Acta Zoologica Academiae Scientiarum Hungaricae 63(3), pp. 361–376, 2017
DOI: 10.17109/AZH.63.3.361.2017

GENETIC PROFILE ANALYSIS OF AQUACULTURE RUSSIAN STURGEON (*ACIPENSER GUELDENSTAEDTII*)

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The aim of this study is to help the conservation effort of the *Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833 population from Lower Danube, ensuring its sustainable development in regards to aquaculture and wild populations. We evaluated the genetic diversity of five aquaculture strains using molecular markers, determining the suitability of employing the individuals in *ex situ* conservation and in restocking programs. The mitochondrial cytochrome *b* gene analysis highlighted that some sequences from aquaculture and wild populations are grouped together representing a single haplotype. Results showed that the five aquaculture strains are grouped in 3 haplotypes with a haplotype diversity of 0.55686. The microsatellite analysis highlights that the majority of loci have a tetrasomic profile that makes the inferring of genotypes and the statistical analyses difficult, only two (AciG198 and Spl106) had a disomic profile that could prove useful in future studies. We can conclude that the molecular analysis is important for the genetic characterization of aquaculture strains, shedding light on the genetics of *A. gueldenstaedtii*.

Keywords: aquaculture, microsatellites, cytochrome *b*, genetic diversity, Romania strains.

INTRODUCTION

Sturgeons, or the representatives of the Acipenseridae family, order Acipenseriformes (Bemis *et al*. 1997), are a group of "living fossils" that diverged from an ancient pre-Jurassic teleost lineage, approximately 200 million years ago (Patterson 1982). The Acipenseridae family consists of 25 species, distributed exclusively in the Northern hemisphere, with the greatest species diversity being found in the Ponto-Caspian region (Bemis *et al.* 1997). Sturgeons are anadromous or potamodromous species and always spawn in freshwater (Bemis & Kynard 1997). Historically, sturgeons have been economically valuable in world trade because of their caviar (Ludwig 2006).

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The Russian sturgeon (*Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833) is one of the four sturgeons that can be encountered in the Lower Danube River. This species is anadromous and migrates from the Black Sea in the Lower Danube for reproduction. Because of some anthropogenic factors like overfishing, poaching, pollution and river regulation, the sturgeon wild populations have been drastically reduced (Jankovic 1993). In 2006 the Romanian Ministries of Environment and Agriculture issued a 10-year ban on wild sturgeon catch and export, measure adopted the next year by Serbia and Bulgaria (Suciu 2008). In 2016 the ban was prolonged by five years in Romania (Order 545/715/2016). *A. gueldenstaedtii* is included in the IUCN Red List as a critically endangered species and is considered to face extinction in the Lower Danube.

mtDNA is useful in the genetic studies in fish, especially because of its rapid rate of evolution and small size compared with the nuclear genome (Ferguson *et al.* 1995). There is a comprehensive array of studies that investigate stock structure in fish including bluefish (Graves *et al.* 1992), sharks (Heist & Gold 1999), snappers (Chow *et al.* 1993) and other species. The D-loop region has been shown to present elevated levels of variation in comparison with protein coding genes such as cytochrome *b* (Brown *et al*. 1993). For sturgeons, the D-loop marker has also presented the phenomenon of length heteroplasmy (Brown *et al.* 1996, Zhang *et al.* 1999, Ludwig *et al.* 2000), which can make the analysis of this region difficult, while the cytochrome *b* gene has been employed in haplotype identification for *A. gueldenstaedtii* (Birstein *et al.* 2000, Jenneckens *et al.* 2000, Çiftci *et al.* 2013). Also, there are several studies revealing the importance of genetic analyses for aquaculture stocks in *ex situ* conservation, as reviewed by Drauch Schreier *et al*. (2012).

For the genetic characterization of aquaculture *A. gueldenstaedtii* strains, two types of molecular markers were employed in this study: mitochondrial cytochrome *b* gene sequence and nuclear microsatellites, which are multiple tandems repeated copies of a short sequence of 2 to 9 base pairs (Wirgin *et al.* 2002, Liu & Cordes 2004). Microsatellites are able to show the ploidy level of the *A. gueldenstaedtii* individuals (Rajkov *et al.* 2014) and the genetic profiles obtained by genotyping can be used in further statistical analysis such as Principal Coordinate Analysis (PCoA), especially to discriminate the origin of sturgeons (Havelka *et al.* 2013) or for illustrating the genetic relationships among white sturgeon *Acipenser transmontanus* Richardson, 1836 (Schreier *et al.* 2012).

