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Validation of 12 species-specific, tetrasomic microsatellite loci from the Russian sturgeon, *Acipenser gueldenstaedtii*, for genetic broodstock management

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Abstract
The Russian sturgeon, *Acipenser gueldenstaedtii*, is a critically endangered fish species. Hatcheries are operated in several countries within its natural range to produce stocking material for release into the wild and also for aquaculture purposes (caviar and meat production). An appropriate genetic broodstock management (plan or strategy) is required to avoid negative effects, e.g., admixture and hybridization of genetically differing stocks or loss of genetic variability due to inbreeding and genetic drift. Therefore, 11 tetrasomic microsatellite loci were newly isolated from the Russian sturgeon genome and arranged together with an already known locus into four multiplex PCR sets. These microsatellites were used to characterize three groups of hatchery juveniles from Germany (aquaculture production), Turkey, and Romania (production of stocking material) as well as a group of wild-caught adults from the Danube River, Romania. Based on the variability within groups, measured by the mean number of alleles per locus and expected heterozygosity, and the differentiation between groups, measured by Nei's G_{ST} and genetic distance D, the ability of the 12 loci to detect unwanted reductions in genetic variability within hatchery juveniles and to differentiate between groups could be demonstrated. This set of loci can also be used to identify those pairs of spawners that transmit the highest possible genetic variability to the next generation.

Keywords *Acipenser gueldenstaedtii* · Broodstock · Genetic diversity · Hatchery · Microsatellite · Ploidy level · Russian sturgeon

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Introduction

The Russian sturgeon (*Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833) is a species of the family Acipenseridae native to the Caspian, Black, and Azov Sea basins. However, aquaculture has resulted in intentional and accidental introductions throughout Europe (Gesner et al. 2010). Originally, the species had freshwater as well as anadromous populations. Freshwater populations existed in the Danube and Volga Rivers—but both are extinct now (Gesner et al. 2010). Also, the anadromous spawning populations in the Azov Sea were lost and only introduced (stocked) individuals remained. Currently, native anadromous spawning populations are only known from the Caspian and Black Sea tributaries; but even these populations have undergone major declines resulting in no confirmed natural reproduction of the Danube River populations over the last 5 years (Suciu, unpubl. data). Consequently, the Russian sturgeon was classified as "critically endangered" in the IUCN Red List of Threatened Species (Gesner et al. 2010). Gesner et al. (2010) also listed the major threats to the species: loss of spawning sites due to dam construction (e.g., 70% of spawning grounds in the Caspian Sea basin since the 1950s), poaching and illegal fishing, which appears to be increasing, and high levels of pollution (from oil and industrial waste), in both the Black and Caspian Sea basins. Genetic pollution is also a potential threat as stocks are moved to different locations (e.g., Caspian Sea stocks moved to the Azov Sea). To compensate the dramatic losses in individual numbers, restocking measures are ongoing. However, stocks are continuing to decline.

With the ever increasing importance of artificial hatchery reproduction both for aquaculture purposes and the production of stocking material, an appropriate broodstock management has to be implemented. From the genetic point of view, the final goal must be to preserve the original genetic structures and variabilities to a degree that avoids (i) the disruption of local adaptations caused by the admixture and hybridization of genetically differing stocks, and (ii) inbreeding, which may result from the small number of breeders usually propagated in hatcheries, in particular if the species is rare. On the other hand, the genetic management should allow genetic change (i.e., natural selection) from pressures that occur in the wild (after release of the stocked fish), but not from pressures in the hatchery. This goal is related to the concept of integrated hatchery programs and conservation hatcheries as defined by Trushenski et al. (2015). To meet these requirements, genetic markers are urgently needed—and have to be developed if not already available—to implement breeding plans for the ex situ measures and for stocking.

