Parentage assignment in the critically endangered European sturgeon (Acipenser sturio) based on a novel microsatellite multiplex assay: a valuable resource for restocking, monitoring and conservation programs

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Author-produced version of the article published in Conservation Genetics Resources, 2016, 8, 313-322
The original publication is available at http://link.springer.com
doi : 10.1007/s12686-016-0538-7

Parentage assignment in the critically endangered European sturgeon (*Acipenser sturio*) based on a novel microsatellite multiplex assay: a valuable resource for restocking, monitoring and conservation programs

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Author-produced version of the article published in Conservation Genetics Resources, 2016, 8, 313-322
The original publication is available at http://link.springer.com
doi : 10.1007/s12686-016-0538-7

SUMMARY: The only remaining population of the critically endangered European sturgeon, *Acipenser sturio,* is located in the Gironde basin (France). A restoration program initiated 20 years ago has allowed more than one and a half million individuals to be stocked. Effective monitoring of this population is a key prerequisite in ensuring the sustainability of this species in the wild. We report the development of a novel microsatellite multiplex assay for genetic monitoring of *A. sturio*. Diversity of a set of 18 loci was low to moderate, with a number of alleles and observed heterozygosity ranging from 4 to 7 and 0.33 to 0.74 respectively, depending on markers. A set of captive-born progeny of known relatives (n=72) was used to examine the efficiency of this assay in assigning parentage to offspring. Three different programs were used. Correct assignment success was generally high (above 90%), but differed between programs. Parentage analysis of individuals captured in the Gironde estuary (n=193) demonstrated that most offspring (91.2%) are unambiguously allocated to parent pairs from the broodstock. Our research provides an efficient and accurate method for the genetic monitoring of the restocking program, but also for others aspects of conservation, including genetic diversity evaluation, effective population size estimation, and inbreeding assessment.

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INTRODUCTION

Sturgeons are among the most threatened groups of fish in the world, with most species at risk of extinction (Rochard et al. 1990, Birstein 1993 and IUCN 2015). Because of some life characteristics (late age at maturity, diadromous life cycle for some species), the conservation of many sturgeon species has relied on long-term breeding and restocking programs – generally over several decades (Carmona et al. 2011, Williot 2011). The genetic monitoring of such species, also characterized by a large number of progeny, therefore depends upon time and cost-effective protocols for routine screening. There is currently a captive breeding stock of European sturgeon (*Acipenser sturio*) located at the Irstea experimental station in Saint-Seurin-sur-l'Isle, France. This stock, first created in 1994, is made up of wild-born adults and juveniles. Its aim is to secure the survival of the species and provide a basis for subsequent releases into the wild (Williot et al. 2007).

The first individuals from this stock were released back into the wild in 1995 (Williot et al. 2002) with further releases taking place 12 years later. Since then, more than one and a half million larvae and juveniles have been released into the Gironde basin. One priority of the restoration program is to characterize the estuarine portion of the population and estimate the efficiency of restocking by monitoring the population in the Gironde estuary (Rochard et al. 2001, Lochet et al. 2004, Acolas et al. 2011). Because the probability of *A. sturio* reproduction in the wild has been low since 1994, an almost closed system is expected, in which most of the captured individuals would belong to the captive-born stock. Since 2007, restocking plans have been devised whereby the progeny of unique parent pairs that are released into the wild at different stages (i.e. larvae, juvenile) and in different locations (Gironde, Dordogne) can be identified. This plan also allows the survival of these different offspring to be assessed based on these criteria. The development of genetic tagging is necessary to provide unambiguous identification of supplemental fish, and to evaluate survival and/or reproductive success in *A. sturio*.

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Of the many different methods used for fish tagging, genetic monitoring would appear to be the most efficient for young stages. This is because it is not constrained by issues of size, as is the case with transponders and external devices (Feldheim et al. 2002, DeHaan et al. 2007). It also has an advantage over mass chemical markers, the effectiveness of which are also reduced when applied to very young sturgeons (Lochet et al. 2011).

Genetic tagging using microsatellites consists of creating a genetic profile for each captured individual, allowing them to be assigned to a given population or parental pair (Lukacs & Burnham 2005). This approach is popular in the management of conservation programs involving aquatic organisms (Hansen et al. 2001, Liu & Cordes 2004, Moghim et al. 2013, Abdul-Muneer 2014). For example, it is often used to analyze the proportion of wild-born individuals and captive-born individuals in the natural environment (Poteaux et al. 1999, Bravington & Ward 2004, Meraner et al. 2014). Microsatellite markers are also powerful tools in captive management (O'Reilly & Kozfkay 2014) because they enable the genetic characterization of captive broodstock (Koljonen et al. 2002, Machado-Schiaffino et al. 2007, Cooper et al. 2009), as well as guiding mating strategies based on relatedness (Kozfkay et al. 2007, Nielsen et al. 2007, O'Reilly & Kozfkay 2014), inbreeding levels (Borrell et al. 2007), and breeding success (e.g., McLean et al. 2007, Hoskin et al. 2015, Sard et al. 2015).

The number of markers needed to obtain reliable results will depend upon the genetic variability of the species studied and the taxonomic level or geographic scale of the analysis required (Frankham et al. 2002, Wan et al. 2004). Restocking and conservation programs generally focus on species characterized by population declines and low genetic diversity. Relatively large numbers of microsatellites are often necessary to gain sufficient reliability and statistical power. Multiplex assays (i.e. the amplification of several microsatellite loci in single PCRs) are therefore relevant for the development of standardized screening protocols for cost-effective genetic analyses (Neff et al. 2000, Renshaw et al. 2006, Morvezen et al. 2013, Panagiotopoulou et al. 2014). Numerous microsatellites have already been developed and used to study wild and captive populations of sturgeon species, for conservation or in commercial breeding programs (May et al. 1997, McQuown et al. 2000, King et al. 2001,

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Henderson-Arzapalo & King 2002, Zane et al. 2002, Welsh et al. 2003, Zhu et al. 2005, Börk et al. 2007, Forlani et al. 2007, Fopp-Bayat & Woznicki 2008, Waldman et al. 2008, Dudu et al. 2011, Moghim et al. 2012, Wozney et al. 2012, Moghim et al. 2013, Zeng et al. 2013, Georgescu et al. 2014, Liu et al. 2014, Panagiotopoulou et al. 2014, Wirgin et al. 2015).

