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Application of mtDNA markers for European huchen (*Hucho hucho* Linnaeus, 1758) management in Poland

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ABSTRACT: Four broodstocks of European huchen (*Hucho hucho*) from Poland, Germany, Slovakia, and Ukraine were investigated using five selected fragments of mtDNA. The mitochondrial DNA sequence analysis was successfully applied to the Polish and German broodstocks of European huchen for the first time. A very low haplotype (h = 0.097) and nucleotide (π = 0.00013) diversity across 3573 bases of mtDNA fragments (partial regions of *NADH-1*, *NADH-5*, *ATPase 6*, *Cytochrome b*, and *D-loop*) evidenced by three closely related mtDNA haplotypes were found. The analysis of pairwise genetic differentiation (*Fst*) displayed a statistically significant differentiation between German (clade A) and the rest of examined broodstocks (clade B), clustering them into two separate groups. Moreover, the applied mtDNA markers did not reveal any differences among fish from the clade B, suggesting that other markers should be used to display a deeper genetic background of the studied broodstocks. The two clusters of European huchen distinguished under the current study should be considered as distinct management units by managers, who may be tempted to transport brood fish or yearlings across the range of European huchen distribution. It means that stocking material and spawners of European huchen from the upper parts of the Danube River (Clade A) should not be mixed with those from the lower parts (Clade B). Moreover, for any supplementation of broodstocks in order to increase their genetic variability only the fishes within the described management units should be used.

Keywords: Salmonidae; broodstocks; conservation; genetic structure; mtDNA

INTRODUCTION

European huchen (*Hucho hucho*), also known as Danube salmon, is endemic to the Danube River drainage in Central Europe, inhabiting cool montane and submontane reaches of large streams and swift rivers (FishBase 2014, http://www.fishbase.org). According to FishBase, European huchen is one of the largest salmonid fish species, reaching up to 1.65 m standard length and 60 kg in weight. Furthermore, this fish species has been listed in annex II and annex V of the Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, and is considered as an endangered species with respect to its global distribution (see the Red List of Threatened Species, http://www.iucnredlist.org), including Poland (Polish Red Data Book of Animals: Vertebrates, http://eunis.eea.europa.eu/references/1782/general). In recent years the distribution range of the species has dwindled and the population size has declined rapidly through

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the effects of anthropogenic habitat alterations, such as river regulation by constructing dams and weirs, siltation of spawning ground, hydropower development as well as both industrial and agricultural pollution (Witkowski et al. 2013a). Additionally, the unique biological features make this fish species a popular target for anglers. As a result, a severe decline of European huchen population causes its dependence on programs of conservation of running waters (Geist et al. 2009). In order to supplement the local populations of European huchen, controlled reproduction is being in progress. According to some authors, due to low natural reproduction success a majority of the wild populations of the European huchen depends on the stocking activities (Witkowski et al. 2013a). In Poland, European huchen inhabits the Dunajec, Poprad, and San Rivers, where it is protected by a fishery supplementation and enacted rigorous fishing restrictions. Currently, there is only one fish farm situated in Lopuszna (southern Poland), which is the only existing source for European huchen stocking material in Poland (Witkowski et al. 2013b). The mentioned broodstock is kept *ex situ* all the time, being maintained only from spawnings. Furthermore, supplementation of the Polish broodstock by fish from natural populations or even from other hatcheries in Europe is not currently carried out and also any breeding program has not been implemented yet (Krzys 2014 – personal communication).