Based on the number of chromosomes (250 ± 8) (Vasil'ev 1985; Vlasenko et al. 1989; both cited in Vasil'ev et al. 2010) and the allele banding patterns of microsatellite loci (Havelka et al. 2013; Rajkov et al. 2014), the Russian sturgeon is considered as an octaploid species at the evolutionary scale, which indicates the maximum ploidy level achieved, and a tetraploid one at the recent scale, which indicates the current functional ploidy level (Rajkov et al. 2014). Rajkov et al. (2014) could identify only one out of 20 microsatellite loci (= 5%) examined as disomic. Among the eight loci newly isolated from Russian sturgeon in their study, three loci (*Ag09*, *Ag28*, and *Ag49*) expressed the tetrasomic pattern expected for a tetraploid species, four loci (*Ag01*, *Ag12*, *Ag14*, and *Ag22*) displayed higher than tetrasomic patterns, and one locus (*Ag18*) was the disomic one mentioned above. This variation in microsatellite loci expression patterns of Russian sturgeon shows that the process of functional genome reduction is still ongoing in species with ~ 250 chromosomes (Ludwig et al. 2001).

Microsatellite loci are popular and efficient DNA markers widely applied in aquaculture genetics (Liu and Cordes 2004). However, they have limitations in the light of the present

development of sequencing technologies (Putman and Carbone 2014) that are underlined by their utilization in polyploid organisms (Dufresne et al. 2014; Meirmans et al. 2018). Although microsatellites might be displaced by single nucleotide polymorphisms (SNPs) for sturgeon conservation in future (Ogden et al. 2013), their suitability for the management of a captive broodstock of another tetraploid sturgeon species, the critically endangered Adriatic sturgeon *Acipenser naccarii*, could clearly be demonstrated (Congiu et al. 2011).

A search in NCBI GenBank (accession date: 13 July 2017) for DNA sequence records of the species *Acipenser gueldenstaedtii* revealed 423 entries. However, there were no other microsatellite loci deposited than the eight described in Rajkov et al. (2014). Therefore, the aim of the present study was doubled: (i) to examine the variability of the three tetrasomic loci described by Rajkov et al. (2014) in a larger number of individuals and populations, and (ii) to isolate additional microsatellites from the Russian sturgeon genome to obtain a sufficient number of markers for improved broodstock management and the characterization of population structures.

Material and methods

Development of new microsatellite loci from the Russian sturgeon genome

Fin clips were collected from 20 juvenile Russian sturgeons at the "Rhönforelle" fish farm in Gersfeld, Germany, to isolate total genomic DNA using the DNeasy Blood & Tissue Kit (QIAGEN) according to manufacturer's protocols. A pool of ten DNA isolates was sent to GenoScreen, Lille, France (www.genoscreen.fr), where 1 μg of the pooled DNA was used for the development of microsatellite libraries through 454 GS-FLX Titanium pyrosequencing as described in Malausa et al. (2011). The bioinformatics program QDD (Meglécz et al. 2010) was used to analyze sequences. QDD performs all steps from raw sequences until obtaining PCR primers: removing adapters/vectors, detecting microsatellites, detecting redundancy and possible mobile element association, selecting sequences with target microsatellites, and designing primers by using the BLAST (Altschul et al. 1990), Clustal W (Larkin et al. 2007), and Primer3 (Rozen and Skaletsky 2000) programs. Among 5752 sequences containing a microsatellite motif, 198 bioinformatically validated primer pairs were designed.

All sequences with validated primer pairs were ranked according to motif type (penta- > tetra- > tri- > di-nucleotide repeats), number of repeats (the higher the better), and PCR product size (> 100 bp) considering only sequences with perfect repeats. From this list, the 60 top-ranking primer pairs were selected for the identification of suitable microsatellites (consistent amplification, ease to score, sufficient variability). Amplification protocols were developed for future use of PCR multiplex kits (QIAGEN) and a peqSTAR 96X Universal Gradient thermocycler (Peqlab). PCR primers were multiplexed (grouped) by the software MultiPLX, version 2.1 (Kaplinski et al. 2005). Three different dye labels (BMN-6, Cyanine 5, and DY-751) were assigned to forward primers. Genotyping of microsatellite loci was performed on an eight-capillary sequencer CEQ 8000 (Beckman Coulter) using the Fragment Analysis module of the GenomeLab™ GeXP Genetic Analysis System, version 10.2 (Beckman Coulter).