For *A. sturio*, there are currently no available specific markers and no standardized multiplex protocols. Only a handful of preliminary studies have described species diversity based on non-specific (Ludwig et al. 2004, Ludwig 2005, Williot et al. 2007, Chassaing 2010, Berrebi & Cherbonnel 2011, Chassaing et al. 2011) or unpublished (Tiedemann et al. 2011) markers. One of the characteristics of the last remaining population of this species is its low genetic diversity (Chassaing 2010), which makes it difficult to design reliable protocols with sufficient resolution power.

In this paper, we present a method based on three multiplexes totaling 18 polymorphic microsatellite loci for its use in the conservation and restocking programs of *A. sturio*. Specifically, the aims of the present study were to (1) Develop and validate an optimized multiplex protocol as a standard genotyping tool for individual genetic tagging (2) Test our assay on 193 individuals captured in the Gironde Estuary (South West of France) in order to assess the feasibility of our methodology in real monitoring condition, and (3) Discuss the usefulness of our assay for the genetic monitoring of both the captive breeding and restocking programs for the long term management of the species.

MATERIALS AND METHODS

Biological material and DNA extraction

We sampled a total of 109 *A. sturio* from the captive broodstock (BROODSTOCK) (N= 19 males and 18 females) composed of 33 wild adults and juveniles captured during the 1970-1994 period and 4 adults from the first cohort born in captivity in 1995, and 72 captive progeny (FARM) issued from 30 known parents for the validation of parentage testing. Additionally, 193 individuals, obtained by trawling in the Gironde estuary (Aquitaine, France) during population monitoring campaigns (2009-2014) were sampled (CAPTURED) (Acolas et

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al. 2011). These captured individuals (juveniles and adults of length between 40-130 cm; mean=70 cm) may come either from captive cohorts of the restocking program that involve a total of 27 known mating pairs between 2007 and 2014, or from reproduction events in the wild. For all individuals, a piece of fin was individually collected and preserved in 95% ethanol.

Selection and test of sturgeon microsatellites in *A. sturio*

DNA extraction was performed using the Chelex extraction method (Walsh et al. 1991).

Because there were no available microsatellite markers specifically developed for *A. sturio*, a total of 118 microsatellite sequences developed for other sturgeon species were screened in the European sturgeon *(see Appendix 1)*. Firstly, simplex PCR amplifications were tested on 24 individuals in a 25 µL reaction volume composed of 0.5 units of Promega Taq polymerase, 1.5 mM $MgCl_2$, 0.2 mM of each deoxyribonucleotide, 1 X buffer and 12.5 pmol of each primer. PCR was performed for all loci with the following thermocycling regime: 95◦C for 5 min, then (95◦C 40 s, 54◦C 40 s and 72◦C 40 s) × 34 cycles, and 72◦C for 10 min. Amplification success was checked by running PCR products on an agarose gel. Loci which successfully amplified were then tested with the same PCR conditions. Forward primers were labelled with a fluorescent dye (6-FAM™, Cy3, NED™ or TET) in simplex or duplex reactions. The PCR products were separated on a denaturing 6% polyacrylamide gel and then scanned on a fluorescence image analyzer, FMBIO II Multi-View (Hitachi Software, Tokyo, Japan). A total of 34 loci *(see Supplementary material S1)* were selected and tested in Multiplex reactions on 24 individuals. PCR amplifications were carried out with the Type-it Microsatellite PCR Kit (Qiagen™) in a 10.5µl reaction volume containing 6.25 µl of Type-it Multiplex PCR Master Mix, 1.25 µl primer mix (each primer at 2 µM) with forward primers labelled at 5' end using fluorescent dyes (6-FAM™, HEX™ and NED™), 3 µl RNase-free water and 4 µl Chelex DNA extraction. Thermal cycling profile followed manufacturer conditions (Qiagen™) and PCR products were separated on a capillary sequencer (either ABI 3130 XL or ABI 3500 XL, Applied Biosystem™). Fragment lengths were assessed with Peak Scanner v1.0 and GeneMapper v4.0 softwares (Life Technologies ™). Selection of the best quality markers was done first, discarding those with more than two alleles per individual or with very low variability. To reduce the probability of genotyping errors, we prioritized those with high

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reliability, clear patterns of the expected range, and high signal intensity. The remaining 18 loci were amplified in three multiplex (MLPX1, MLPX2 and MLPX3; see Table 1).

Statistical analysis and optimization of a microsatellite multiplex assay

Basic genetic parameters including allele frequencies, numbers of alleles per locus (A) and observed (Ho) and expected (He) heterozygosity were calculated for BROODSTOCK individuals using CERVUS v.3.0 (Kalinowski et al. 2007). Linkage disequilibrium between pairs of loci was tested with GENEPOP 4.0 (Rousset 2008) using the Markov chain method (10 000 dememorization steps, 100 batches, 5000 iterations) and Fisher's exact test; a sequential Bonferroni correction for multiple testing was applied (Rice 1989). Null allele frequency estimates were computed using CERVUS 3.0. The success of amplification was calculated as the percentage of positive PCR for each locus over the whole sample. The 18 selected loci were classified in increasing order of probability of identity (PID) (Waits et al. 2001) which is a good predictor of the true probability of correct parentage assignment. PID values were calculated using the GIMLET software (Valière 2002) under "sibs" and "unrelated" relatedness scenarios. The polymorphic information Content (PIC) for each locus, as well as the combined non-exclusion probabilities over loci, for first parent (NE-1P), second one (NE-2P), parent pair (NE-PP), unrelated individuals (NE-unrel) or siblings (NE-sibs) were calculated using CERVUS v.3.0 (Kalinowski et al. 2007).