Although salmonids are one of the most extensively studied fish group, genetic information for some rare species are limited. Genetic data on European huchen are still sparse and mostly restricted to phylogenetic analysis of a few specimens in higher order systematic studies (e.g. Crespi and Fulton 2004; Shedko et al. 2013) or research focused on the congener taxa, such as taimen (*Hucho taimen*) (Froufe et al. 2005; Maric et al. 2014a), lenok (*Brachymystax lenok*) (Xia et al. 2006; Liu et al. 2015), Sichuan taimen (*Hucho bleekeri*) (Wu 2006; Wang et al. 2011), and Japanese huchen (*Hucho perryi*) (Oleinik and Skurikhina 2008). Some previous genetic studies on the phylogeographic structure associated with multiple broodstocks and wild populations of European huchen among major drainages provided fragmentary information about their genetic structure and variability (Geist et al. 2009; Weiss et al. 2011; Maric et al. 2014b; Kucinski et al. 2015). It should be emphasized that one of the most important factors determining the efficiency of conservation management of endangered fish species is the origin and quality of stocking material. Baseline genetic data are essential during well-developed restitution programs, enabling proper identification and selection of stocking material for supplementing wild populations, originating from proper stocks, being characterized by a high level of genetic diversity (Razpet et al. 2007; Snoj et al. 2009). Therefore, studies on the genetic structure of stock or population are crucial for conservation management of endangered fish species protected by a rehabilitation measure, such as European huchen. Despite the conservation, the management of European huchen urgently needs information on the genetic structure and differentiation, these data are still sparse for the Polish broodstock of the species.

The major objective of the present study was the assessment of the current genetic structure of four European huchen broodstocks from Poland, Germany, Slovakia, and Ukraine by means of selected mitochondrial DNA fragments analysis. The results of the research will provide baseline data for the improvement of existing conservation program management of the European huchen broodstock in Poland.

MATERIAL AND METHODS

Sample collection and DNA extraction. Fin clips from a total of 119 European huchen specimens were non-invasively sampled from four European huchen broodstocks and used for the genetic analysis. Fish tissues collected during 2011–2013 were derived from the following fish farms: Poland (Restocking Centre and Trout Hatchery Lopuszna), Germany (Fish farm Lindbergmuehle, Bavaria), Slovakia (Fish farm Pribovce, Martin province), and Ukraine (Fish farm "Ishkhan" Baniliv, Chemivtsi province). Small (< 1 cm^2) pelvic or pectoral fin clips were placed in Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and kept in 96% ethanol at a temperature of 4°C until DNA extraction. Genomic DNA was extracted from collected fin clips using Genoplast Tissue Genomic DNA Extraction Mini Kit (Genoplast, Pruszkow, Poland) following the manufacturer's protocol.

Amplification and sequencing. Five fragments of mtDNA genes (*NADH-1* (*ND1*), *NADH-5* (*ND5*), *ATPase 6* (*ATP6*), *Cytochrome b* (*Cytb*), and *D-loop* (*CR*)) were amplified using polymerase chain re-

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action (PCR) (Table 1). Reaction mixtures were prepared in a total volume of 25 µl with a 0.7 µl DNA template (4.6 ± 0.5 µg/ml), 5.0 µl of 10× PCR reaction buffer (100mM KCl, 100mM $(NH_4)_2SO_4$, 200mM Tris-HCl pH 8.5, 20mM $MgSO_4$, 1% Triton X-100), 1.0 µl of each primer, 0.5 µl (500µM) of each deoxynucleotide triphosphate (dNTP), and 0.3 unit TaqDNA polymerase RUN (A&A Biotechnology, Gdynia, Poland). Re-distilled water was used to bring the reaction mixture to the desired final volume. Amplification was performed with a Mastercycler gradient thermocycler (Eppendorf AG) under the following conditions: initial denaturation at 94°C for 3 min, followed by 34 cycles at 94°C for 30 s, annealing at 52–59°C (Table 1) for 45 s, elongation at 72°C for 2.5–3 min, and final elongation step at 72°C for 10 min. Subsequently, amplified DNA templates were purified using DNA CleanUp Kit (A&A Biotechnology). All sequencing reactions were prepared using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) following the manufacturer's protocol with slight modifications. The amplified, fluorescently labelled and terminated DNA was salt-precipitated with BigDye® XTerminator Purification Kit (Applied Biosystems) and analyzed on an Applied Biosystems 3130 Genetic Analyzer. The obtained sequences of *ND1*, *ND5*, *ATP6*, *Cytb,* and *CR* genes were deposited in GenBank under Accession Nos. KM593284–KM593293.

