

First Data on Genetic Diversity of Burbot (*Lota lota* L.) in the Western Siberian¹

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Abstract—Analysis of genetic diversity of burbot (*Lota lota* Linneus, 1758) mitochondrial control region (mtCR) haplotypes from geographically distant localities in the Ob-Irtysh River basin in comparison with distribution of known burbot haplotypes was conducted. mtCR fragments from burbot samples, obtained in two localities (longitudinal part of the Irtysh near Tobolsk and the Sob River, a left-bank tributary of the Ob River), were sequenced.

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Today, the use of genetics and molecular biology tools along with conventional morphological methods is required for the research on biodiversity and intraspecies differentiation. Indeed, genetic and molecular biology techniques are not only employed to establish genetic variability, but also to determine phylogenetic relationships between geographically separate populations and to shed the light on the history of postglacial re-colonization in Eurasia and North America. Burbot (*Lota lota* Linneus, 1758) is a circumpolar fish species, which serves as an ideal model organism for such research focus [1, 2]. Burbot genetic variability is assumed to be connected with geological history of separate Holarctic regions, while the formation of *L. l. lota* subspecies is related with the development of the modern river systems.

The distribution of burbot mitochondrial haplotypes, revealed in previous studies, suggests that Western Siberia most likely played an important role of being a transit region, through which burbot could spread from Europe to Siberia during the post-glacial period [1]. Thus, in Western Siberia burbot haplotypes most likely belong to the Eurasian clade (Fig. 1).

On the other hand, paleontological data points to the importance of Western Siberia as one of the key regions in the formation of Eurasian biota during the Quaternary period [3, 4]. The finding of burbot

ancient bones at Chembackchino-94A (lower reach of Irtysh River, age— 650000 ± 110000 TL) [5] is one of the oldest findings in Eurasia. This indicates that the Ob-Irtysh River basin could play much more important role in the formation of *Lota* biodiversity compared to the mere extrapolation of genetic data, obtained for the localities in Europe and Eastern Siberia.

We have sequenced the mitochondrial control region fragment from 44 samples of burbot: 20 samples from the Irtysh River in the vicinity of Tobolsk (Lat: $58^{\circ}37' N$, Lon: $68^{\circ}31' E$) and 24 samples from the Sob River (Lat: $66^{\circ}39' N$, Lon: $65^{\circ}33' E$), a left tributary of the Ob river (hereinafter referred to as Tobolsk and the Sob, respectively). DNA was isolated from fish muscle tissue samples, fixed in 96% ethanol, employing salt extraction protocol [6]. Amplification was conducted using primers LProF [7] and 12S5R [8] in 30 μ L of the reaction mixture containing 10 to 100 ng DNA, PCR buffer, 1.25 mM $MgCl_2$, 50 μ M dNTPs, 0.5 μ M of each primer and 0.5 units of Taq polymerase (SibEnzim, Novosibirsk) according to the following protocol: denaturation 1 min at $95^{\circ}C$, 36 cycles of PCR product synthesis ($95^{\circ}C$ —10 s, $58^{\circ}C$ —20 s, and $72^{\circ}C$ —50 s); final elongation—10 min at $72^{\circ}C$. The PCR products were purified according to the protocol of Velikov [9].

Sequencing was conducted with the help of BigDye v.3.1 Terminator Cycle Sequencing Ready Reaction Kit at standard conditions using primers, employed for the PCR. After purification, the fragments were sequenced at the ABB 130 Genetic Analyzer (Applied Biosystems, United States). The direct and reverse sequences were edited using BioEdit program [10]. Median Joining Network was obtained employing Network 4.6.1.2 [11].

¹ The article was translated by the authors.