The microsatellite variability was initially examined in the 20 juveniles from the German hatchery (broodstock and progenies kept for aquaculture purposes) but later on extended to 20 juveniles from the Romanian hatchery of Isaccea (F1 half-sibs of wild spawners used for restocking into the Danube River), 22 juveniles from a Turkish hatchery (F1 progeny of wild

females and hatchery males used for enhancement of natural resources) and 11 wild-caught adults from the Romanian part of the Danube River. Due to the scarcity of disomic loci in Russian sturgeon, the focus of the study was to identify suitable tetrasomic loci showing consistent amplification and sufficient polymorphism.

Data analysis

The difficulty of analyzing codominant tetrasomic microsatellite loci of tetraploid species is to identify the true genotypes for partial heterozygotes. For instance, the genotype ABBC produces the microsatellite phenotype ABC, and cannot be reliably differentiated from genotypes AABC or ABCC. Jenneckens et al. (2001) suggested to estimate the number of allele copies on the basis of peak heights and areas in the electropherograms as exemplified by the microsatellite locus *LS-39* in the Siberian sturgeon *Acipenser baerii*. However, this approach seems not to be fully reliable: Congiu et al. (2011) found that the four peaks of a complete heterozygous genotype did not always show the expected same height in Adriatic sturgeon, and the same discrepancy was observed in the present study of Russian sturgeon (data not shown). Instead, Congiu et al. (2011) proposed a band sharing approach for which microsatellite data were considered as presence/absence of bands, disregarding the number of alleles present in each individual. Another and probably more convenient solution of the problem is provided by software specifically designed for the analyses of (allo-)tetraploid microsatellite loci: TETRASAT (Markwith et al. 2006), TETRA (Liao et al. 2008), and ATETRA (Van Puyvelde et al. 2010). Van Puyvelde et al. (2010) compared the three programs and concluded that TETRASAT and ATETRA have a comparable precision but are both more precise than TETRA. Since ATETRA is able to calculate more genetic variables and can handle an infinite number of partial heterozygotes, this program was chosen for the present study.

ATETRA was used with default parameters for Monte-Carlo simulations to calculate (i) the Hardy-Weinberg expected heterozygosity or Nei's genetic diversity H_E (Nei 1987) as well as values corrected for sample size $H_{E,c}$, (ii) the populational differentiation or Nei's G_{ST} (Nei 1973), and (iii) Nei's genetic distance D (Nei 1972, 1978). The matrix of pairwise genetic distances between the four Russian sturgeon groups was then used to construct a neighbor-joining tree (Saitou and Nei 1987) with MEGA5 (Tamura et al. 2011).

Data availability The DNA sequences of the 11 newly isolated *Agu* microsatellite loci were deposited in NCBI GenBank (for accession numbers see Table 1).

Results and discussion

Out of the three tetrasomic microsatellites chosen from Rajkov et al. (2014) for testing two loci displayed pentasomy (*Ag09* in two out of 40 samples, and *Ag28* in two out of eight samples) and were therefore discarded from further use. Only one locus (*Ag49*) displayed the expected tetrasomic pattern consistently and was included in further analyses. Out of the 60 new Russian sturgeon PCR primer pairs chosen for testing, 11 amplified microsatellite loci matched our selection criteria, in particular consistent amplification, tetrasomic pattern, and sufficient variability. These 11 loci were combined with locus *Ag49* into four

Table 1 Characteristics of the 12 polymorphic, tetrasomic microsatellite loci tested in a total number of 73 Russian sturgeons