Data analysis. DNA sequences were aligned using the computer program ClustalX (Version 2.1, 2007) and checked by eye. Haplotype (*h*) and nucleotide diversity (π) as well as average number of nucleotide differences (*Kt*) were assessed employing DNAsp software (Version 5.1, 2009). The number of transitions and transversions within the protein coding genes of selected mitochondrial DNA fragments and the genetic differentiation (*Fst*) among European broodstocks were obtained using Arlequin software (Version 3.5, 2010). Pairwise *Fst* values were calculated employing the Kimura's two-parameter algorithm (gamma correction = 0.09), which accounts for differing rates of transition and transversion substitutions and 10 000 permutations. The same software was used for the analysis of molecular variance (AMOVA), also based on the Kimura's two-parameter distance matrix. The UPGMA dendrogram based on pairwise genetic differentiation (*Fst*) matrix was constructed using MEGA6 software (Version 6.0.5, 2013).

RESULTS

In the present study, altogether 3573 bp fragments of mitochondrial genome were sequenced in European huchen. In total 7 (0.2%) nucleotide positions were polymorphic, including 5 parsimony informative positions and 2 singleton polymorphic positions. The final alignment for all the examined specimens included 910 bp for *ND1* gene, 746 bp for *ND5*, 664 bp for *ATP6*, 463 bp for *Cytb,* and 790 bp for *CR*. Three haplotypes were found for the *ND1* gene, two for *ND5*, *ATP6* as well as *Cytb,* and one for *CR*. Combining the sequences, three haplotypes emerged in total. One haplotype was dominant and found in all broodstocks (Haplo_1), whereas two haplotypes (Haplo_2 and Haplo_3) were fixed only in the German broodstock. The haplotypes distribution among the broodstocks for all genes is shown in Table 2. The values for total

Table 1. Characterization of five fragments of mitochondrial DNA genes applied in the study of European huchen: gene designation, primer sequences, optimal annealing temperature (T_a), and source reference

Structural gene	Primer sequence (5'→3')	T_a (°C)	Reference
NADH-1	B1NDF: TAAGGTGGCAGAGCCCGGTA B1NDR: TTGAACCCCTATTAGCCACGC	59	Froufe et al. 2005
NADH-5	ND5-F:CTCTTGGTGCAAATCCAAGT ND5-R: GTGCTGGAGTGTAGTAGGGC	52	Wang et al. 2011
ATPase 6	L8558: AGCTTCTTCGACCAATTTATGAG H9208: TATGCGTGTGCTTGGTGTGCCA	54	Giuffra et al. 1994
Cytochrome b	L14735: AAAAACCACCGTTGTTATTCAACTA H15149: GCCCCTCAGAATGATATTTGTCCTCA	55	Wolf et al. 2011
D-loop	ND5/CytbF: TTCTTCCCCGCTATCATCCACCG CR2R: GCTGAAAGGAAAAAAAAAAAAG	58	Wang et al. 2011, present study

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Table 2. List of haplotypes and their frequencies for five fragments of mtDNA genes (*ND1, ND5, ATP6, Cytb*, and *CR*) across four sampled broodstocks of European huchen (n = 119 individuals examined)

Genes and haplotypes	Broodstocks (n of individuals)			
	Poland	Germany	Slovakia	Ukraine
ND1				
Hh_ND1_1	30	24	30	29
Hh_ND1_2		5		
Hh_ND1_3		1		
ND5				
Hh_ND5_1	30	25	30	29
Hh_ND5_2		5		
ATP6				
Hh_ATP6_1	30	24	30	29
Hh_ATP6_2		6		
Cytb				
Hh_tRNAGlu-Cytb_1	30	25	30	29
Hh_tRNAGlu-Cytb_2		5		
CR				
Hh_Cytb-CR_1	30	30	30	29
Combined haplotypes				
Haplo_1	30	24	30	29
Haplo_2		5		
Haplo_3		1		