Parentage testing and applicability for the restocking program

To validate the best parentage assignment protocol, our microsatellite multiplex assay was first tested on 72 progeny of known parents (FARM) with three commonly used programs that perform different algorithms: CERVUS v3.0 (Marshall et al. 1998, Kalinowski et al. 2007); PASOS v1.0 (Duchesne et al. 2005) and PAPA 2.0 (Duchesne et al. 2002). CERVUS and PASOS allow for incomplete parental sampling (i.e. open systems) while PAPA is better designed for parentage assignment in closed systems (i.e. all parents are sampled). To select the minimum number of loci necessary for reliable assignment, all three programs were tested using several sets of an increasing number of markers, classified in the order of PID values.

CERVUS uses a likelihood-based approach to assign parental origin combined with simulation of parentage analysis to determine the confidence of parentage assignments. Likelihood

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score ratios (LOD or Delta) estimate the likelihood that the candidate parent is the true parent divided by the likelihood that the candidate parent is not the true parent. Before proceeding to the parentage assignment, simulations were run in CERVUS to determine the distribution of the critical values of Delta or LOD score for 80% and 95% confidence levels. The following simulation parameters for 100,000 offspring were chosen for our studied organism: numbers of candidate parents (BROODSTOCK) incremented by a 10-15% corresponding to putative non-sampled parents (i.e. 20 candidate mothers and 22 candidate fathers), 10% of candidate parents with a relatedness coefficient of 0.1, 98% of candidate parents sampled, 99% loci typed with a 1% error rate. Confidence levels obtained from simulations were used for true paternity screening of the 72 progeny (FARM). In PASOS and PAPA, likelihoods are calculated for each potential parental pair and the allocation of a descendant is based on the search for the most likely pair among all potential pairs of known parents. For the analysis with those two programs, we allowed one locus genotype mismatch between parents and offspring (i.e. offset=1) as suggested by the author (P. Duchesne, Personal Communication). Assignments of FARM samples were compared to the true parent's pairs, which was defined as true assignment (i.e. TRUE). The difference between both assignments is considered as the error rate associated with the method. Assignment success, true assignment, and error rates were also compared among the three programs.

To test the feasibility of our assay in tracking the parental origins of cohorts released into the wild, parentage analysis was conducted on 193 individuals captured in the Gironde Estuary (CAPTURED).

RESULTS

Microsatellite variability

Of the 118 primer pairs tested, 80 (68%) gave an amplification product, and among those, 33 (41%) were polymorphic (i.e. two or more alleles per locus) (*Appendix 1*). To decrease the putative error rate associated with allele scoring, we decided to exclude 15 loci based on unclears signal on the capillary sequencer (AfuG113, AoxB34, AoxD241, AciG198, Afu19, and

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AoxD297), or low reliability (AfuG184, Ag16). Other seven markers were omitted because of their reduced variability (Afu54, Ag20, Aox23, Aox27, AoxD54, AS002, Spl101). Statistics for the 18 selected loci are given in Table 1. No overlapping of allele size ranges was observed within any of the three multiplexes and the amplification success was high for all loci (Table 1). No significant linkage disequilibrium was detected between pairs of markers after Bonferroni corrections. Overall polymorphism was moderate to low, with the number of alleles (Na) varying from two (Ag39) to seven (Ag10) alleles per locus, He from 0.46 to 0.90 and Ho from 0.33 to 0.74. Null allele frequency was displayed for a single marker, but at a very low rate (AoxD188; ≤ 0.05). PIC values varied between loci, between 0.37 and 0.77. Although the mean number of alleles per locus was low (n=4), the potential of these 18 loci used in combination to distinguish individuals was high, as indicated by the low cumulative probability of identity (PID) over loci of respectively of 5E-12 or 1E-05, assuming all individuals are unrelated (PID-unrel) or siblings (PID-sibs). Values of non-exclusion probabilities for unrelated (NE-unrel) and siblings (NE-sibs) were also low, at 1.18E-12 and 4.62E-06, respectively, as well as the combined non-exclusion probabilities for the first parent, second parent and parent pairs of 1.6E-02, 4.9E-05 and 2.5E-06, respectively. Amplification success was high for all loci (Table 1).

Validation of parentage assignment in *A. sturio*

Simulations in CERVUS result in high assignment rates, with 98% assignments of parent pairs at both strict (95%) and relaxed (80%) levels. Only 2% of simulated offspring remained unassigned. Individual genetic tagging was successful in identifying the parental origins of most individuals originating from the breeding program (FARM). The parentage assignments of these offspring using the three methods (CERVUS, PASOS, and PAPA) are shown in Figure 1. Overall, assignment success was high and increased as the number of loci used went up, but for all programs the highest level of assignment success was obtained for 17 loci (Figure 1A). For FARM, parentage analysis using CERVUS generated 100% parent pair assignments (Figure 1B). The results of assignment tests in CERVUS showed that most individuals (95.8%) were assigned to the parent pair matching the parent database information (i.e. named TRUE assignment) at high (strict, 95%) or lower (relaxed, 80%) level of confidence, but that a few individuals (N=3 out of 72, 4.2%) were wrongly assigned. Using PASOS, 93.1 % of offspring were unambiguously assigned to their true parents (N=67 out of 72), while four

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(5.5%) had a parentage assignment corresponding to “uncollected” mother or father (Duchesne et al. 2005) and one was assigned to wrong parental pair (i.e. 1.4%). PAPA gave 65 correct assignments (90.3 %) (N=65 out of 72) and 2 wrong assignments (2.7%), while the assignment of the remaining individuals was ambiguous (i.e. the highest likelihood is shared by several parental pairs, see Duchesne et al. 2002), and is therefore the least powerful program in parentage testing for *A. sturio*. These results indicate that error rates associated with the methodology (i.e. the difference between assignment and TRUE assignment) are low to moderate and range from 9.7% to 4.2%, depending on the program used.

