in the bioelements C, O, H, N and S. Isotopes are atoms of the same element

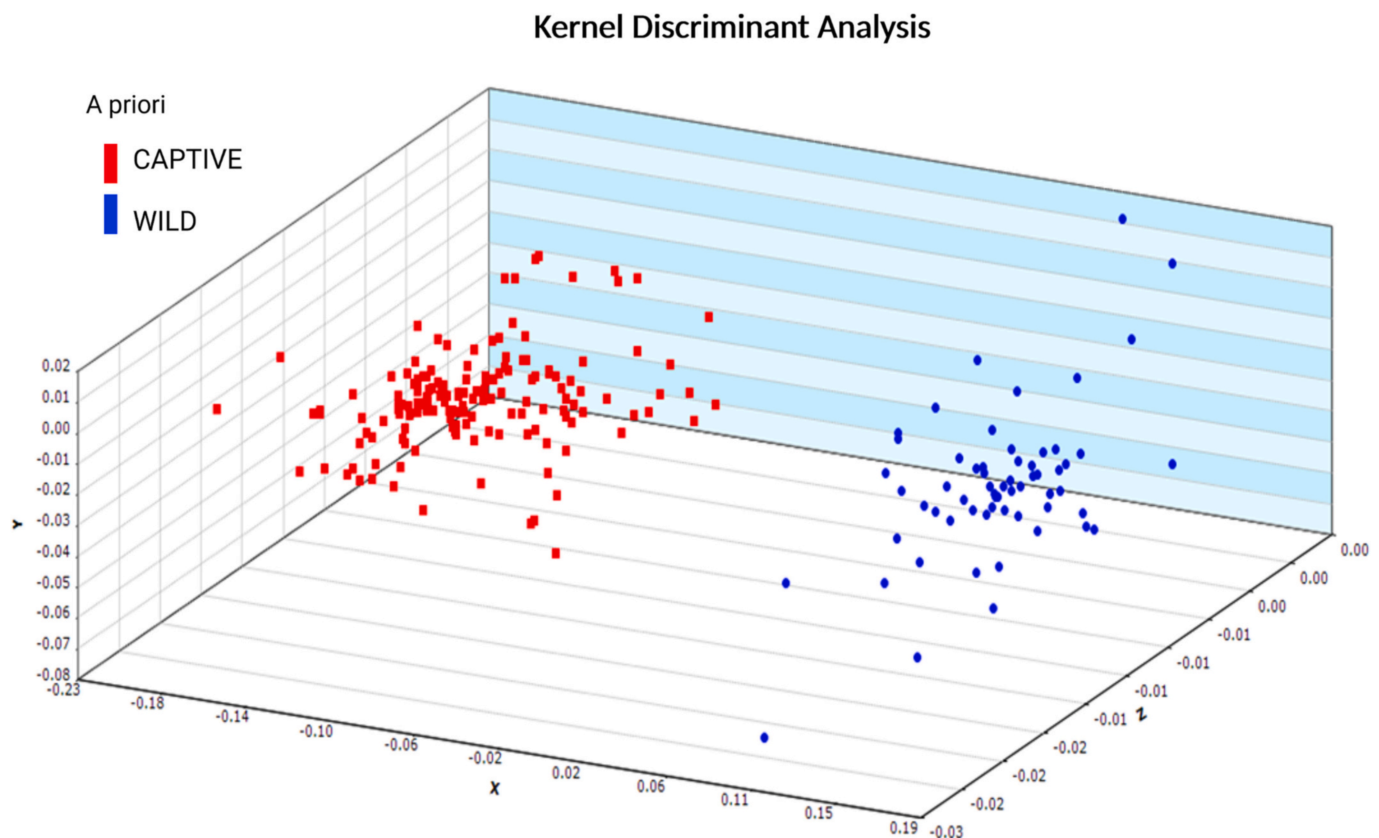


**Fig. 4.** Example of fatty acids analyses. Differentiation of caviar from wild and farmed origin using KDA Kernel discriminant analysis of the fatty acid chemical fingerprint from the DART-TOF analysis performed on 54 samples, 38 of farmed and 16 of wild origin. All analyses were performed 4 times and results are independently reported in the graph. (Created with BioRender.com).