PCR multiplex set (annealing temperature)	Locus name/GenBank accession no.	Forward primer dye label	Primer concentration (μM)	Primer sequence (5′-3′)	Repeat type	Total number of alleles	Allele size range (bp)
1 (63 °C)	*Agu56*/MG956817	BMN-6	0.200	F: TGATTGGTAATAATGCCTGCC R: TGTCACAGGGGACAGATTCA	TC	5	148–158
	Agu51/MG956815	DY-751	0.180	F: TACATCCACAGCACCTTCCA R: CCAGAGCTGGCTGTATGTGA	CT	5	141–159
	Agu38/MG956812	BMN-6	0.175	F: ACTGGGGTTGAAGGACAGTG R: TCCGTCTCATGTCCAAGGTA	GA	4	108–114
2 (63 °C)	*Agu54*/MG956816	Cyanine 5	0.055	F: GGAGCCAGTATCCCCTCAAT R: CTTCGCACGCAACTTAACAA	TG	5	188–214
	Agu36/MG956810	DY-751	0.195	F: GCAAACTGGGCTAGAACCTG R: TCCCCTCTCCTCTTCTCCTC	AG	11	104–128
	Agu37/MG956811	BMN-6	0.195	F: ACATGGTAGCAAAATCCCAA R: CAGCAAGCTTAGATGCATGG	CT	5	128–136
3 (63 °C)	*Ag49*[a]/HG931711	Cyanine 5	0.075	F: TGTTATCTGCTCTGATATTGATTCG R: CGTTTTAAAGTTTGAACGGCA	TTC	7	198–219
	Agu15/MG956808	DY-751	0.500	F: CGCAGCACAACTGCACAT R: CTGCCAGTGCGGTATCTACA	AGG	13	120–165
	Agu59/MG956818	BMN-6	0.170	F: TCGAAACCATAAGCGTTGAA R: TGACACTTGTGAAGCAACAGC	TG	5	210–280
4 (57 °C)	*Agu34*/MG956809	BMN-6	0.150	F: CGCAGCATGTGAAGGTTTTA R: CTCACAAACTTTGGATGCCT	GA	6	116–130
	Agu46/MG956814	DY-751	0.160	F: TGTACTGGAGTGTTTTTGAAAAGG R: CATTTGTTGGATACATTCAGCTTT	TC	5	103–113
	Agu41/MG956813	BMN-6	0.180	F: AAGACAAACAGTGGCCCAAC R: CAATGGCAGGTGCTACTGAA	AG	14	178–218

[a] Locus name, Genbank accession number, primer sequences, and repeat type information: supplementary Table S1 of Rajkov et al. (2014)

multiplex PCR sets (Table 1). Optimized PCR reaction mixes consisted of 5.0 μl of master mix and 1.5 μl Q-solution (QIAGEN), 1.0 μl DNA isolate, primers with concentrations as stated in Table 1, and PCR-grade water up to a final volume of 10.0 μl. The PCR program based on QIAGEN recommendations included an initial denaturation at 95 °C for 15 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C (multiplex set 4), or 63 °C (multiplex sets 1 to 3) for 90 s, and extension at 72 °C for 60 s. A final extension at 60 °C lasted for 30 min.

The observed total number of alleles per locus ranged from 4 to 14 (Table 1) with an average of 7.08. The hatchery juveniles possessed a mean number of alleles per locus ranging from 3.67 to 4.25; the wild-caught adults were more variable with a mean number of 5.67 (Table 2). However, the differences in mean number of alleles per locus were only significant

Table 2 Variability of the 12 tetrasomic microsatellite loci in the four Russian sturgeon groups (*A* number of alleles, H_E expected heterozygosity, $H_{E,c}$ expected heterozygosity, corrected for sample size)