Genetic monitoring of the stocked population

The results of analyses of the captured individuals (CAPTURED, N=193) were compared between CERVUS and PASOS, the two most powerful programs. Parentage testing of CAPTURED using CERVUS generated 99 % parent pair assignments. A single juvenile was not assigned with a sufficient level of confidence (<80 % confidence). Using PASOS, assignment success for parent pairs was 96%. Eight individuals were assigned to one unsampled father or mother. When successful assignments were compared to the 23 known mating pairs from the breeding program, 17 individuals (8.8%) and 18 individuals (9.3%) were assigned to non-existing parental pairs, for CERVUS and PASOS, respectively. Among those, 15 individuals were common among both programs. These 15 assignments (7.8 %) might be the result of errors associated with the method (estimated above between 6.9% and 4.2%), as well as a possible wild origin.

DISCUSSION

Parentage assignment in endangered species – which are generally genetically impoverished – calls for a difficult balance to be struck between having enough polymorphic markers to provide the necessary resolution power and managing the time and expense associated with prolonged laboratory work (Harrison et al. 2013). In this study, we aimed to test and estimate a minimum number of loci for accurate parental assignment in *A. sturio* and optimize a multiplexing approach for routine screening. Because no specific markers were available for the species, we tested more than a hundred markers developed for other sturgeon species (i.e. "crosspriming"). We found that more than 40% of the markers were

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polymorphic and that successful crosspriming concerned markers developed for either very close (i.e. *A. oxyrinchus*) or more distant (*A. gueldenstaedtii*) species (Appendix 1). Crosspriming has proved to be effective among sturgeon species (Rajkov et al. 2014) and its use has been largely extended in previous studies (May et al. 1997, King et al. 2001, Moghim et al. 2012).

Assignment success with microsatellites also relies on accurate genotyping. The high mutation rate of microsatellites is often associated with scoring errors (Pompanon et al. 2005), as well as frequent mismatches of offspring genotypes with those of their parents, which may lead to incorrect assignment and this will even increase while increasing the number of markers used. Generally, the number of typed loci should be chosen as a compromise between the probability of identity (more resolution) and the probability of error (loss of correct assignment). This stresses the need for the technique to be optimized and validated prior to its application to ensure accuracy in parent-offspring assignments (Nielsen et al. 2001). Although our results support the previous documented low genetic diversity for *A. sturio* (Chassaing et al. 2010, 2016), the final optimized assay of three multiplexes allowed efficient and reliable amplification of 18 loci with PID and non-exclusion probabilities sufficiently low to assure proper individual and parental identification. Our multiplex assay was successful in identifying the parents of a set of 72 progeny, whose parental origin was known (Figure 1). The analysis of cumulative loci revealed that 17 loci of the 18 were required in order to meet the maximum assignment rate. This has been observed elsewhere for individual assignment (Roques et al. 1999) and illustrates that adding extra loci may increase the proportion of erroneous genotypes, thus leading to a larger proportion of incorrect allocations. This also pointed out that Aox45 might be a poorly segregating locus. Considering the best assignment obtained with CERVUS, only three individuals were assigned to the wrong parental pairs (i.e. 4.2%), which is a satisfying result given the very low genetic diversity of the species, some level of relatedness among parents and the high probability of scoring errors. This value could be considered the maximum error rate associated with the present genetic assay (i.e. corresponding to laboratory, sample handling, or data transcription's errors) and should be taken into account in future analyses. These results support the potential of these programs to establish parent-offspring

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313 relationships even when some genotypes are incomplete, incorrect or missing (Kalinowski et
314 al. 2007).

315 This study further clearly highlights the power of this multiplex assay for the monitoring of
316 the *A. sturio* restocking program. Genetic tagging proved to be a reliable tool for species
317 conservation programs requiring a high number of individuals to be monitored (Andreou et
318 al. 2012). For *A. sturio* population monitoring, tagging systems such as PIT (passive
319 integrated transponder) or WOT tags (external loop tags, wire on tag) are efficient methods,
320 but are limited by the size of fish (Acolas et al. 2011). Genetic tagging clearly avoids the
321 problem of tag loss, and can be used at all stages. Furthermore, genetic monitoring is a
322 widely used tool to estimate successful reintroduction, establishment and reproduction of
323 individuals released into the wild (e.g. Perrier et al. 2013, Sard et al. 2015, Schreier et al.
324 2015). Our results provide compelling evidence that most individuals captured in the Estuary
325 descended from captive breeders (91.2%) and thus give positive signs of reintroduction
326 success of captive-born *A. sturio* into the wild. In addition, according to our previous error
327 estimation (4.2%) a small estimated proportion (i.e. 100-(91.2+4.2) =4.6 % corresponding to
328 9 individuals) might be issued from reproduction in the wild which need to be confirmed by
329 further investigations. This indicates that *A. sturio* survival will depend mostly upon
330 successful captive breeding and stocking, and stresses the importance of such genetic assay
331 for the careful and intensive ex-situ management of this species.