haplotype and nucleotide diversity were h = 0.097 and π = 0.00013, respectively, and differed in individual broodstocks (h = 0.342, π = 0.00045 for the German broodstock; h = 0.000, π = 0.00000 for the rest of the examined broodstocks). The average number of nucleotide differences was Kt = 0.454. In the case of protein coding genes, the transition : transversion ratio was 1 : 2 for *ND1*, 2 : 0 for *ND5*, 1 : 0 for *ATP6,* and 1 : 0 for *Cytb*. The overall genetic differentiation (*Fst*) among European huchen broodstocks was 0.1353. The analysis of pairwise *Fst* displayed a statistically significant differentiation between the German and the rest of the examined broodstocks, clustering them into two separate groups (Table 3, Figure 1.).

Table 3. Pairwise genetic differentiation (*Fst*) between four European huchen broodstocks analyzed (below diagonal) and significance of these values (above diagonal)

Broodstock	Poland	Germany	Slovakia	Ukraine
Poland		*	ns	ns
Germany	0.1353		*	*
Slovakia	0.0000	0.1353		ns
Ukraine	0.0000	0.1353	0.0000	

*significant for α = 0.01, ns = not significant

The analysis of the genetic structure of the studied broodstocks with AMOVA method revealed that 13.47% of genetic diversity was distributed among broodstocks and 86.53% occurred among individuals within the broodstocks.

DISCUSSION

Mitochondrial DNA is widely used as a marker for evolutionary and population studies due to its compact size, nearly complete maternal inheritance, and fast evolutionary rate. Moreover, mitochondrial DNA markers were also applied for identifying both farmed and wild populations/stocks of fish,

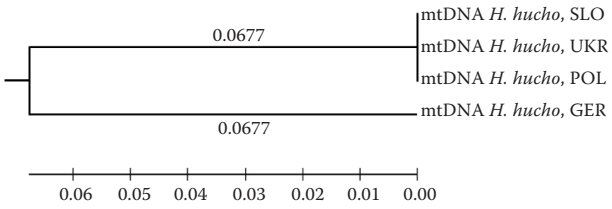

Figure 1. UPGMA dendrogram based on pairwise genetic differentiation (*Fst*) matrix illustrating the relationships between four European huchen broodstocks under the current study

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estimating the relative contribution of individual stocks in the multi-population catches, quantifying the range of genetic variation in stocks as well as identifying the species or interspecific hybrids (Sunnucks 2000). In the present study, the selected fragments of mitochondrial DNA were sequenced for the first time in European huchen from Poland and Germany. The genetic analysis, based on sequenced fragments of mitochondrial DNA, provided new information on genetic diversity of the studied European huchen broodstocks.

The previous assessment of the phylogeographic structure of European huchen left some blank spots in the characteristics of its genetic structure across the Danube River Basin, which is known as perhaps the most important refuge of freshwater organisms in Europe. To date, two main groupings between the western part (Clade A: Austria and Slovenia) and the eastern part of the Danube catchment (Clade B: Slovakia, Ukraine, Bosnia-Herzegovina, Montenegro, and Serbia) have been distinguished for wild populations of huchen in Europe (Weiss et al. 2011; Maric et al. 2014b). The present analysis based on the *ATP6*, *ND5,* and *Cytb* genes in four European huchen broodstocks confirms the division, previously observed by Weiss at al. (2011) and Maric et al. (2014b) for *CR* and *ND1* sequencing results, into two subclades: (1) Germany (Clade A) and (2) Poland, Slovakia, and Ukraine (Clade B). The current results seem to evidence that the examined broodstocks reflect the genetic structure of wild populations of European huchen across the Danube River Basin. Moreover, similar genetic differentiation has also been recorded in another Danubian salmonid species, European grayling (*Thymallus thymallus*) on the basis of microsatellite and mitochondrial DNA sequence data, which probably originated in the course of two independent post-Pleistocene colonization events (Maric et al. 2011).

