

## Phylogenetic Relationships of Burbot (*Lota lota* L., 1758) of the Volga–Kama River Basin Inferred from the Analysis of Mitochondrial DNA Markers

L. E. Yalkovskaya<sup>a,\*</sup>, Y. Y. Khrunyk<sup>b</sup>, M. A. Krokhalova<sup>a</sup>, Correspondent Member of the RAS V. D. Bogdanov<sup>a</sup>, P. A. Sibiryakov<sup>a</sup>, S. B. Rakitin<sup>a</sup>, and A. V. Borodin<sup>a</sup>

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**Abstract**—The phylogenetic relationships of burbot (*Lota lota* L., 1758) of the Volga–Kama River basin are reconstructed for the first time. The sequences of the gene cytochrome *b* and the mtDNA control region obtained for 44 samples from the Kama River and Mezhevaya Utkha River (the Volga River tributaries) are studied. New haplotypes of both markers were revealed. The results of phylogenetic reconstructions based on cytochrome *b* and control region mtDNA do not contradict the existing ideas about the phylogenetic structure of the species, and indicate inclusion of burbot from the Volga–Kama basin in Eurasian haplogroup. According to obtained data, the Volga–Kama River basin could play an important role in shaping the genetic diversity of burbot in Europe, and during certain periods it served as a corridor connecting the river systems of the European and Asian parts of the species range.

**Keywords:** mitochondrial genome, phylogeography, genetic differentiation, freshwater ichthyofauna, circumpolar range, postglacial recolonization

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Genetic studies of widespread species on the basis of analysis of mitochondrial markers are successfully used in solving problems of intraspecific differentiation, taxonomy, and phylogeny, as well as for the reconstruction of the evolutionary history of individual taxa and the biome as a whole. The burbot (*Lota lota* L., 1758) is the only freshwater representative of the family Gadidae. The circumpolar range of this species is represented by rivers and lakes of Eurasia and North America up to 40° N (Fig. 1). Studies of the genetic structure of *L. lota* using cytochrome *b* (*cyt b*) and the mtDNA control region (CR) cover the territories of both continents [1–6]. The existence of two major mitochondrial lineages corresponding to two subspecies was shown. The North American lineage (*L. l. maculosa*) occupies the southeastern part of the range of the species in North America, and the Eurasian–Beringian (*L. l. lota*) lineage occupies the northwestern part of North America and the entire territory of Eurasia and has a circumpolar range (Fig. 1).

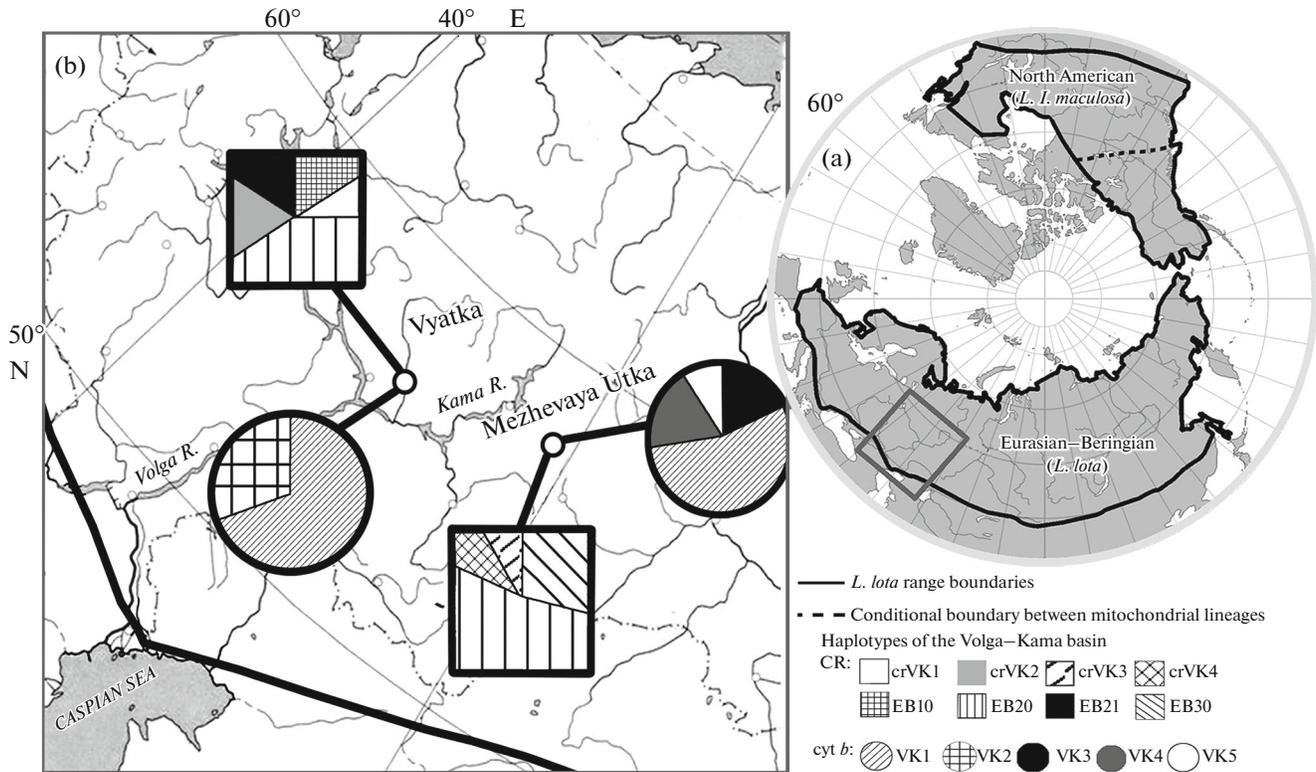
The analysis of genetic diversity and demographic history of the Eurasian–Beringian lineage indicates