332 Our genetic assay further enables the study of other aspects of genetic management such as
333 monitoring levels of genetic variability in the captive stock, estimating inbreeding
334 coefficients or breeding success. For example, the comparison of the genetic composition
335 and levels of inbreeding between the captive stock and the released cohorts are important
336 clues in conservation breeding programs (Wilson et al; 2012, Schreier et al. 2015). The
337 captive breeding stock of *A. sturio* in Europe is shared through a joint conservation program
338 between IRSTEA in Bordeaux and IGB (Institute of Freshwater Ecology and Inland Fisheries)
339 in Berlin, and sturgeons from France are regularly transferred to be released into the Elbe
340 and to complement the German captive stock in Germany (Kirschbaum et al. 2011). This
341 set of multiplex assays has the potential to become a promising tool to standardize genetic
342 information and coordinate scientific tools to assist in genetic management of *A. sturio* in
343 the future.

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ACKNOWLEDGEMENTS

We wish to thank the regional funders of this study: Agence de l'Eau Adour Garonne, DREAL (Direction régionale de l'environnement, de l'aménagement et du logement ; Aquitaine), région Aquitaine, Conseil Régional Gironde. This study was also supported by the National French Action Plan in favor of *A. sturio* restoration. We thank Pierre Duchesne for help in assignment analyses using PASOS and PAPA programs, Frédérique Cerqueira and Erick Desmarais for their help in data acquisition. Data used in this work were (partly) produced through the Genotyping and Sequencing facilities of ISEM (Institut des Sciences de l'Evolution-Montpellier) and Labex Centre Méditerranéen Environnement Biodiversité. We also thank technicians from Migado association and from Irstea for fins sampling within the captive stock.

FIGURES

Figure 1. Comparison of assignment success (in percentage) of captive-born progeny (FARM) using three programs (PASOS, CERVUS, and PAPA). A) TRUE assignment rates for several sets of increasing number of markers (from 12 to 18) classified in increasing order of probability of identity (PID). TRUE refers to assignment success when compared to known data of parental pairs. B) Assignment, TRUE assignment and error rates for the multiplex assay of 17 markers.

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Figure

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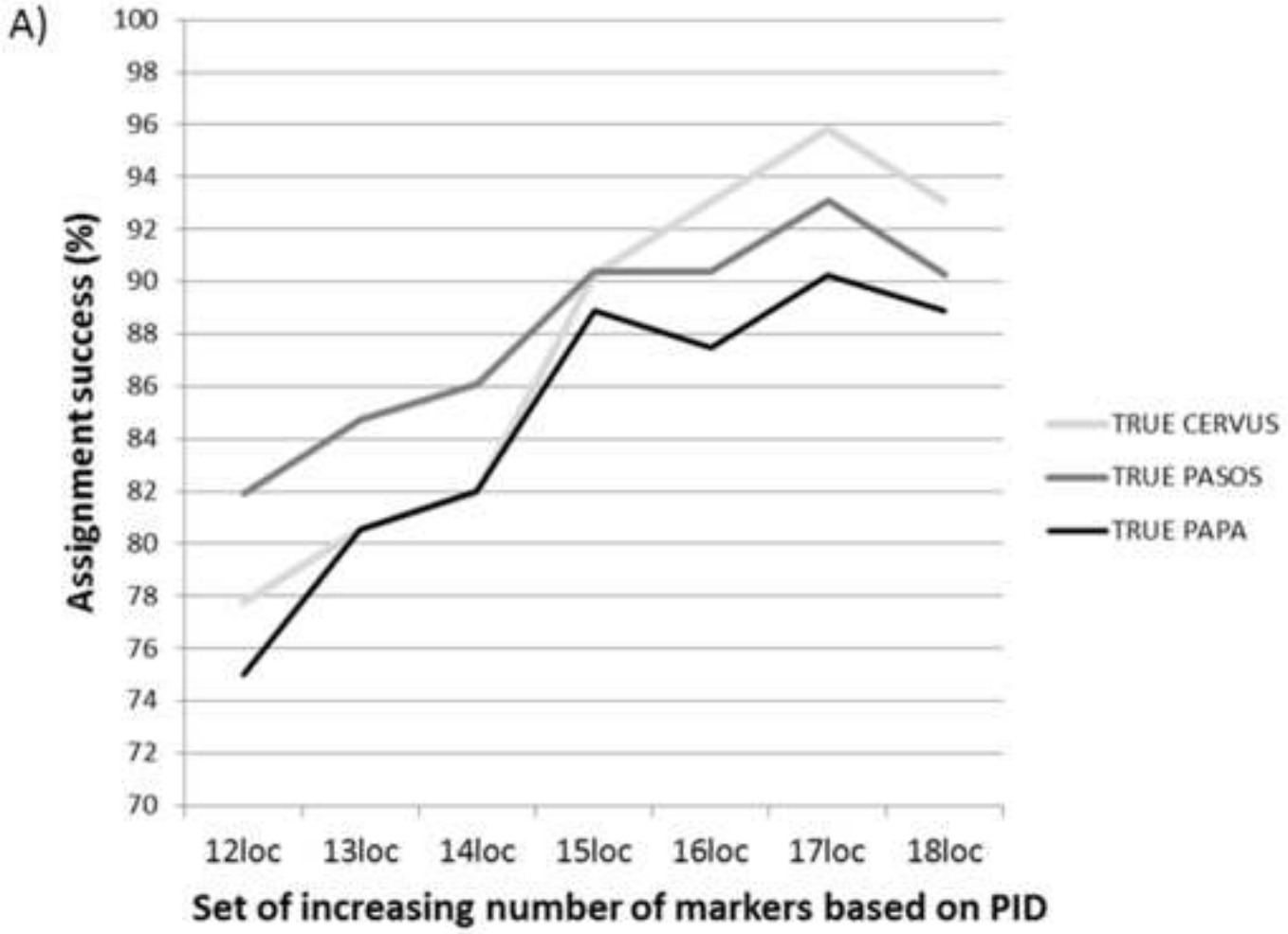