The examined broodstocks of European huchen exhibited a very low overall haplotype (*h*) and nucleotide (π) diversity. The current results are in accordance with data on interspecific variability in European huchen (Weiss et al. 2011; Maric et al. 2014b), where only four haplotypes for *ND1* and two haplotypes for *CR* have been found in wild populations across Europe. Similarly, low values of these indices have been also reported for other close related species such as taimen (Froufe et al. 2003, 2005; Balakirev et al. 2013; Liu et al. 2015) and Sichuan taimen (Wu 2006). The highest haplotype (*h*) and nucleotide (π) diversity observed in German broodstock of European huchen is congruent with previously published microsatellite data (Kucinski et al. 2015). In the case of huchonine fish species, Froufe et al. (2003, 2005) hypothesized that their lower mtDNA nucleotide diversity compared to other salmonids may be associated with a slower molecular clock. Furthermore, on the basis of comparative phylogeographic data the mentioned authors found a much greater mtDNA sequence divergence within the genus *Thymallus* (> 4%) than in *Brachymystax lenok* and *Hucho taimen* across the same region in Asia. Various biological characteristics of huchonine subfamily, such as long life span, big body size, slow metabolic rate, and low body temperature would support this hypothesis (Gillooly et al. 2005). However, the low historical effective population size, founder effect, and more recent human-caused bottlenecks may explain the limited genetic diversity within the studied European huchen broodstocks. In turn, earlier presented genetic data on European huchen broodstocks seem to confirm the occurrence of the bottleneck effect or founder effects in the past (Kucinski et al. 2015).

Our results showed a certain genetic diversity between broodstocks (13.47%), however, a majority of the diversity was observed among individuals within the broodstocks (86.53%). Similar genetic diversity (14%) was observed among Atlantic salmon (*Salmo salar*) populations from the Bay of Fundy in southeastern Canada (Verspoor 2002). A higher genetic diversity was reported among *Brachymystax lenok tsinlingensis* populations from the streams of the Qinling Mountains (26.02%) and from the water system of eastern China (24.17–41.26%) (Xia et al. 2006; Liu et al. 2015). According to the current results, the overall genetic differentiation (0.1353) between German and the rest of examined broodstocks was moderate (Balloux and Lugon-Moulin 2002). The presented data evidences that despite the low overall levels of mtDNA haplotype and nucleotide diversity, a substantial genetic differentiation between the tested broodstocks of European huchen is observed and is similar with genetic data previously reported for the microsatellite DNA analysis (Kucinski et al. 2015).

Based on the current results, two subclades of European huchen were distinguished: (1) Germany and (2) Poland, Slovakia, and Ukraine. These two groups should be considered as management units by managers, who may be tempted to transport

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brood fish or yearlings across the range of European huchen distribution. It means that stocking material and spawners of European huchen from the upper parts of the Danube River (Clade A) should not be mixed with those from the lower parts (Clade B). Moreover, for any supplementation of broodstocks in order to increase their genetic variability, only fishes within the described management units should be used. It is considered that breeding programmes, reintroductions, supplementation, and removal of migration barriers without any genetic structure knowledge may promote the extinction of populations of the already endangered fish species (Geist 2011). Moreover, increasing population size by restocking activities may result in outbreeding depression (Huff et al. 2011). On the other hand, stocking activities by inbreeded material pose the risk of a total diminution of wild populations genetic variability. The after-effects of these interventions are highly unpredictable, particularly when small and fragmented populations, such as European huchen, are involved (Frankham et al. 2002). Unfortunately, the long-term results assessment of historic stocking of huchen in Europe is very poorly known. Thus, the influence of hatchery fish on wild populations remains an open question and a topic of major concern. For European huchen, stocking activities have not been well documented, thus no official information is available regarding the number of currently existing broodstocks, purpose of stocking, cross-basin transport, and the origin or the numbers of stocked fish. The obtained results clearly evidence a strong need to map in a higher resolution the genetic structure of huchen natural populations across the Danube River Basin, which will enable us to assess the impact of restocking by material from artificial reproduction on wild populations of huchen in Europe.