the formation of the boundaries of its present area of distribution in the postglacial period. According to the phylogenetic reconstructions, groups of haplotypes are differentiated within the lineage. The distribution of this differentiated groups is limited to the peripheral parts of the range, and their basal haplotypes derived from the haplotypes of the widespread Eurasian group, which occupies the territory of Western and Northern Europe as well as Western and Eastern Siberia [1–3, 6]. The formation of the Eurasian group is associated with the existence of several refugia. For Europe, this might be the Danube River basin [1–3]. However, our studies of the genetic diversity of burbot of the Ob–Taz basin [6] showed that the reconstructions of the evolutionary history of the burbot cannot be considered justified without taking into account the possible contribution of the major river systems in Central and Eastern Eurasia, data for which are practically absent. For example, in the genetic studies of the burbot, the Europe’s largest Volga–Kama river basin, which flows into the undrained (inland) Caspian Sea and the formation of which involves both the European as well as Asian rivers, is not represented. According to the geological history of the basin, there were periods of connections with both the European and Siberian hydrological systems in its recent past [7]. In this regard, we studied the phylogenetic relationships of the burbot of the Volga–Kama river basin

<sup>a</sup> Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, Russia

<sup>b</sup> Yeltsin Ural Federal University, Yekaterinburg, Russia

\*e-mail: lida@ipae.uran.ru



**Fig. 1.** The range of *L. lota* and an approximate boundary between mitochondrial lineages (a), sampling sites in the Volga–Kama river basin, and the occurrence of *cyt b* and CR haplotypes (b).

on the basis of analysis of two mitochondrial markers—*cyt b* and CR.

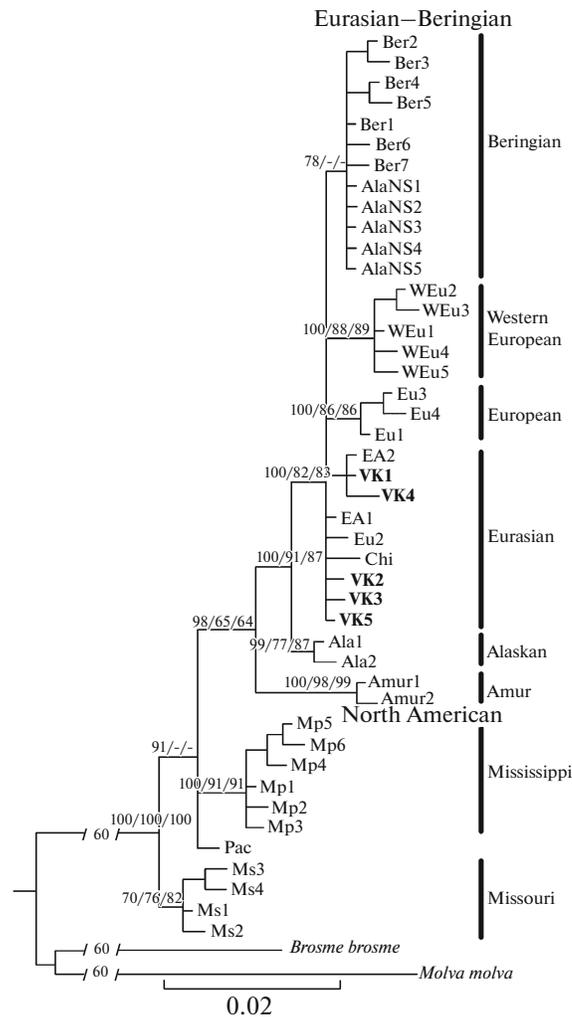
The study was performed with muscle tissue samples fixed in 96% ethanol from 44 burbot individuals from the Volga tributaries of the second and third order—the Vyatka River (57°38' N, 59°04' E) and the Mezhevaya Utka River (56°07' N, 51°20' E), respectively (Fig. 1). DNA isolation, amplification (PCR conditions were the same for both markers), sequencing of mtDNA fragments containing *cyt b* and CR, processing of sequences, genetic diversity assessment, and phylogenetic reconstructions were performed by the methods described in detail previously [6]. For amplification of *cyt b* we used primers L14299Gm and H14942Gm [1]; for CR, primers LProF and 12S5R [6].