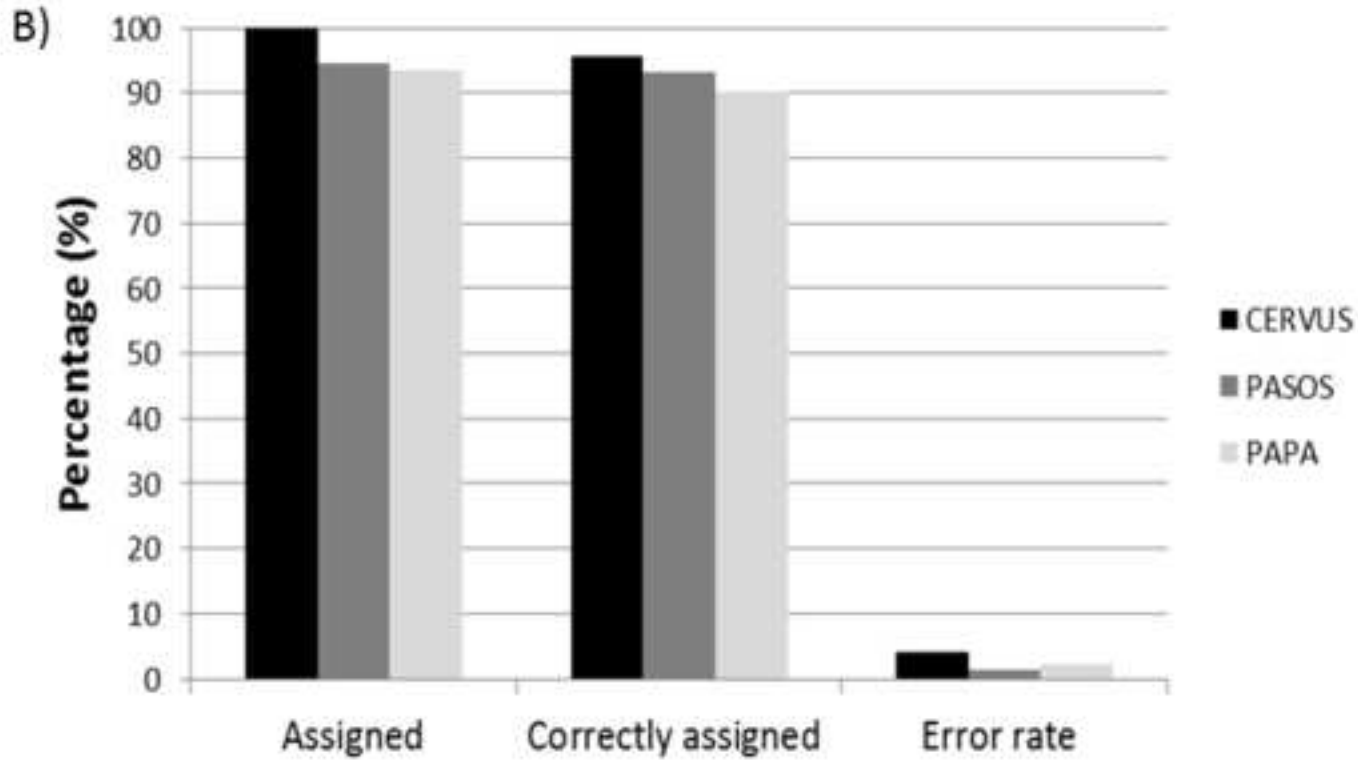

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Table 1: 18 microsatellites selected for *Acipenser sturio* multiplex assay and diversity in N=5(Annealing temperature (Ta),fForward (F*, labelled with fluorescent dye) and reverse (R) prim estimates (F null), rates of amplification success (in percentage), expected (Hexp) and obser

Name	Fluorescent dye	MLPX	Ta (oC)	Primer sequence F* (5'–3')
AOXD64	FAM	MPLX1	55	TTGTCCAATAGTTTCCAACGC
AOXD165	FAM	MPLX1	55	TTTGACAGCTCCTAAGTGATACC
AG10	FAM	MPLX1	55	AACAAGTTCTTACCTCGATTTTGG
AG47	NED	MPLX1	55	GCGAAACGACTCCCTTAACA
AOXD32	NED	MPLX1	55	CAGATTTAAGTAAGATAAGCATCAGC
AOXD44	HEX	MPLX1	55	ACCGAGTTTCAAATCAAATAGC
AOXD234	HEX	MPLX1	55	AACTGGCTTTGTGATTGATCC
AG14	FAM	MPLX2	55	GCTGTCCCATTAGCTGATCC
AOXD188	FAM	MPLX2	55	TGAAGTCATTGGTGATGTGTATG
AOXC55	NED	MPLX2	55	GCAAGGTGTATTAAACTGGACC
AOXD161	HEX	MPLX2	55	GTTTGAAATGATTGAGAAAATGC
AOX45	HEX	MPLX2	55	TTTGTGTAGGGAAATACCCTTG
AG38	FAM	MPLX3	55	AAACAGGTATAAAAATGTTGCTTGTG
SPL106	FAM	MPLX3	55	CACGTGGATGCGAGAAATAC
AG28	NED	MPLX3	55	CCATCAGCAGCTTCAACTCA
AG39	HEX	MPLX3	55	CATCTGGGAAGCAAGTGGAG
AFU68	HEX	MPLX3	55	TTATTGCATGGTGTAGCTAAAC
AN20	HEX	MPLX3	55	AATAACAATCATTACATGAGGCT
Overall mean				