CONCLUSION

In conclusion, the lack of information on the genetic structure of European huchen broodstock in Poland essentially limits the sustainable conservation of this species. Moreover, the Polish broodstock of this fish species is characterized by insufficient values of breeding indicators, such as low rate of growth (10 years spawners reach the mass of less than 5 kg), low fry survival (30–40% from hatching to fry), and high frequency of deformations within raised juvenile specimens (Krzys 2014 – personal communication). Additionally, within broodstock only 50% of individuals are fertile and their sexual products (eggs and melt) are characterized by still lower quality with each next spawning season (Ciereszko 2014 – personal communication). In order to avoid these negative effects, the species conservation management protocol based on genetic analysis, morphological observations, and optimum selection of the spawners should be elaborated and implemented in the European huchen broodstocks in Poland and other countries. Baseline genetic data are crucial to guide future population specific conservation programs and research efforts on European huchen in Poland. The presented results suggest that the newly applied tree fragments of mtDNA (*ATP6*, *ND5,* and *Cytb*) can be useful for future investigations and conservation of European huchen stocks.

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REFERENCES

Balakirev E.S., Romanov P.B., Mikheev P.B., Ayala J. (2013): Mitochondrial DNA variation and introgression in Siberian Taimen Hucho taimen. PLOS ONE, 8, e71147.

Balloux F., Lugon-Moulin N. (2002): The estimation of population differentiation with microsatellite markers. Molecular Ecology, 11, 155–165.

Crespi B.J., Fulton M.J. (2004): Molecular systematic of Salmonidae: combined nuclear data yields a robust phylogeny. Molecular Phylogenetics and Evolution, 31, 658–679.

Frankham R., Briscoe D.A., Ballou J.D. (eds) (2002): Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK.

Froufe E., Alekseyev S., Knizhin I., Alexandrino P., Weiss S. (2003): Comparative phylogeny of salmonid fishes (Salmonidae) reveals late to post-Pleistocene exchange between three now-disjunct river basins in Siberia. Diversity and Distributions, 9, 269–282.

Froufe E., Alekseyev S., Knizhin I., Weiss S. (2005): Comparative mtDNA sequence (control region, ATPase 6 and NADH-1) divergence in Hucho taimen (Pallas) across four Siberian river basins. Journal of Fish Biology, 67, 1040–1053.

Geist J. (2011): Integrative freshwater ecology and biodiversity conservation. Ecological Indicators, 11, 1507–1516.

Geist J., Kolasha M., Gum B., Kuehn R. (2009): The importance of genetic cluster recognition for the conservation

doi: 10.17221/8599-CJAS

of migratory fish species: the example of the endangered European huchen Hucho hucho (L.). Journal of Fish Biology, 75, 1063–1078.

Gillooly J.F., Allen A.P., West G.B., Brown J.H. (2005): The rate of DNA evolution: effects of body size and temperature on the molecular clock. Proceedings of the National Academy of Sciences of the United States of America,102, 140–145.

Giuffra E., Bernatchez L., Guymord R. (1994): Mitochondrial CR and protein coding genes sequence variation among phenotypic forms of brown trout Salmo trutta from northern Italy. Molecular Ecology, 3, 161–171.

Huff D.D., Miller L.M., Chizinski C.J., Vondracek B. (2011): Mixed-source reintroductions lead to outbreeding depression in second generation descendants of a native North American fish. Molecular Ecology, 20, 4246–4258.

Kucinski M., Fopp-Bayat D., Liszewski T., Svinger V., Lebeda I., Kolman R. (2015): Genetic analysis of four European huchen (Hucho hucho Linnaeus, 1758) broodstocks from Poland, Germany, Slovakia and Ukraine: implication for conservation. Journal of Applied Genetics, 56, 469–480.