For sturgeons, the genetic characterisation of aquaculture strains is important for conservation and restocking programs, maintaining the locally adapted gene pools (Doukakis *et al.* 1999). Moreover, such type of analysis is necessary for designing proper breeding programs in aquaculture with the purpose of avoiding inbreeding and maintaining the genetic health along several generations.

The aim of this study is to genetically investigate the *A. gueldenstaedtii* aquaculture strains from four Romanian fish farms, highlighting the importance of this type of analysis in maintaining the genetic health of stocks and in species conservation in the Lower Danube.

MATERIAL AND METHODS

Sampling and DNA isolation

In this study we analysed samples from 51 individuals belonging to five aquaculture strains, from four Romanian hatcheries as shown in Table 1. All the aquaculture farms from which the biological samples were collected have facilities for the entire process of breeding, hatching and rearing and they buy and trade sturgeon fry. The fish farms do not exclusively rear Russian sturgeons, but also other sturgeon species. The breeding program used in this case for the analysed aquaculture stocks is the typical one for Romanian sturgeon farms, in which gametes from one female and multiple males are used for assisted breeding. We consider a strain a group of individuals with a common ancestor, having the same geographic origin or representing the result of the same breeding schema in aquaculture. The analysed individuals were obtained by crossing wild genitors from the Lower Danube.

The samples, represented by anal fin clips, were collected in 96% ethanol without sacrificing the animal, transported to the laboratory and stored at 4°C until DNA extraction. For DNA isolation we used the standard phenol-chloroform protocol as described by Taggart *et al*. (1992).

mtDNA analyses

The entire cytochrome *b* gene (1141 bp) was amplified for all individuals by using two primer pairs (Glu-F 5′-gaagaaccaccgttgttattcaa-3′, Cytb-R 5′-tctttatatgagaartangggtg-3′ and Cytb-F 5′-cacgaracrgrtcnaayaa-3′, Thr-R 5′-acctccratctycggattaca-3′). The PCR reactions were conducted with the GeneAmp 9700 PCR System (Applied Biosystems), in 25 μL final volume with 50 ng DNA template, 1X AmpliTaq Gold Reaction Buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTPs, 0.4 μM of each primer and 1 U of AmpliTaq Gold Polymerase (Applied Biosystems). For details about amplification see Dudu *et al*. (2012). PCR products' sequencing was conducted with the ABI Prism 3130 Genetic Analyser (Applied Biosystems), using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the BigDye

Table 1. Sampling sites and GenBank accession numbers for unique haplotypes.

Strain	n	Sampling location	Age (yr)	GenBank
A	20	Fish farm, locality Tămădău, Calarași County	1	KT232131
B	5	Fish farm, locality Ianca, Olt County	4	KT232132
C	6	Fish farm, locality Horia, Tulcea County	8	KT232133
D	10	Fish farm, locality Ianca, Olt county	1	KT232131
E	10	Fish farm, locality Constanța, Constanța County	8	KT232133

n – number of individuals

Table 2. GenBank cytochrome *b* reference sequences.

Sampling location	GenBank accession number
Russia, Caspian Sea	AJ563388, AJ563389, AJ563395
Romania, Danube River	GU647227
Turkey, Black Sea	KC130093, KC130094, KC130095, KC130100
Romania, Lower Danube	AF238682, AF238684, AF238690, AF238689, AF238687, AF238686, AF238683, AF238688

XTerminator Purification Kit (Applied Biosystems). The raw sequences were edited using BioEdit Sequence Alignment Editor 7.1.9 (Hall 1999), and aligned with ClustalX 2.0 (Larkin *et al.* 2007). The sequences representing unique haplotypes were deposited in GenBank under the following accession numbers KT232131, KT232132 and KT232133.

Apart from the sequences resulted from the analysed individuals, similar sequences from GenBank database isolated from *A. gueldenstaedtii* individuals of the Caspian, Black Sea and Lower Danube populations, were considered for phylogenetic analysis (Table 2). In this study we did not consider individuals from the Azov Sea, because 90% of the captured individuals are from hatcheries (Vecsei 2001), so they are not representatives of native populations.

