The following table provides an overview on the different methods described in the study *Identification of species and hybrids, source and geographical origin of sturgeon and paddlefish (Acipenseriformes spp.) specimens and products in trade* (SC74 Doc. 47). It explains the scope of each method (which forensic technique can be used to identify the sturgeon species or hybrid, its relatedness, its geographic origin or the production methods (wild caught versus farmed)). It provides advantages and restrictions, estimates on costs and technical capacity needed.

Summary of forensic methods, their advantages, restrictions and costs implications

Method	Application for sturgeon identification (scope)	Advantages (in general or in comparison)	Restrictions/ limitations	Cost / sample	Equipment needed + associated costs	Required skills of personnel	Comments
Stable isotope composition analysis	Verification of geographic origin: stable isotopes of hydrogen and oxygen are used to determine geographic provenance in freshwater fish. Verification of production method wild caught versus farmed: Nitrogen and Carbon isotopes to determine wild versus farmed origin; Sulphur has highest diagnostic power for discrimination wild versus farm .	Already tested and applied for caviar and sturgeon meat Accepted as analytical proof in court cases (e.g. in Germany, Europe*)	Stable isotope analysis of seizures/samples is only available in a few laboratories world-wide. Results take 5-10 working days; rush samples (48hrs) are highly challenging; Discrimination wild caught versus farmed actually identifies the feed, not the fish itself	Price for analysis of 1 sample remains expensive (100-400 €)	EA (Elementanalyser) in combination with IRMS (Isotopic Ratio Mass Spectrometry) Manufacturer: Thermo Finigan, Elementar, Nu Instruments IRMS: 100.000 -> 140.000 € EA: 40.000 to 80.000 €	Analysis requires high degree of expertise.	Currently the leading standard method for authentication due to its application to a wider range of other products (food, Ivory, timber, ..). Several official food laboratories in Europe are using IRMS to detect fraud samples in the market. e.g. Germany: 6 official labs. Stable isotopes are recommended in European guidelines to track the origin (e.g. timber). Furthermore UNODC guidelines like ivory, timber note IRMS for origin tracking.
Fatty acid profiling	Distinction of production method (wild versus framed) depends upon feed and feed composition	Standardised Gas Chromatography method.	Fatty acid composition of eggs or tissue depends on the residuals of feed composition, can cause unclear results in extensive rearing on natural diets.); Species specific variability in composition not fully	Depending upon lab specialization and throughput	Specialized equipment (HPLC, GCMS) needed, medium expensive 30,000-80,000€	Trained personnel with experience in the method required, intercalibration between labs useful, harmonization of methods required	Still research needed, which additional fatty acids could be relevant

			known; Can be manipulated by addition of FA mix after processing; Sensitive to decay during storage.				
	DART-TOF-MS Direct Analysis in Real Time (DART), coupled with a time-of-flight (TOF) mass spectrometer (MS)	Almost no sample preparation is required to obtain the chemical profile of a suitable sample and it can be obtained within seconds	See above	Lower costs per sample (20-40 €)	High investment costs (approx. 250.000.- € for laboratory system (DART MS)	Analytical chemistry	DART-MS was successfully applied for the first-time to analyse the profile of fat extract from caviar. Routinely used to verify the species of timber in USA
Overview of DNA analyses	<u>Determination of sturgeon species (populations, hatchery stocks, individuals)</u>	Genetic code is uniform; DNA can be extracted from all types of tissues of living specimen even processed caviar and sturgeon meat; DNA is thermally stable (processed samples); only very small samples are needed; results are reliable and verifiable between laboratories	Calibration between labs is recommended for nuclear DNA; References are available in GeneBank	Costs are rather low (depending on the question of interest between 20-100€)	High investment costs (approx. 250.000.- € for laboratory system (Sanger or NGS sequencer; PCR equipment)	Advanced knowledge of molecular genetic methods; trace lab experience	Routinely used to verify the species of origin and hybrid status since years; Used in species identification not only in sturgeon worldwide; Huge panel of experience
Mitochondrial DNA analysis	<u>Determination of the maternal sturgeon species</u> from tissue of live specimen, or processed samples (meat/caviar)	Complete reference mitochondrial genomes are available for 22 out of 27 sturgeon and paddlefish species; Cheap, fast and reliable; Produces often results even in samples of poor quality; Worldwide accepted method by court	DNA sequencing needs good and reliable reference databases: No complete reference genomes have been archived for two important caviar producers NAC and PER; it is not possible to distinguish GUE and PER based on genetic markers ;	low costs 10-20€	High investment costs (approx. 250.000.- € for laboratory system (Sanger or NGS sequencer; PCR equipment) Notable, equipment is the same in any of genetic methods.	Advanced knowledge of molecular genetic methods; trace lab experience	Prime standard of species identification in trade control not only in sturgeon and paddlefish; Diagnostic reference sequences of all sturgeon and paddlefish species are available in public data bases