that have different masses, due to the variable numbers of neutrons in the core of the atom. In nature, different patterns of these isotopes occur in the tissue samples depending on geographic origin. These geographical patterns of isotopes composition can be used to trace the geographical origin of a sample through Isotope Ratio Mass Spectrometers (IRMS), allowing to verify the authenticity of materials (Primrose et al., 2010). There is a wide array of publications available on their use to determine the provenance of various products such as agricultural and animal products (see Divelos and Constantinos, 2012 for review).

The remarkable sensitivity of the method also allows for depicting temporal variations within individuals if applied to continuously growing tissues, for example to study animal migration patterns (Hobson, 1999). It was also applied to investigate interspecific trophic relationships and aquatic food webs (Middleburg, 2014). Given the great geographic variability in isotopic patterns in water (Bowen and Wilkinson, 2002), water isotopes are largely used to assess the geographical origin of organisms. In fact, the location-dependent water isotope signatures are reflected in the tissue water of plants and animals (Boner and Förstel, 2004) and in their products (Lesley et al., 2010). Most studies using stable isotopes for authentication purposes focus on terrestrial plants and animals. This is due to the fact that the first applications focused on the analysis of hydrogen and oxygen which have informative patterns in freshwater. In fact, $^{2}H/^{1}H$ and $^{18}O/^{16}O$ stable isotope ratios have been successfully used to verify the provenance of freshwater fish (Soto et al., 2016). The sea water shows reduced variability of these elements world-wide and their use is therefore less effective in sea organisms. As most commercial caviar is obtained from animals reared in freshwater aquaculture, the stable isotopes of oxygen and hydrogen can be applied to it. The comparison performed on >50 reference samples from 9 different countries is shown in Fig. 5A, revealing a good distinction among caviar from different areas.

Further information can be obtained from the analyses of stable strontium isotopes ($^{87}Sr/^{86}Sr$) which have been successfully used to track the origin of salmon (Barnett-Johnson et al., 2008). Unfortunately,