The 51 aquaculture individuals used in this study, along with sequences like GU647227 and the ones from Birstein *et al.* 2000 (AF238682-84, AF238686-90) were analysed using DnaSP 5.10 (Librado & Rozas 2009) for assessing the number of haplotypes, the haplotype diversity index (Hd), Wright's Fixation Index (F_{st}) and $Gamma_{st}$ index. The number of nucleotide differences (k) and nucleotide diversity (Pi) were also assessed. For a more robust analysis, the 859 bp sequences were aligned to the 1141 bp ones and the gaps were considered as fifth state. Prior to phylogenetic analysis the 1141 bp sequences were aligned with the 859 bp ones and the gaps were considered for the analysis. The dataset was then tested in jModelTest 2.1.7 (Darriba *et al.* 2012) using BIC (Bayesian Information Criterion), obtaining the best-suited evolution model. The ML (Maximum Likelihood) phylogenetic tree with 1000 bootstrap replicates was built using MEGA 6.0 (Tamura *et al.* 2013). The MCMC (Markov chain Monte Carlo) Bayesian inference phylogenetic tree with 10 million iterations and samples recorded every 1000 generations was built using BEAUTi and BEAST 1.8.1 (Drummond *et al.* 2012). The tree was visualized as a maximum clade credibility tree with 10% initial burn-in using the TreeAnnotator 1.8.1 software (Drummond *et al.* 2012) and edited using FigTree (http://tree.bio.ed.ac.uk/software/figtree). Effective sampling size (ESS) was confirmed for each parameter using the Tracer 1.5 software as ESS>500 (Drummond *et al.* 2012).

Microsatellite marker analyses

For microsatellite analysis we considered the following 12 loci: AciG93, AciG198 (Börk *et al.* 2007), AnacC11, AnacE4 (Forlani *et al.* 2008), Aox27 (King *et al.* 2001), AoxD234 (Henderson-Arzapalo & King 2002), As002 (Zhu *et al.* 2005), LS19, LS34, LS39, LS54 (Krueger *et al.* 1997), and Spl106 (McQuown *et al.* 2000). The reactions were prepared in 25 μL final volume with 30 ng DNA template, 1X AmpliTaq Gold Reaction Buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTPs, 0.24 μM of each primer and 1U of AmpliTaq Gold Polymerase (Applied Biosystems). The amplification was done on the GeneAmp 9700 PCR System (Applied Biosystems) with the following PCR program: 95°C for 10 minutes, then 35 cycles

of 95°C for 30 seconds, specific annealing temperatures for different loci (46-60°C), 72°C for 1 minute and a final extension at 72°C for 60 minutes. For genotyping we used the GeneScan-500 LIZ Size Standard (Applied Biosystems) with ABI Prism 310 Genetic Analyzer (Applied Biosystems), followed by the analysis with GeneMapper ID v3.1 (Applied Biosystems). We generated a matrix of pairwise distances between individuals by using cmdscale (http://stat.ethz.ch/R-manual/R-patched/library/stats/html/cmdscale.html) and the Polysat 1.4-1 R package (Clark & Drauch Schreier 2015), with two options provided by the package: Bruvo`s distance (Bruvo *et al.* 2004) which takes mutational distance between alleles into account and Lynch`s distance (Lynch 1990) which is a simple band-sharing measure (Clark 2015). With the same Polysat 1.4-1 R package we performed a PCoA (Principal Coordinate Analysis) on the matrix, with the first two principal coordinates plotted and each strain represented by a different symbol.