Locus	Parameter	German hatchery	Romanian hatchery	Turkish hatchery	Romanian wild-caught
Agu56	*A*	3	4	1	5
	H_E	0.626	0.675	0.000	0.570
	$H_{E,c}$	0.634	0.684	0.000	0.583
Agu51	*A*	3	2	3	3
	H_E	0.429	0.496	0.488	0.507
	$H_{E,c}$	0.434	0.502	0.494	0.519
Agu38	*A*	3	2	3	4
	H_E	0.494	0.095	0.515	0.644
	$H_{E,c}$	0.500	0.096	0.521	0.659
Agu54	*A*	4	3	4	4
	H_E	0.746	0.542	0.733	0.669
	$H_{E,c}$	0.755	0.549	0.742	0.685
Agu36	*A*	6	4	4	7
	H_E	0.754	0.603	0.394	0.768
	$H_{E,c}$	0.764	0.611	0.399	0.786
Agu37	*A*	5	4	4	5
	H_E	0.755	0.380	0.566	0.698
	$H_{E,c}$	0.764	0.385	0.572	0.715
Ag49	*A*	3	6	6	7
	H_E	0.649	0.798	0.778	0.763
	$H_{E,c}$	0.657	0.808	0.787	0.780
Agu15	*A*	7	7	5	8
	H_E	0.700	0.773	0.600	0.771
	$H_{E,c}$	0.709	0.783	0.607	0.789
Agu59	*A*	2	2	2	5
	H_E	0.476	0.495	0.495	0.631
	$H_{E,c}$	0.483	0.501	0.501	0.646
Agu34	*A*	5	3	4	4
	H_E	0.745	0.512	0.664	0.676
	$H_{E,c}$	0.754	0.519	0.672	0.692
Agu46	*A*	4	2	4	5
	H_E	0.647	0.179	0.697	0.685
	$H_{E,c}$	0.655	0.181	0.705	0.701
Agu41	*A*	6	5	7	11
	H_E	0.758	0.674	0.830	0.852
	$H_{E,c}$	0.767	0.682	0.840	0.872
Mean values	*A*	4.25	3.67	3.92	5.67
	H_E	0.648	0.519	0.563	0.686
	$H_{E,c}$	0.656	0.525	0.570	0.702

Table 3 Populational differentiation G_{ST} (Nei 1973) (above diagonal) and genetic distance D (Nei 1972, 1978) (below diagonal) between the four groups of Russian sturgeon

	German hatchery	Romanian hatchery	Turkish hatchery	Romanian wild-caught
German hatchery		0.113	0.074	0.033
Romanian hatchery	0.412		0.143	0.066
Turkish hatchery	0.263	0.491		0.062
Romanian wild-caught	0.150	0.202	0.220	

between the wild-caught adults and the hatchery juveniles from Romania ($P = 0.02$; two-sided t test) and Turkey ($P = 0.04$; two-sided t test), respectively. Mean expected heterozygosities H_E were also lower in the hatchery juveniles, ranging from 0.519 to 0.648, than in the wild-caught adults with a value of 0.686 (Table 2). A significant difference was only found between the two Romanian groups ($P = 0.02$; two-sided t test). The correction for sample size resulted in only slight changes; again only the difference between the two Romanian groups was statistically significant ($P = 0.02$; two-sided t test).

Pairwise population differentiation was lowest between German hatchery juveniles and Romanian wild-caught adults ($G_{ST} = 0.033$) and highest between Romanian and Turkish hatchery juveniles ($G_{ST} = 0.143$) (Table 3). Accordingly, the lowest genetic distance was observed between German hatchery juveniles and Romanian wild-caught adults ($D = 0.150$) and the highest between Romanian and Turkish hatchery juveniles ($D = 0.491$) (Table 3). The neighbor-joining algorithm does not simply join the two groups displaying the lowest genetic distance (i.e., German hatchery juveniles and Romanian wild-caught adults) but also takes into consideration the genetic distances to all other groups. Hence, on the neighbor-joining tree (Fig. 1) the German and Turkish hatchery juveniles ($D = 0.263$) were placed together into one group separated from a Romanian group consisting of the hatchery juveniles and wild-caught adults ($D = 0.202$).