Phylogenetic constructions were performed using the data from GenBank. For *cyt b* we used 39 haplotypes (AY226383-444 [1], KM201364 [8], KM363244 [9], KC44053, KU244689-92, and KT327178 (unpublished data)), the majority of which are represented by sequences 1063 bp long: 567-bp fragment from the 5' end and 496-bp fragment from the 3' end [1]. For CR, we used 104 haplotypes (AY656840-915 [2], DQ630963-1411 [3], EU873154-65 [4], KX017626-48 [6], KM201364 [8], KM363244 [9], KJ381204 [10], KC44053, EF191381-84, and EF191387 (unpublished data)) 409 bp in length from the 5' end, which allowed

us to include in the analysis the maximum amount of data available in GenBank.

Complete *cyt b* sequences (1140 bp) of the burbot of the Volga–Kama basin were obtained from 21 individuals: 10 individuals from the Vyatka River and 11 individuals from the Mezhevaya Utka River. Five haplotypes were described and deposited in GenBank (NCBI) with accessions MN708969–MN708973, four of which are new. Haplotype VK1 was detected in the majority of individuals in both rivers (seven and six individuals in Vyatka and Mezhevaya Utka rivers, respectively), VK2 (three individuals) was found only in the Vyatka River, and VK3–VK5 were found in the Mezhevaya Utka river (VK3 in two individuals, VK4 in two individuals, and VK5 in one individual) (Fig. 1).

Phylogenetic reconstructions on the basis of data for *cyt b* (Fig. 2) showed that haplotypes of the burbot from the Volga–Kama basin, as expected from the notion of the distribution of mitochondrial lineages, belong to the Eurasian–Beringian lineage, whose structure generally corresponds to the previously proposed schemes [1, 5]. The haplotypes of the Volga–Kama basin do not form a separate group and belong to the Eurasian haplogroup, which is widespread from Sweden, Finland, and Lake Onega to Lake Baikal and Lena River basin (Fig. 2).