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0 captive individuals. Microsatellites are amplified in three multiplex (MLPX1, MLPX2, MLPX3);
ıer sequences; Number of alleles per locus (A), allelic range in bps (range, bp), null allele freque
ved (Hobs) heterozygosity and probabilty of identity under unrelated (PID unrel) and sibling (PI

Primer sequence R (5'–3')	A	Range (bp)	F (null)	Amplification success rate (%)	Hobs
TGTGCTCCTGCTTTTACTGTC	5	103-145	-0.126	99.7	0.58
AAAGCCCTACAACAAATGTCAC	4	174-190	-0.072	100	0.73
GAGATTTGAACAAGACAGGAGGA	7	227-287	-0.075	97.2	0.80
ACAGGTCTTGGGCTTTCCTC	6	110-122	-0.126	100	0.59
AAAGCAGCTTGACATAACGG	4	156-204	-0.098	98.9	0.70
TGAAACTGCTGTGCAATAAGAG	3	144-160	-0.044	100	0.60
TGAAGCAAAGGGTATTATTTGAG	4	197-221	-0.111	97.9	0.60
GACAGAGGATGTTTCTGTGAGC	5	188-204	-0.167	100	0.70
ATGGAAATGTTTTATGGTAATGTG	3	244-252	0.049	99.7	0.57
CGACCCTGTAAAGGAGTAAGC	4	117-141	-0.023	100	0.67
TGAGACAGACACTCTAGTTAAACAGC	5	157-173	-0.114	100	0.72
TGAGTGCAGCCCTACTGCTC	4	198-222	-0.037	98.9	0.42
TCAGAAAGAGTTTAGTACGCATGG	5	190-202	-0.087	99.3	0.59
GGGGAGAAAACTGGGGTAAA	3	227-235	-0.002	99.7	0.51
ACATGCACGTATGCACGC	5	152-172	-0.105	96.6	0.44
CTCGATGGAACCCAGAAAAG	2	123-125	-0.183	99.7	0.47
AGCCCAACACAGACAATATC	4	142-168	-0.138	99.3	0.48
TGGTCAGTTGTTTTTTTATTGAT	5	186-214	-0.168	99.7	0.47
	4			**99.2**	**0.57**

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ıncy
D sibs) scenarios .

Hexp	PID unrel	PID sibs	References
0.60	1.21E-08	3.92E-04	Henderson-Arzapalo and King 2002
0.80	8.74E-03	1.54E-01	Henderson-Arzapalo and King 2002
0.90	7.00E-02	3.69E-01	Rajkov et al. 2014
0.72	9.21E-07	2.91E-03	Rajkov et al. 2014
0.74	2.48E-05	1.25E-02	Henderson-Arzapalo and King 2002
0.66	5.15E-08	7.55E-04	Henderson-Arzapalo and King 2002
0.68	2.17E-07	1.48E-03	Henderson-Arzapalo and King 2002
0.90	1.66E-04	2.84E-02	Rajkov et al. 2014
0.50	8.75E-10	1.10E-04	Henderson-Arzapalo and King 2002
0.70	4.45E-06	5.77E-03	Henderson-Arzapalo and King 2002
0.88	1.14E-03	6.52E-02	Henderson-Arzapalo and King 2002
0.52	1.88E-12	5.53E-06	Henderson-Arzapalo and King 2002
0.72	3.10E-09	2.04E-04	Rajkov et al. 2014
0.54	3.01E-10	6.39E-05	McQuown et al. 2005
0.46	1.29E-11	1.42E-05	Rajkov et al. 2014
0.66	5.06E-12	8.74E-06	Rajkov et al. 2014
0.64	1.06E-10	3.82E-05	Welsch et al. 2003
0.62	3.83E-11	2.32E-05	Zane et al. 2002
0.65	**1.88E-12**	**5.53E-06**	

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doi : 10.1007/s12686-016-0538-7

Appendix 1: List of 118 sturgeon loci tested in this study

POLYM: positivie amplification and more than one allele per locus, UNCLI
SIZE: Size exceeding 500 bps; NO AMP: no amplification

Locus	Amplification	Species
AfuG113	POLYM	Acipenser fulvescens
Ag01	UNCLEAR	Acipenser gueldenstaedtii
Ag12	DUPLI	Acipenser gueldenstaedtii
Ag15	DUPLI	Acipenser gueldenstaedtii
AoxB34	POLYM	Acipenser oxyrinchus
AoxD241	POLYM	Acipenser oxyrinchus
AciG22	UNCLEAR	Acipenser transmontanus/Acipenser me
AfuG198	MONOM	Acipenser fulvescens
Afug51	MONOM	Acipenser fulvescens
Ag02	MONOM	Acipenser gueldenstaedtii
Ag08	MONOM	Acipenser gueldenstaedtii
Ag09	MONOM	Acipenser gueldenstaedtii
Ag19	MONOM	Acipenser gueldenstaedtii
Ag21	MONOM	Acipenser gueldenstaedtii
Ag22	MONOM	Acipenser gueldenstaedtii
Ag24	MONOM	Acipenser gueldenstaedtii
Ag25	MONOM	Acipenser gueldenstaedtii
Ag26	MONOM	Acipenser gueldenstaedtii
Ag35	MONOM	Acipenser gueldenstaedtii
Ag40	MONOM	Acipenser gueldenstaedtii
Ag45	MONOM	Acipenser gueldenstaedtii
AoxD242	MONOM	Acipenser oxyrinchus
AS004	MONOM	Acipenser sinensis
AS021	MONOM	Acipenser sinensis
AS043	MONOM	Acipenser sinensis
Psp-26	MONOM	Polyodon spathula
Psp-29	MONOM	Polyodon spathula
Spl-163	MONOM	Scaphirhynchus platorynchus
AciG 56	NO AMP	Acipenser transmontanus/Acipenser me
AciG 93	NO AMP	Acipenser transmontanus/Acipenser me
AciG110	NO AMP	Acipenser transmontanus/Acipenser me
AciG142	NO AMP	Acipenser transmontanus/Acipenser me
AciG48	NO AMP	Acipenser transmontanus/Acipenser me
AciG4	NO AMP	Acipenser transmontanus/Acipenser me
AciG76	NO AMP	Acipenser transmontanus/Acipenser me
AfuG115	NO AMP	Acipenser fulvescens
Ag03	NO AMP	Acipenser gueldenstaedtii
Ag04	NO AMP	Acipenser gueldenstaedtii
Ag06	NO AMP	Acipenser gueldenstaedtii
Ag07	NO AMP	Acipenser gueldenstaedtii
Ag17	NO AMP	Acipenser gueldenstaedtii
Ag18	NO AMP	Acipenser gueldenstaedtii