Comparable studies on Russian sturgeon or other tetraploid sturgeon species are still relatively rare. Most of them dealt either with the isolation and characterization of new microsatellite loci or their use as nuclear markers for sturgeon species and hybrid identification and to examine genome duplication events (Table 4). Comparisons of general microsatellite variability might be biased by ploidy level and sample size. However, if only tetrasomic loci were considered then the ranges of numbers of alleles per locus are rather similar across different loci and sturgeon species (Table 4): Russian sturgeon—4-14 (present study), 6–16 (Ludwig et al. 2001), 7–19 (Havelka et al. 2013); Adriatic sturgeon—8-16 (Congiu et al. 2011); lake sturgeon—8-10 (McQuown et al. 2003); Siberian sturgeon—11-18 (Barmintseva and Mugue 2017). Heterozygosity estimates are only available for disomic loci of the lake sturgeon (Table 4). Nevertheless, they are also at similar levels as the expected heterozygosities

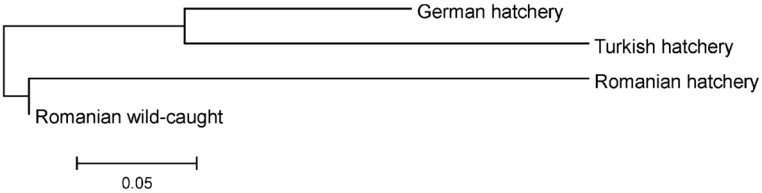

Fig. 1 Neighbor-joining tree of the four Russian sturgeon groups based on genetic distance D (Nei 1972, 1978)

Table 4 Use of microsatellite loci in tetraploid sturgeon species (n.d. no data reported)

Sturgeon species	Sample size	Origin of microsatellite loci	Number of loci (allelic pattern)	Number of alleles per locus	Heterozygosity (H_O observed, H_E expected)	Purpose of the study	Reference
Russian sturgeon (*A. gueldenstaedtii*)	99	Lake sturgeon (*A. fulvescens*)	5 (4 tetrasomic, 1 octosomic)	6–16 (tetrasomic loci); 28 (octosomic locus)	n.d.	Examination of genome duplications in sturgeons	Ludwig et al. (2001)
	16	Lake sturgeon (*A. fulvescens*), Atlantic sturgeon (*A. oxyrinchus*), and Adriatic sturgeon (*A. naccarii*)	8 (some tetrasomic)	1–8	n.d.	Nuclear markers for detection of Danube sturgeons hybridization	Dudu et al. (2011)
	35	Lake sturgeon (*A. fulvescens*), Atlantic sturgeon (*A. oxyrinchus*), white sturgeon (*A. transmontanus*), and shovelnose sturgeon (*Scaphirhynchus platorynchus*)	11 (7 tetrasomic, 4 octosomic)	7–19 (tetrasomic loci); 19–26 (octosomic loci)	n.d.	Examination of genome duplications in sturgeons	Havelka et al. (2013)
Adriatic sturgeon (*A. naccarii*)	20	Adriatic sturgeon (*A. naccarii*)	7 (higher than disomic)	3–19	n.d.	Isolation and characterization of microsatellites	Zane et al. (2002)
	42	Adriatic sturgeon (*A. naccarii*), lake sturgeon (*A. fulvescens*), Atlantic sturgeon (*A. oxyrinchus*), and shovelnose sturgeon (*Scaphirhynchus platorynchus*)	24 (12 tetrasomic, 4 disomic, 7 with max. 3 alleles, 1 with max. 7 alleles)	2–8 (disomic loci); 8–16 (tetrasomic loci)	n.d.	Managing polyploidy in ex situ conservation genetics	Congiu et al. (2011)
	20	Adriatic sturgeon (*A. naccarii*)	24 from genomic library, 33 from transcriptomic	2–7; 2–16	n.d.; n.d.	Isolation and characterization of microsatellites	Boscari et al. (2015)