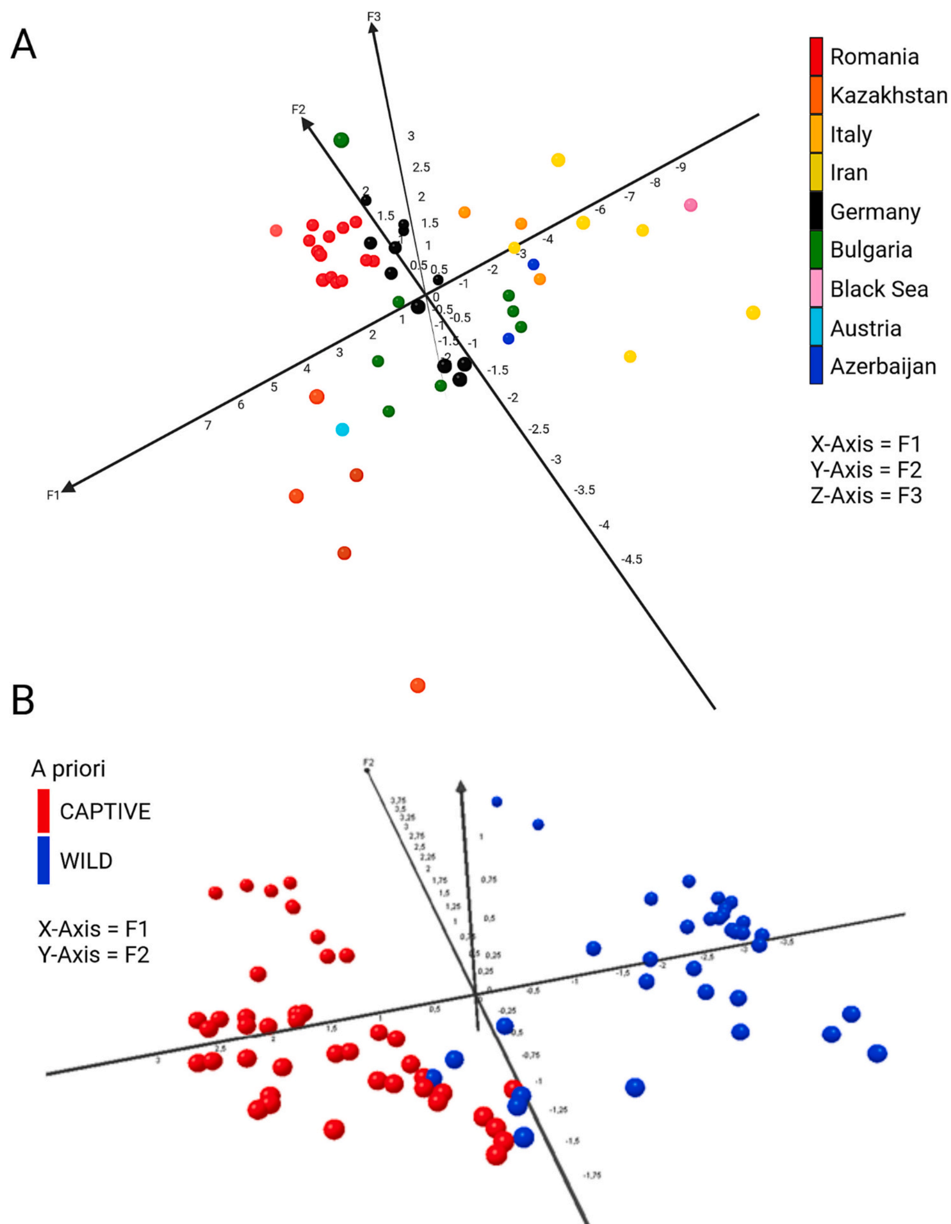


**Fig. 5.** Example of stable isotope ratio analysis (SIRA) A. Agroisolab database of sturgeon caviar: $\delta^{2}H$ and $\delta^{18}O$ isotopic value (v.s. Vienna Standard Mean Ocean Water) of the tissue water and the organic fraction. B. PCA-Differentiation of caviar from wild / farmed origin using $\delta^{34}S$, $\delta^{15}N$ and $\delta^{13}C$ isotopic values. (Created with BioRender.com).

strontium isotopes are highly affected by the processing of sturgeon and caviar (e.g., salt effect). Nevertheless, new models have been developed to minimize the processing effect and finally to link the sturgeon sample with the origin (Tchaikovsky et al., 2019). Further stable isotopes of other bio elements such as carbon, nitrogen, and sulphur measured in animal tissue reflect indirectly the origin of the animals and of their products. For instance, carbon and nitrogen isotope ratios allowed to differentiate salmon of wild, organic, and conventional-farmed origin (Moltekin et al., 2007) and shrimps from wild-caught and aquaculture production systems (Gamboa-Delgado et al., 2014). Unfortunately, these elements seem to have a limited potential for sturgeon identification with respect to the sturgeon farming system probably due to the complexity of sturgeon feed, as well as the varying feeding conditions. However, their combination with sulphur isotopes, even if still rarely used in aquatic studies (Hesslein, 1991; Kiyashlo et al., 2011), results in a high discrimination power between wild and farmed origin (Fig. 5B). Preliminary results based on 76 reference samples of caviar (34 wild and 42 from aquaculture) Analyzed by Agroisolab (Jülich, Germany), showed that the current separation probability using discriminate analysis (DA) by $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S of wild and farm caviar is over 93%.