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The original publication is available at http://link.springer.com
doi : 10.1007/s12686-016-0538-7

Ag27	NO AMP	Acipenser gueldenstaedtii
Ag29	NO AMP	Acipenser gueldenstaedtii
Ag30	NO AMP	Acipenser gueldenstaedtii
Ag32	NO AMP	Acipenser gueldenstaedtii
Ag33	NO AMP	Acipenser gueldenstaedtii
Ag37	NO AMP	Acipenser gueldenstaedtii
Ag42	NO AMP	Acipenser gueldenstaedtii
Ag43	NO AMP	Acipenser gueldenstaedtii
Ag44	NO AMP	Acipenser gueldenstaedtii
Ag46	NO AMP	Acipenser gueldenstaedtii
Ag50	NO AMP	Acipenser gueldenstaedtii
An16	NO AMP	Acipenser naccarii
AnacE4	NO AMP	Acipenser naccarii
AoxB28	NO AMP	Acipenser oxyrinchus
AoxD170	NO AMP	Acipenser oxyrinchus
LS34	NO AMP	Acipenser fulvescens
Psp-12	NO AMP	Polyodon spathula
Psp-20	NO AMP	Polyodon spathula
Psp-21	NO AMP	Polyodon spathula
Spl-100	NO AMP	Scaphirhynchus platorynchus
Spl-104	NO AMP	Scaphirhynchus platorynchus
Spl123	NO AMP	Scaphirhynchus platorynchus
Spl-168	NO AMP	Scaphirhynchus platorynchus
Spl170a	NO AMP	Scaphirhynchus platorynchus
AfuG123	UNCLEAR	Acipenser fulvescens
AfuG72	UNCLEAR	Acipenser fulvescens
Ag05	UNCLEAR	Acipenser gueldenstaedtii
Ag13	UNCLEAR	Acipenser gueldenstaedtii
Ag36	UNCLEAR	Acipenser gueldenstaedtii
Ag41	UNCLEAR	Acipenser gueldenstaedtii
Ag48	UNCLEAR	Acipenser gueldenstaedtii
Ag49	UNCLEAR	Acipenser gueldenstaedtii
AnacC11	UNCLEAR	Acipenser naccarii
AnacD3	UNCLEAR	Acipenser naccarii
Aox12	UNCLEAR	Acipenser oxyrinchus
AoxD161	POLYM	Acipenser oxyrinchus
AoxD242	UNCLEAR	Acipenser oxyrinchus
AciG198	POLYM	Acipenser transmontanus/Acipenser me
Afu19	POLYM	Acipenser fulvescens
Afu39	UNCLEAR	Acipenser fulvescens
Afu54	POLYM	Acipenser fulvescens
Afu68	POLYM	Acipenser fulvescens
AfuG184	POLYM	Acipenser fulvescens
Afug41	UNCLEAR	Acipenser fulvescens
Ag10	POLYM	Acipenser gueldenstaedtii
Ag14	POLYM	Acipenser gueldenstaedtii
Ag16	POLYM	Acipenser gueldenstaedtii
Ag20	POLYM	Acipenser gueldenstaedtii

Author-produced version of the article published in Conservation Genetics Resources, 2016, 8, 313-322
The original publication is available at http://link.springer.com
doi : 10.1007/s12686-016-0538-7

Ag28	POLYM	Acipenser gueldenstaedtii
Ag31	UNCLEAR	Acipenser gueldenstaedtii
Ag38	POLYM	Acipenser gueldenstaedtii
Ag39	POLYM	Acipenser gueldenstaedtii
Ag47	POLYM	Acipenser gueldenstaedtii
An20	POLYM	Acipenser naccarii
Aox23	POLYM	Acipenser oxyrinchus
Aox27	POLYM	Acipenser oxyrinchus
Aox45	POLYM	Acipenser oxyrinchus
AoxC55	POLYM	Acipenser oxyrinchus
AoxD165	POLYM	Acipenser oxyrinchus
AoxD172	UNCLEAR	Acipenser oxyrinchus
AoxD188	POLYM	Acipenser oxyrinchus
AoxD242	MONOM	Acipenser oxyrinchus
AoxD234	POLYM	Acipenser oxyrinchus
AoxD297	POLYM	Acipenser oxyrinchus
AoxD32	POLYM	Acipenser oxyrinchus
AoxD44	POLYM	Acipenser oxyrinchus
AoxD54	POLYM	Acipenser oxyrinchus
AoxD64	POLYM	Acipenser oxyrinchus
AS002	POLYM	Acipenser sinensis
Spl101	POLYM	Scaphirhynchus platorynchus
Spl-106	POLYM	Scaphirhynchus platorynchus
Spl-113	UNCLEAR	Scaphirhynchus platorynchus
Ag11	SIZE	Acipenser gueldenstaedtii
Ag23	SIZE	Acipenser gueldenstaedtii
Ag34	SIZE	Acipenser gueldenstaedtii
An77	SIZE	Acipenser naccarii