Table 4 (continued)

Sturgeon species	Sample size	Origin of microsatellite loci	Number of loci (allelic pattern)	Number of alleles per locus	Heterozygosity (H_O observed, H_E expected)	Purpose of the study	Reference
			library (some higher than tetrasomic)			from the genome and transcriptome	
Lake sturgeon (*A. fulvescens*)	210 (15–40) from 7 locations	Lake sturgeon (*A. fulvescens*), Atlantic sturgeon (*A. oxyrinchus*), and shovelnose sturgeon (*Scaphirhynchus platorynchus*)	7 (4 disomic, 3 presumptive tetrasomic)	5–12 (disomic loci); 8–10 (presumptive tetrasomic loci)	$H_O = 0.46–0.66$ (based on the 4 disomic loci)	Genetic comparison of populations	McQuown et al. (2003)
	943 (1–136) from 27 locations	Lake sturgeon (*A. fulvescens*), Atlantic sturgeon (*A. oxyrinchus*), and shovelnose sturgeon (*Scaphirhynchus platorynchus*)	12 (all disomic)	2–12	$H_E = 0.46–0.63$	Genetic assessment of population structure in the Great Lakes	Welsh et al. (2008)
	316 (13–66) from 10 locations	Lake sturgeon (*A. fulvescens*)	14 (all disomic)	2–12	$H_O = 0.514–0.563$; $H_E = 0.522–0.664$	Genetic assessment of population structure in the Ottawa River	Wozney et al. (2011)
Siberian sturgeon (*A. baerii*)	151 (20–48) from 5 locations	Lake sturgeon (*A. fulvescens*), Atlantic sturgeon (*A. oxyrinchus*), and Adriatic sturgeon (*A. naccarii*)	5 (all tetrasomic)	11–18	n.d.	Natural genetic polymorphism and phylogeography	Barmintseva and Mugue (2017)

calculated in the present study. The magnitude of genetic population differentiation strongly depends on the population status (wild or farmed) as well as the geographical scale of sampling (i.e., a stronger differentiation can be expected if the whole distribution range is covered compared to sampling within a single river, lake, or sea basin). Therefore, a comparison with available population genetic studies on lake sturgeon (McQuown et al. 2003; Welsh et al. 2008; Wozney et al. 2011) and Siberian sturgeon (Barmintseva and Mugue 2017) would be suspect and was not attempted.

Conclusion

Although the sample size of the four Russian sturgeon groups examined in the present study was relatively small, the 12 tetrasomic microsatellite loci showed a level of polymorphism that allowed to differentiate between groups and to detect unwanted reductions in genetic variability within hatchery juveniles. The genotypic data obtained with this set of microsatellites can therefore be used to improve the genetic management of captive broodstocks reared for aquaculture purposes (caviar and meat production) or restoration programs (production of stocking material). In particular, these microsatellites can be applied to identify those combinations of spawners that transmit the highest possible genetic variability to the next generation. The process of selecting such suitable spawners can be aided by the software Genassemblage (Kaczmarczyk 2015).

Moreover, the neighbor-joining tree indicates that both the German and Turkish hatchery Russian sturgeons may originate most probably from the Caspian Sea basin, while the Romanian ones are from the Black Sea/Danube River population. Because of its implications for Russian sturgeon supportive stocking programs in the Black Sea basin, this assumption urgently requires more detailed investigations, in particular by including Russian sturgeon samples from the Caspian Sea basin.

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Compliance with ethical standards All sturgeon samples were collected in conformity with national legislations.**Conflict of interest** The authors declare that they have no conflict of interest.

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