RESULTS

In the case of mitochondrial cytochrome *b* gene sequences, we identified three haplotypes with individuals from aquaculture and the GU647227 individual (hap_1 – strain A and strain D, hap_2 – strain B and GU647227, hap_3 – strain C and strain E) and three haplotypes from the wild (hap_4 – AF238682 and AF238684, hap_5 – AF238683, AF238686, AF238687, AF238689 and AF238690, hap_6 – AF238688), with a Hd, between all haplotypes, of 0.67175. The Hd between haplotypes from aquaculture is 0.55686 and 0.69444 for the wild haplotypes. The genetic variation within aquaculture strains is non-existent, each strain presenting a haplotype diversity of 0. The polymorphism assessment with DnaSP 5.10 of the *cyt b* sequences truncated to 859 bp in order to accommodate to GenBank sequences typical for Lower Danube population showed only nine polymorphic (segregating) sites with a k value of 1.95254 and Pi value of 0.00227. The genetic differentiation between aquaculture strains and wild individuals was inferred by F_{st} with a score of 0.86997 and $Gamma_{st}$ index with a score of 0.85464. The jModelTest analysis showed that the best suited BIC model is HKY (Hasegawa–Kishino–Yano) (Hasegawa *et al.* 1985). The phylogenetic trees (Fig. 1) were constructed by using the sequences for aquaculture individuals that we determined in this study along with GenBank sequences characteristic to the Black Sea, Caspian Sea and Lower Danube wild populations. In the phylogenetic analysis we included all the sequences for each aquaculture strain. Both ML and MCMC tests show similar segregation of sequences.

The microsatellite results show that 67% of loci have a maximum number of alleles per locus (MNA) of 4 (AciG93, AnacC11, Aox27, AoxD234, LS19, LS34, LS39, and LS54), 8% have a MNA of 8 (As002), 8% have a MNA of 3 (AnacE4), and 17% have a MNA of 2 (AciG198 and Spl106) (Table 3). The graphical representations of the strains, depicted by the PCoA analysis (Fig. 2), show that the strains A and D are both forming a cluster, similar to the situation of the strains E and C, while strain B is placed separately. The axis 1