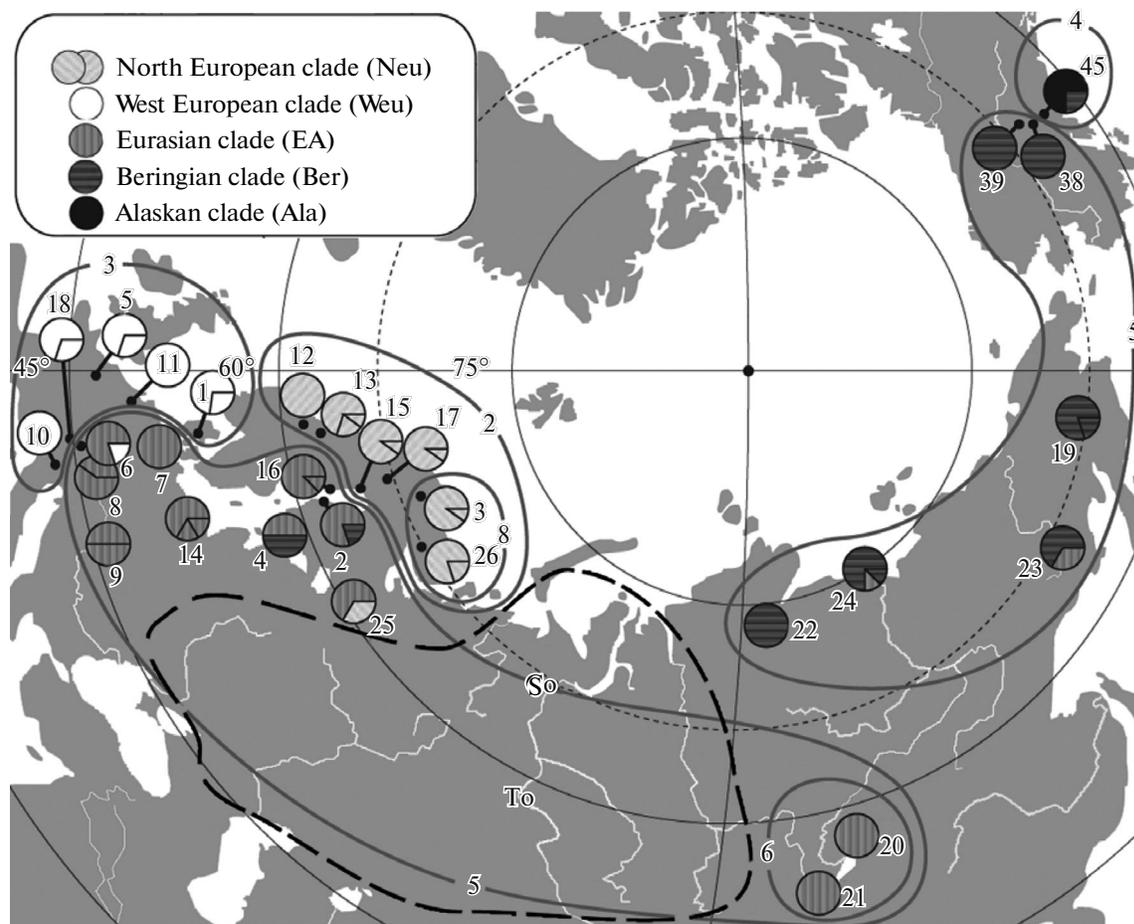


Fig. 1. Literature data on genetic diversity and phylogeny of burbot (*L. lota*) and burbot sampling sites. The pies depict the geographical distribution of the various mitochondrial clades; the pie numbers stand for burbot sampling sites [1]; the group structure defined by the Samova is delineated by dark grey lines for the EB phylogroup; the associated number refers to the grouping level [1]. The area, where genetic data on burbot haplotypes is missing, is highlighted by dotted line. Burbot sampling sites in the Western Siberia: So—the Sob River; To—the Irtys River, Tobolsk town.

Due to the fact that 90% of polymorphism within the control region is observed in the left hypervariable half of this fragment [12], and the majority of control region haplotypes, deposited to the NCBI, are not completely sequenced, we used the fragment of 407 nucleotides for the comparative analysis. Eighty-six sequences including the ones from Tobolsk and Sob burbot samples were analyzed.

Median joining network encompassing burbot control region haplotypes from two lineages, *L. l. lota* and *L. l. maculosa*, together with sequences, derived from the Ob-Irtys River burbot populations, demonstrated a high level of *L. l. maculosa* differentiation, which enabled us to exclude this subspecies from the further analysis. A separate cluster includes haplotypes from the Amur River basin. Two resulting clusters are separated by three mutation steps. The second cluster is mostly composed from various Eurasian populations and has a star-like structure, in the center of which

most frequent haplotypes are placed. It is subdivided in a few haplogroups (Fig. 2).

Most of the control region haplotypes, revealed in the Ob-Irtys River basin, fall into Eurasian and Beringian haplogroups. Our data show that 28 from 44 control region sequences represent haplotypes, described earlier (see table). The majority of samples belong to the EB30 haplotype, which is one of the central Eurasian haplotypes and the habitat of which covers not only European (the Isar, the Vistula), but also Asian (the Lena) rivers, as well as Lake Baikal [12]. The ratio of this haplotypes is 39%. Besides, five samples from Tobolsk and Sob have Beringian haplotype EB41, which is found in the Kurenjoki and Porkkala Bay in Finland. This haplotype, also named as Xj1, was observed in large quantities in the Irtys River in the North-Western China [13]. Another Beringian haplotype, EB44 (also named as Xj2) [13], was also found in that area, and is represented in samples from the Sob.