### 3.3. Framing pros and cons of isotope utilization for forensic analysis

To conclude, stable isotopes deliver a wide range of information about the geographical origin and the production method (wild/farm). Although a routine implementation of stable isotope analyses remains challenging and expensive, it is at the moment the leading standard method for authentication due to its potential for universal application to a broad range of products and has also been accepted in various court cases as an analytical proof (Camin, 2017).

The main restriction in application is that stable isotope technology is discriminative only if the diet of fish in the wild is different to commercial feed used in aquaculture. Therefore, it is important to keep in mind that the method cannot actually discriminate wild-caught from captive-bred sturgeon but rather sturgeon fed with natural versus commercial feed (e.g. American Paddlefish from an aquaculture facility in Ukraine was determined as wild-caught as it feeds on natural food only).

Finally, it should be stressed that the stable isotope applications require dedicated reference databases: the first made up by geolocated samples for the identification of the geographical origin, and the second with known wild and farmed samples for the distinction of the two origins in unknown individuals.

## 4. Implications for conservation

Methods that allow distinguishing wild and farmed animals have direct implications for conservation as they provide the possibility to detect illegal trade, or the illegal exploitation of natural populations and stable isotope methods have already been accepted as analytical proof in court cases (e.g. in Germany).

Furthermore, the possibility of correctly identifying sturgeon species and hybrids has a great conservation value. Most sturgeon populations are on the verge of extinction and many conservation programs include the support of restocking programs. The animals used for restocking are often produced in captivity through controlled reproduction. In many cases, the broodstock originates from aquaculture facilities. These fish should be checked genetically to assess their relatedness and their purity in terms of the species they belong to and the population of origin. Given the thriving production of different kinds of hybrids and exotic sturgeon species in aquaculture and since animals descending from a hybridization followed by different rounds of backcrosses cannot be morphologically distinguished from pure ones, the probability of accidental inclusion of impure individuals in the broodstock and the resulting risk of massive genetic pollution of already reduced natural populations is significant (Boscari et al., 2014b; Congiu et al., 2011). With the aim of selecting individuals whose purity is confirmed at as many loci as possible, the application of whole genome genotyping approaches is increasingly used. An enormous amount of information can be collected by these approaches dramatically increases the chances of detecting informative loci on which developing multilocus diagnostic tests of rapid and economic applicability. This is the direction that will be followed in the coming years for the development of diagnostic markers in sturgeons.

## 5. Conclusion

The illegal trade in sturgeons is continuously putting remaining wild populations at risk.

CITES listing of sturgeons in 1997 and the subsequent mandatory labeling of caviar in international trade provided a first set of tools to increase control of illegal trade. Despite this, recent investigations (Jahrl et al., 2021) show that illegal trade still comprises significant proportions of the commercial utilization of sturgeons on domestic markets, including poached products, fraud, as well as consumer deceit.

Improved regulations allowing the clear discrimination of legality of products and a consistent labelling technique must be put in place also on domestic markets, supported by systematic controls of the commodities.

The available methods for these controls have been outlined in this paper to provide a sound guidance on the information to be gained by these analyses. To make them effective tools to stop illegal trade in all its forms, national reference laboratories should be trained and intercalibrated to deliver meaningful and jurisdictionally sound results acceptable by courts.

In addition, more communication and information efforts are necessary by public bodies but also by caviar producers to allow the consumers to make educated decisions, being aware about the conservation relevance of their behavior.

## CRediT authorship contribution statement

**Leonardo Congiu:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Beate Striebel-Greiter:** Conceptualization, Writing – review & editing. **Joern Gessner:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Elisa Boscari:** Investigation, Writing – original draft, Writing – review & editing. **Markus Boner:** Investigation, Writing – original draft, Writing – review & editing. **Jutta Jahrl:** Conceptualization, Writing – review & editing. **Stefano Dalle Palle:** Formal analysis, Writing – review & editing. **Arne Ludwig:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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