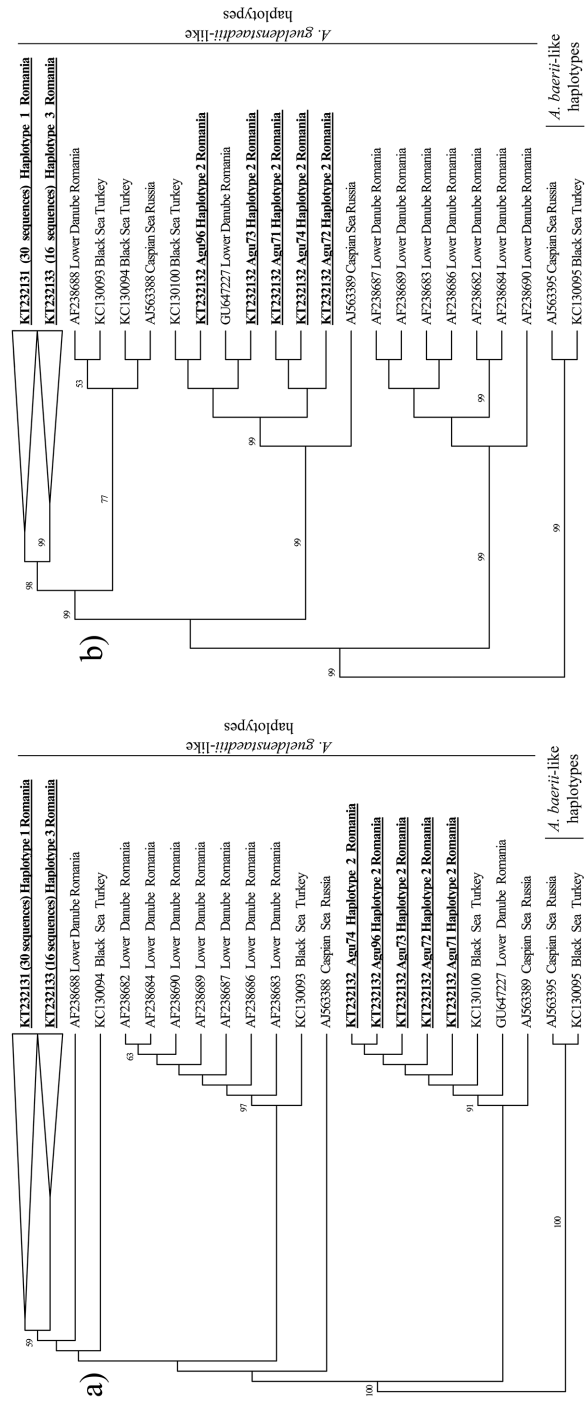

Fig. 1. **a)** Molecular phylogenetic tree inferred from 1141 bp aligned with 859 bp *A. gueldenstaedtii* cytochrome *b* gene sequences analysis by ML based on HKY model. 1000 bootstrap replicates. Values for partitions reproduced in less than 50% bootstrap replicates are not shown. The node numbers represent the confidence bootstrap values. The GenBank accession number is followed by sampling location for the sequences retrieved from the database. For our own sequences the GenBank accession number is followed by sample code, haplotype and sampling location. Haplotype 1 and 3 are collapsed. **b)** Molecular phylogenetic tree inferred from 1141 bp aligned with 859 bp *A. gueldenstaedtii* cytochrome *b* gene sequences analysis by MCMC based on HKY model with 10 million iterations, samples at 1000 generations and 10% burn-in. The branch numbers represent posterior probability. Values for posterior probability under 50% are not shown. The identification number of the individuals is followed by sampling location. Haplotypes 1 and 3 are collapsed.

Table 3. The characteristics for the 12 microsatellites used in this study.

Microsatellite	Size	Maximum number of alleles per locus
AciG93	383–399 bp	4
AciG198	184 bp, 188 bp	2
AnacC11	144–204 bp	4
AnacE4	320–356 bp	3
Aox27	110–142 bp	4
AoxD234	188–268 bp	4
As002	92–140 bp	8
LS19	115–154 bp	4
LS34	121–151 bp	4
LS39	85–157 bp	4
LS54	117–237 bp	4
Spl106	234–246 bp	2

(PC1) from the PCoA with Bruvo distance explained 15.78% of the variance, while the axis 2 (PC2) explained 9.54% of the variance; these scores are similar to the ones obtained from the PCoA with Lynch distance, where the first axis (PC1) explained 14.86% of the variance and the second axis (PC2) explained 8.76% of the variance.

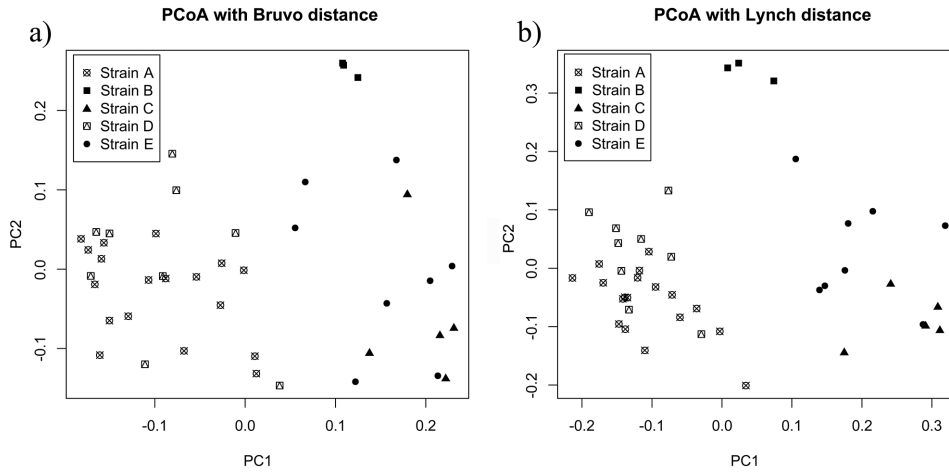

Fig. 2. Principal Coordinate Analysis (PCA) with Bruvo (a) and Lynch (b) distance. **a)** PC1 explained 15.78 % of the variance, PC2 explained 9.54 % of the variance; **b)** PC1 explained 14.86 % of the variance, PC2 explained 8.76 % of the variance.

DISCUSSION

For *A. gueldenstaedtii* the monitoring of the Romanian Danube sector and the northwest part of the Black Sea has yielded a low number of breeding individuals and a disrupted age class structure (Paraschiv *et al.* 2007, Suciu 2008). This situation, along with the "Allee" effect which led to the almost extinction of *A. gueldenstaedtii* in the Lower Danube (Gessner *et al.* 2010, Georgescu *et al.* 2014), makes it difficult to obtain samples from the wild for a genetic analysis study.

