

Supplementary Material File S1: Brown trout haplogroups determination

Within the brown trout, *Salmo cf trutta*, Bernatchez et al (2001) defined five main haplogroups, based on the control region (CR) of mitochondrial DNA (mtDNA). Those are: Atlantic (AT), Danubian (DA), Adriatic (AD), Marmoratus (MA) and Mediterranean (ME) haplogroups. Control region or D-loop is Approximately 1000 base pair (bp) long product, but for haplogroup determination, only its first part is used (until thymine homopolymer that starts at 561 bp). Differences in the nucleotide sequences between these haplogroups are given in Table 1. Note that AT and DA haplogroups differ always on four nucleotide sites (2, 26, 236, and 389).

Table S1. Polymorphic sites in five main haplogroups of brown trout CR mtDNA: Atlantic (AT), Danubian (DA), Adriatic (AD), Marmoratus (MA) and Mediterranean (ME). Firstly described haplotypes (At1, Da1, Atcs1, MAcs1, MEcs1) of each haplogroup were compared.

Haplotypes	Polymorphic sites													
	2	26	113	146	196	236	262	389	390	403	530	542	543	548
At1	T	T	/	G	A	T	G	C	T	T	T	G	G	C
Da1	C	A	/	-	-	G	-	T	C	-	C	A	C	T
ADcs1	-	C	/	-	-	-	C	T	C	C	-	-	-	-
MAcs1	-	C	A	-	-	-	A	T	C	C	-	-	-	-
MEcs1	-	C	/	A	C	-	-	T	C	C	-	-	-	-

Second marker used in this research is nuclear lactate dehydrogenase gene (*LDH-C**). This gene is 440 bp long and has two alleles (*LDH-C*90* and *LDH-C*100*) in brown trout that give products of different lengths when exposed to restriction enzyme BseII. Allele *LDH-C*90* is linked to AT, while *LDH-C*100* is linked to DA mtDNA haplogroup. Restriction Fragment Length Polymorphism (RFLP) analysis was performed to determine potential hybridization between individuals of AT and DA haplogroups.

Results of the mtDNA and RFLP analysis of the *LDH-C** locus were combined to define pure AT and DA individuals and their hybrids. Individuals that were homozygous for *LDH-C*90* allele and had AT mtDNA were defined as AT, those who were homozygous for *LDH-C*100* and belong to the DA mtDNA haplogroup were defined as DA and heterozygotes for *LDH-C** gene locus that carried both *LDH-C*90* and *LDH-C*100* alleles were defined as hybrids. Further, hybrids were individuals that carried DA haplotypes and two *LDH-C*90* alleles, and those who carried AT haplotypes, but with two *LDH-C*100* alleles.

References

- Bernatchez L. 2001. The Evolutionary History of Brown Trout (*Salmo trutta* L.) Inferred From phylogeographic, Nested Clade, and Mismatch Analyses of Mitochondrial DNA Variation. *Evolution* 55: 351–379. <https://doi.org/10.1111/j.0014-3820.2001.tb01300.x>