			hybrids and introgressed species (historical hybridisation) cannot be identified based on mDNA only				
Nuclear DNA markers 2 classes of markers that can be used individually or in combination:	Determination of the sturgeon species (both parental species) Used to recognize the genetic contribution of both parents such as relatedness analyses (strain identification) or detection of hybrids	The possibility to characterize paternal contribution allows to test for hybridization and for relatedness, not feasible with mitochondrial DNA only	Limited number of nuclear markers available but their number increases fast	Cost depends on complexity of hybrids (2 or more species involved; back crosses with paternal species etc.) but ranges between 20-100 €)	High investment costs (approx. 250.000.- € for laboratory system (Sanger or NGS sequencer; PCR equipment)	Advanced knowledge of molecular genetic methods; trace lab experience	Frequent hybridisation in sturgeon species opened new frontiers to commercial frauds in which, for instance, the less valuable hybrid caviar is sold as product obtained from the pure maternal species, in order to cheat standard controls exclusively based on mtDNA. By the same time, caviar from poached sturgeon is offered as “hybrid” blurring the origin.
Microsatellites	Markers of genetic differentiation at different levels of diversity (relatedness, population analyses, and species identification, hybrid detection)	High variability (number of alleles per locus) makes microsatellites very informative for the study of genetic diversity; Sturgeons have different levels of ploidy. These differences can be used for species/hybrid detection based on microsatellites.	Lower comparability of results between different laboratories Intercalibration highly recommended; misidentification due to allelic dropout; null alleles or wrong allele dosage estimation	20-50 €	High investment costs (approx. 250.000.- € for laboratory system (Sanger or NGS sequencer; PCR equipment)	Advanced knowledge of molecular genetic methods; trace lab experience	Should not be used as stand-alone method for species identification but produces valuable data for hybrid detection and for validation of mt and SNP analyses. All laboratories relying on this approach should include some reference samples with known genotype in their sample set.

Single Nucleotide Polymorphisms (SNPs)	Prime standard for hybrid detection but also useful at different levels of diversity, (relatedness, population analyses, and species identification)	Advent of massive sequencing methods allow simultaneous analysis of hundreds of samples good transferability and replicability among laboratories	As regards their applicability in routine forensic analyses, SNPs with diagnostic power ("private" polymorphisms) must be identified in order to then develop fast and cheap single locus analyses	20 – 100 € depending on number of SNPs	High investment costs (approx. 250.000.- € for laboratory system (Sanger or NGS sequencer; PCR equipment)	Advanced knowledge of molecular genetic methods; trace lab experience	Gains increasing importance because Next-Generation Sequencing techniques produce tons of SNPs annually; Validation of private SNPs difficult sometimes because of secure reference samples
Morpho-logical identification	Identification of sturgeon species	Easy, low-cost and time efficient; can be applied if sturgeons are encountered on a vessel, on the bank of a river, in a farm or in trade; Mainly applicable for intact fish	Not reliable for parts of fish; not suitable for meat or caviar Only reliable for specimen older than 45 days, difficult for some species (e.g. Beluga sturgeon) until sexual maturity Morphometric and some morphological characteristics can be difficult to be obtained in live animals or are meaningful only in statistical comparison. Therefore, a larger number of individuals of the same species is ideal to safely determine species; Identification of hybrids occurring in nature and in aquaculture is complicated	Only personal salary	Measuring tape, sliding calliper, magnifying glass, stereo photography	The identification of a sturgeon species by morphology, even under optimal conditions, and especially of hybrids requires an experienced specialist	CITES ID Guide in EN, FR, ES (EEEC) and UA (WWF) Only reliable for adult fish; Entire fish is necessary Not useful for hybrids because variation of morphological traits in hybrids is unknown

	Wild caught versus farmed: discrimination due to typical malformations that are encountered regularly in farmed individuals		Malformation may but do not have to occur in aquaculture and can only serve as a hint but no proof				Unreliable, limited to the utilization as a pre-screening