The genetic differentiation between strains inferred by Wright's Fixation Index (F_{st}), which can range from 0 (no genetic differentiation) to 1 (complete differentiation between populations) (Nei 1973), shows the score of 0.86997 between aquaculture and wild individuals. The $Gamma_{st}$ value, which is a more reliable interpretation of genetic differentiation data, that corrects for errors associated with incomplete sampling of populations and that is more suitable for mitochondrial haplotype data (Weir & Cockerham 1989), shows the score of 0.85464. The results from the comparison of our sequences (aquaculture) to the ones from Birstein *et al.* (2000) and GU647227 lead to identifying a high value for these indices, indicating a high genetic differentiation between the wild and aquaculture individuals. There are multiple strains within the same haplotype because only one female genitor is usually used for reproduction and for obtaining a strain in aquaculture (Chebanov *et al.* 2011), so, the same female could have been used for the rearing of different strains. Even though several male genitors can be used, they do not show up on mitochondrial DNA analysis of the offspring due to mitochondrial maternal inheritance (Sato & Sato 2013). Even though in sturgeon aquaculture the present trend, including in Romania, is to use a single female and more males for sturgeon stock (Sulak *et al.* 2014), we consider that the use of more females should assure higher genetic diversity within aquaculture stocks (Petrova *et al.* 2001). Unfortunately, this schema is not feasible because only few breeders are captured in the Danube River and then included in reproduction programs (Paraschiv *et al.* 2007, Suciu 2008).

Figure 1 shows that the *A. gueldenstaedtii*-like haplotypes clade is formed out of cytochrome *b* sequences of individuals identified as *A. gueldenstaedtii* by Birstein *et al*. (2000), Birstein *et al*. (2005) and by Çiftci *et al*. (2013). By examining the trees topology, it is shown that the aquaculture haplotypes were distributed in the *A. gueldenstaedtii*-like clade, in different subclades, which is a good indication of their genetic difference. In the two cladograms (Fig. 1) we can observe a distinct clade of *A. gueldenstaedtii* sequences that are similar to Siberian sturgeon (*Acipenser baerii* Brandt, 1869) mitochondrial cytochrome *b* sequences, forming the *A. baerii*-like haplotypes clade (Birstein *et al.* 2005, Çiftci *et al.* 2013). The Romanian aquaculture haplotypes are a part of

the *Acipenser gueldenstaedtii*-like haplotypes clade, ensuring the usefulness of these aquaculture strains in future restocking programs and *ex situ* conservation because of the lack of ambiguity in respect to species identification. The phylogenetic trees support the possibility of checking if new characterized strains fit into the already observed *A. gueldenstaedtii*-like haplotypes clade. The haplotype two (strain B) is distributed in a distinct monophyletic group together with a haplotype that was previously determined for a wild individual caught in the Lower Danube (GU647227) and a sequence characteristic to an individual from the Black Sea. The other two haplotypes (one and three), clustered together in related groups and alongside haplotype two, are a part of the *A. gueldenstaedtii*-like haplotypes clade.

The nuclear microsatellite analysis can give information about the genetic relationships between sturgeons but also about the ploidy level of *A. gueldenstaedtii* genome, which has a high probability of being tetraploid.

The microsatellite analysis revealed that out of 12 loci that were tested, only two (AciG198 and Spl106) had a disomic profile with a maximum of two alleles per locus in each individual and are suitable for genetic diversity studies. Unfortunately, the number of microsatellites that could be used for a reliable genetic analysis is drastically reduced. Another drawback is that AciG198 has just two fixed alleles, which makes it unsuitable for genetic diversity studies. The other loci had a polysomic profile. The problems that arise with using polysomic loci in population genetics analyses are thoroughly reviewed by Dufresne *et al.* (2014), and basically they are not recommended for depicting descriptive statistics indices, as also stated by Dudu *et al*. (2011). Jenneckens *et al*. (2001) described a method for interpreting the genotyping results in order to identify the correct genotypes in sturgeons when using polysomic loci, which implied the ratio of peak heights and areas from the electropherograms. We applied the scoring method for the tetrasomic loci and correlated it with the maximum number of alleles per locus observed overall. We observed that for AciG93 the ratio was 1:1 and the maximum number of alleles per locus was 4, for AnacC11 the ratio was 1:2 and the maximum number of alleles per locus was 4 which indicated that we cannot imply with exact precision the genotype. The ratio for Aox27 was 2:2:1:1, but we observed a maximum number of 4 alleles per locus, so the ratio does not support the tetrasomic profile of the locus. For AoxD234 we obtained the ratio 1:1:1 and we observed a maximum number of 4 alleles, which does not support the tetrasomic profile of the locus. The same situation was observed for both LS19, and LS34. For LS39 and LS54 the genotype could be inferred by this method, each having a maximum number of 4 alleles with the ratio of 1:1:1:1. Thus LS39 and LS54 are tetrasomic loci, but they are too few for a reliable genetic analysis (Fig. 3 & Table 4). In conclusion, we could not test for potential presence of genotyping errors, null alleles and allelic dropouts and neither population genetics analy-