Liu H., Li Y., Liu X., Xiong D., Wang L., Zou G., Wei Q. (2015): Phylogeographic structure of Brachymystax lenok tsinlingensis (Salmonidae) populations in the Qinling Mountains, Shaanxi, based on mtDNA control region. Mitochondrial DNA, 26, 532–537.

Maric S., Razpet A., Nikolic V., Simonovic P. (2011): Genetic differentiation of European grayling (Thymallus thymallus) populations in Serbia, based on mitochondrial and nuclear DNA analyses. Genetics Selection Evolution, 43, 2.

Maric S., Alekseyev S., Snoj A., Askeyev O., Akseyev I., Weiss S. (2014a): First mrDNA sequencing of Volga and Ob basin taimen Hucho taimen: European populations stem from a late Pleistocene expansion of H. taimen out of western Siberia and are not intermediate to Hucho hucho. Journal of Fish Biology, 85, 530–539.

Maric S., Razpet A., Nikolic V., Snoj A., Simonovic P. (2014b): Analysis of genetic structure of huchen (Hucho hucho) in Serbia inferred from mitochondrial and nuclear DNA. Acta Veterinaria – Beograd, 64, 236–244.

Oleinik A.G., Skurikhina L.A. (2008): Phylogenetic relationships of Sakhalin taimen Parahucho perryi from PCR-RFLP analysis of mitochondrial DNA. Russian Journal of Genetics, 44, 767–776.

Razpet A., Susnik S., Jug T., Snoj A. (2007): Genetic variation among trout in the River Neretva basin, Bosnia and Herzegovina. Journal of Fish Biology, 70, 94–110.

Shedko S.V., Miroshinchenko I.L., Nemkova G.A. (2013): Phylogeny of salmonids (Salmoniformes: Salmonidae) and its molecular dating: analysis of mtDNA data. Animal Genetics, 49, 718–734.

Snoj A., Maric S., Berrebi P., Crivelli A.J., Shumka S., Sunsik S. (2009): Genetic architecture of trout from Albania as revealed by mtDNA control region variation. Genetics Selection Evolution, 41, 22.

Sunnucks P. (2000): Efficient genetic markers for population biology. Trends in Ecology and Evolution, 15, 199–203.

Verspoor E., O'Sullivan M., Arnold A.L., Knox D., Amiro P.G. (2002): Restricted matrilineal gene flow and regional differentiation among Atlantic salmon (Salmo salar L.) populations within the Bay of Fundy, eastern Canada. Heredity, 89, 465–472.

Wang Y., Guo R., Li H., Zhang X., Du J., Song Z. (2011): The complete mitochondrial genome of the Sichuan taimen (Hucho bleekeri): repetitive sequences in the control region and phylogenetic implications for Salmonidae. Marine Genomics, 4, 221–228.

Weiss S., Maric S., Snoj A. (2011): Regional structure despite limited mtDNA sequence diversity found in the endangered Huchen, Hucho hucho (Linnaeus, 1758). Hydrobiologia, 658, 103–110.

Witkowski A., Bajic A., Treer T., Hegedis A., Maric S., Sprem N., Piria M., Kapusta A. (2013a): Past and present of and perspectives for the Danube huchen, Hucho hucho (L.), in the Danube basin. Archives of Polish Fisheries, 21, 129–142.

Witkowski A., Goryczko K., Kowalewski M. (2013b): The history of huchen, Hucho hucho (L.), in Poland – distribution, restoration and conservation. Archives of Polish Fisheries, 21, 161–168.

Wolf C., Hubner P., Luthy J. (1999): Differentiation of sturgeon species by PCR-RFLP. Food Research International, 32, 699–705.

Wu X.C. (2006): The loss of genetic diversity in Sichuan taimen as revealed by DNA fingerprinting. Biochemical Genetics, 44, 177–185.

Xia Y.Z., Chen Y.Y., Sheng Y. (2006): Phylogeographic structure of lenok (Brachymystax lenok Pallas) (Salmoninae, Salmonidae) populations in water systems of eastern China, inferred from mitochondrial DNA sequences. Zoological Studies, 45, 190–200.

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