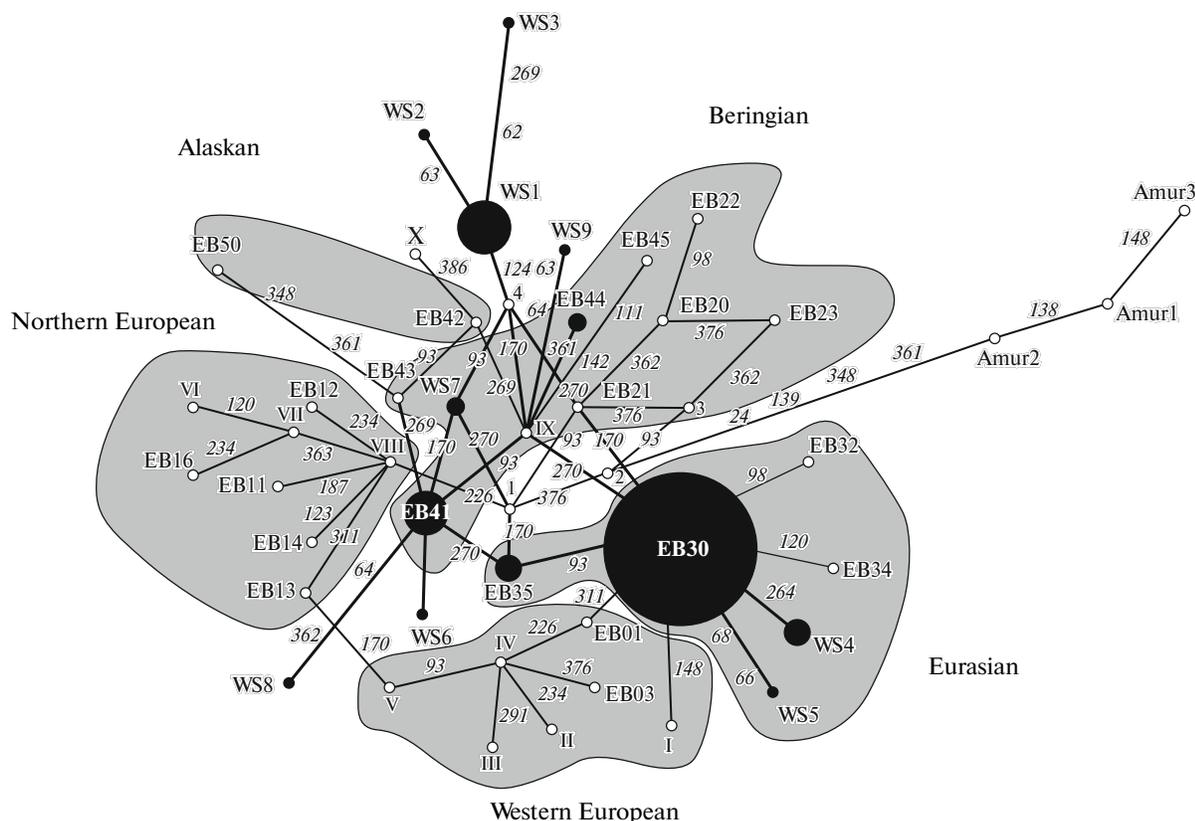


Fig. 2. Median joining network 4.6.1.2 representing mitochondrial control region haplotypes of burbot (*L. l. lota*). Haplotypes from the Ob-Irtysh River basin are highlighted in black; surfaces of circles are proportional to their abundance. Circles of haplotypes, obtained from the NCBI data base, are highlighted in white: Amur1-3, I-X, EB 01-50. Haplotypes, revealed for the first time, are named as WS1–WS9. Numbers 1–4 show hypothetical haplotypes. Haplogroups are shadowed in grey [1]. Numbers on branches mark mutation sites.

Nine new haplotypes from the Ob–Irtysk River basin were named as WS1–WS9 (Western Siberian) (see table). One of those nine haplotypes is the most

Identified haplotypes based on the mtDNA control region and the number of samples for each haplotype (N) of analyzed burbot populations inhabiting Western Siberia

Haplotype*	N	
	Tobolsk	Sob
EB30	5	12
EB35	1	2
EB41	4	1
EB43	1	—
EB44	—	2
WS1	4	2
WS2	1	—
WS3	1	—
WS4	2	—
WS5	—	1
WS6	—	1
WS7	—	2
WS8	1	—
WS9	—	1

* EB—earlier described haplotypes [12, 13]; WS—original haplotypes.

widely distributed and was found in samples from the Sob and Tobolsk. It appears that derivatives from WS1 haplotypes WS2 and WS3 are younger (Fig. 2). All three new haplotypes present a separate haplogroup: haplotypes WS7, WS6, and WS8 are close to Beringian haplotype EB41, while the Eurasian haplotype EB30 is the closest to haplotypes WS4 and WS5.

Thus, a high level of burbot genetic diversity was revealed both along longitudinal part of the Irtysh (center of the Western Siberian Plain) and in the Lower Ob (polar part of the Western Siberian Plain). Besides dominating haplotypes of Eurasian and Beringian clades 36% of revealed control region sequences belong to nine new haplotypes, three of which form a separate haplogroup. Our data indicate that studies on burbot phylogeography nominotypical subspecies *L. L. lota* are incomplete and require further research, focused on genetic analyses of burbot inhabiting central part of Eurasia, in particular the Western Siberian Plain.

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