Table 4. Example of peak data of tetrasomic microsatellites in *A. gueldenstaedtii*.

Sample	Marker	Allele 1	Area 1	Allele 2	Area 2	Allele 3	Area 3	Allele 4	Area 4
Agu179	AciG93	383 bp	3530	387 bp	2851				
Ratio		~1		~1					
Agu179	AnacC11	168 bp	45163	180 bp	85249				
Ratio		~1		~2					
Agu179	Aox27	122 bp	9806	130 bp	8502	132 bp	6365	142 bp	5618
Ratio		~2		~2		~1		~1	
Agu179	AoxD234	208 bp	19315	236 bp	19304	240 bp	16778		
Ratio		~1		~1		~1			
Agu209	LS19	127 bp	43303	133 bp	50924	139 bp	40340		
Ratio		~1		~1		~1			
Agu179	LS34	136 bp	57533	139 bp	47178	151 bp	53655		
Ratio		~1		~1		~1			
Agu179	LS39	124 bp	8539	139 bp	7266	148 bp	6816	157 bp	5866
Ratio		~1		~1		~1		~1	
Agu179	LS54	177 bp	8223	201 bp	6796	213 bp	6397	225 bp	5899
Ratio		~1		~1		~1		~1	

ses, like allelic richness, expected and observed heterozygosities, along with fixation index for inbreeding.

For *A. gueldenstaedtii*, Rajkov *et al*. (2014), showed that out of the 20 loci that were tested, 75% were tetrasomic, 20% were polysomic (more than four alleles per locus) and only 5% were disomic. Some of the loci from Rajkov *et al*. (2014) were also tested in our study, and we observed the same profiles (tetrasomic for AnacC11, Aox27, and AoxD234). Moghim *et al*. (2012) observed that 72% of the loci tested were tetrasomic and 28% were octasomic. Our own results show that 67% of loci have a tetrasomic profile, 8% are octasomic, and 17% are disomic. AnacE4 was shown to be tetrasomic for *Acipenser naccarii* (Bonaparte, 1836) in case of Congiu *et al*. (2011) while our results for *A. gueldenstaedtii* showed a maximum number of alleles per locus of 3. The data suggests that there is a high degree of transition from an octaploid state to a tetraploid one and that the genome for *A. gueldenstaedtii* has a low degree of functional diploidization. This qualifies *A. gueldenstaedtii* as a tetraploid sturgeon as proposed by Fontana *et al*. (2001), the trend being first indicated by the presence of chromosomal rearrangements, shown by telomeric sequences scattered along two chromosomes (Fontana *et al.* 1998).

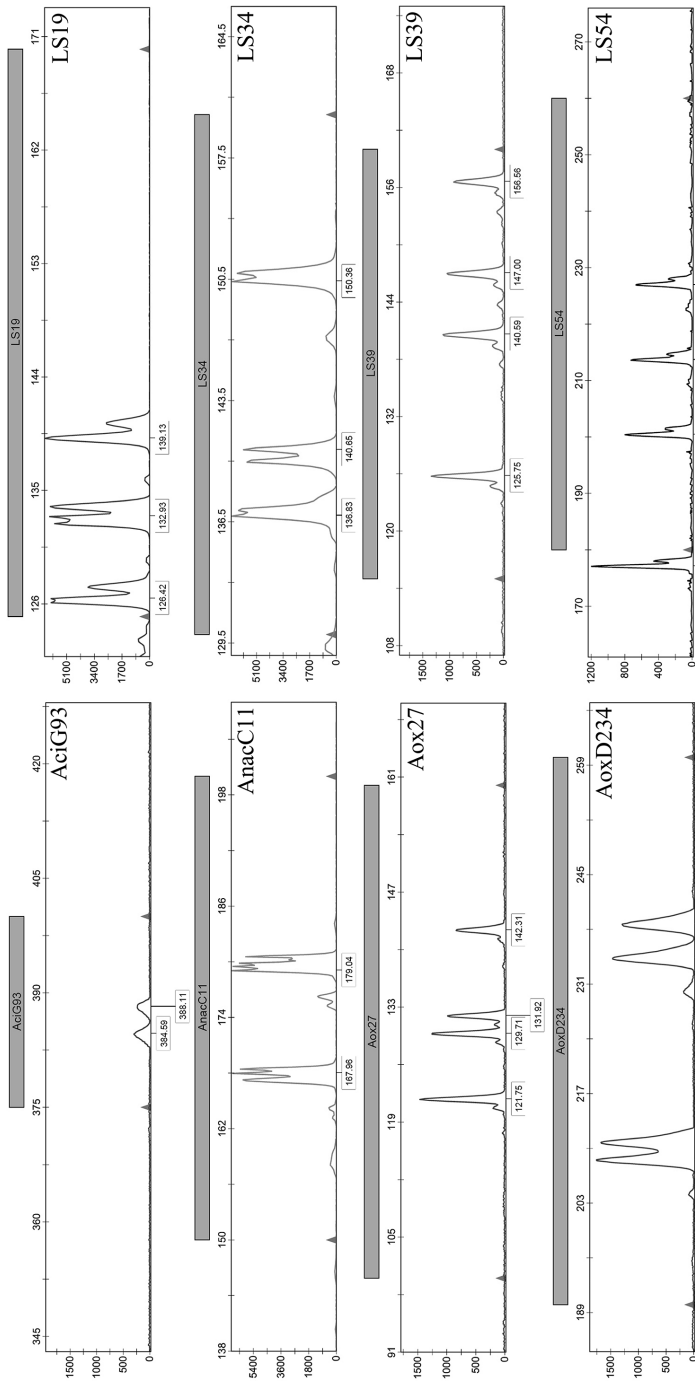

Fig. 3. Example of electrophoregrams of tetrasomic microsatellites in *A. gueldenstaedtii*. In the image the raw data for the following microsatellites: Acig93, AnacC11, Aox27, AoxD234, LS19, LS34, LS39 and LS54 is presented

The sequencing results regarding the three haplotypes are backed up by the PCoA analysis of the nuclear markers in terms of the three clusters observed in figure 2, corresponding to strain A and D, E and C, while the strain B is separated. As mentioned by Clark (2015) the Bruvo distance takes mutation into account, while the Lynch distance does not, suggesting that the latter measure works better for distinguishing populations, or strains in our case. Genetically we inferred the presence of three aquaculture strains. An explanation for this lower than we started with number of strains is the fact that the individuals of the same age are the result of the same breeders captured in the Danube River. In the years when the strains were obtained, the fish farms were involved in the program of population restocking and because only few individuals (breeders) were caught they were all used in multiple reproduction stations (http://ddni.ro/sturgeons/).

The genetic analyses from this study represent a first step of a more complex approach with high importance in future restocking and *ex situ* conservation programs. There is a further need for genetic characterization of the aquaculture strains due to a lack of uncovered nuclear disomic loci. For conservation measures, it is important to also take into consideration the genetic fitness and morphometric adaptation of individuals to the environment (Neff *et al.* 2011).

Molecular markers are important in the genetic characterization of individuals from the wild and from aquaculture and future finding of nuclear markers such as SNPs through Next Generation Sequencing can help in further understanding of *A. gueldenstaedtii* genetics and in proper conservation of this species along the Danube River.

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Acknowledgements – This work was supported by the National Authority for Scientific Research, PN-II-PT-PCCA 116/2012 "Genetic evaluation and monitoring of molecular and biotechnological factors that influence productive performance of Danube sturgeon species bred in intensive recirculating systems" and PN-III-P2 53PTE/2016 – "Technology for selection and genetic improvement in order to increase profitability of sturgeon's aquaculture".

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Received April 8, 2016, accepted September 1, 2016, published July 21, 2017