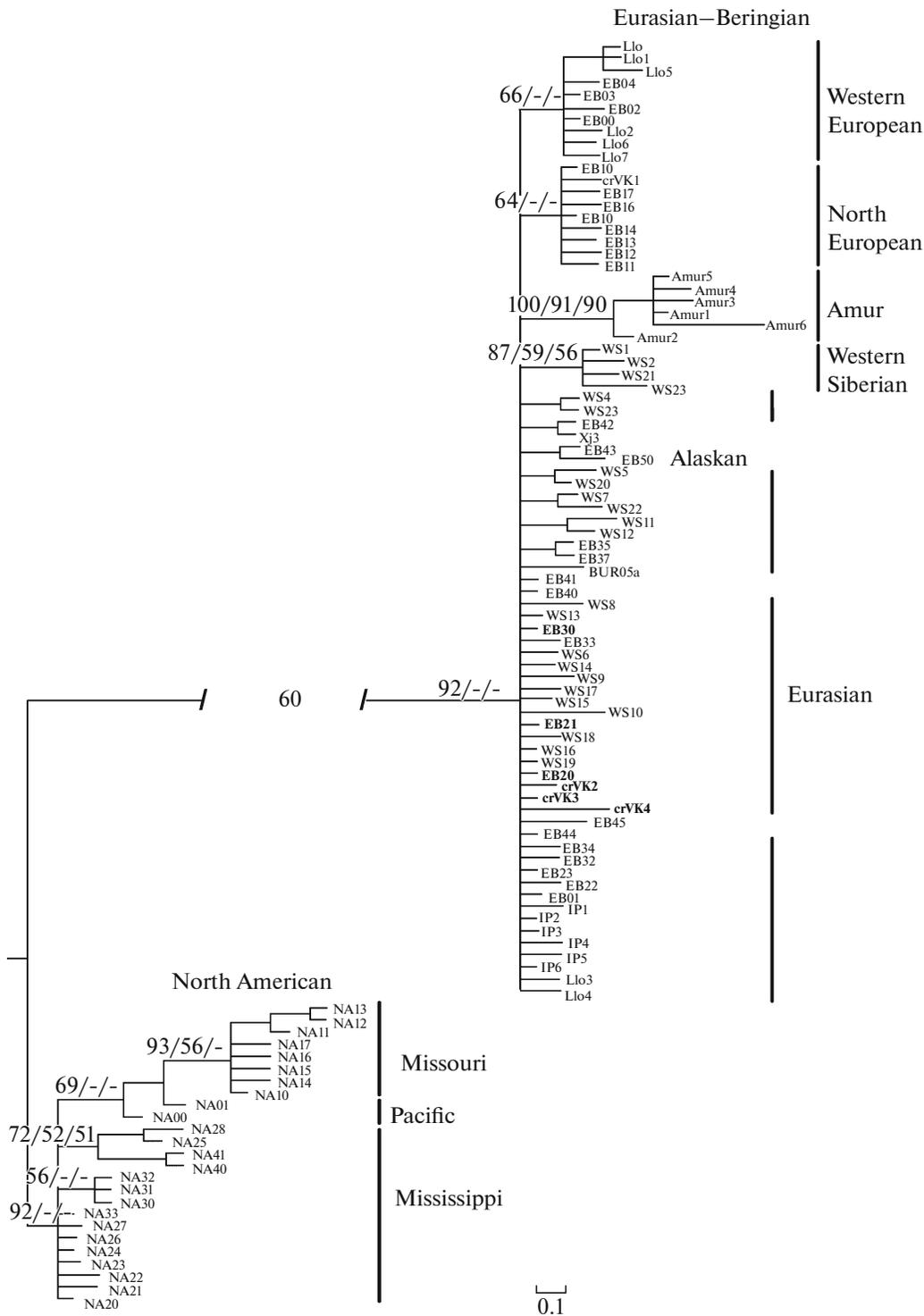


**Fig. 2.** Phylogenetic tree (Bayesian analysis (BI)) for the *cyt b* haplotypes (1063 bp) of *L. lota*. The haplotypes found in the Volga–Kama river basin are shown in bold. Values over the branches indicate a posteriori probabilities (BI) > 60/maximum likelihood method (ML) > 50/neighbor joining method (NJ) > 50, dash is the low probability. The breaks in branches indicate reduction of the branch length (%).

For 23 burbot individuals from the Volga–Kama basin (12 individuals from the Vyatka River and 11 from the Mezhevaya Utkha River), the complete CR sequences were sequenced (804 bp) and 11 haplotypes were described, among which eight were new and were deposited in the GenBank (NCBI) database with accessions MN685694–MN685704. The use of only a small CR region (409 bp) for the phylogenetic reconstructions resulted in a decrease in the total number of haplotypes from 11 to 8: four haplotypes (crVK1–crVK4) were new and four haplotypes (EB10, EB20, EB21, and EB30) were described previously within the Eurasian–Beringian lineage [2]. The EB20 haplotype is found in both rivers (in five and six individuals from the Vyatka and Mezhevaya Utkha rivers, respectively). Haplotypes EB10 (two individuals), EB21 (one individual), crVK1 (one individual), and crVK2 (one individual) were found only in the Vyatka River, and haplotypes EB30 (three individuals), crVK3 (one individ-

ual), and crVK4 (one individual) were found only in Mezhevaya Utkha River (Fig. 1).

The results of the phylogenetic reconstruction on the basis of CR sequences (Fig. 3) are consistent with the data obtained on the basis of *cyt b* sequences and do not contradict the existing notions of the phylogeographic structure of the species [2, 5, 6]. The new haplotypes found by us (crVK1–crVK4) do not form a separate clade. Among the haplotype described previously, three (EB20, EB21, and EB30) belong to the Eurasian haplogroup and are widespread: EB20 in the Danube River basin, Lake Onega, and Kyrönjoki River (Finland); EB21 in the Danube River basin, Vistula River, and Ob River; and EB30 in the Central Europe, Western and Eastern Siberia. The latter is considered one of the basal haplotypes for the entire Eurasian–Beringian lineage [2]. The fourth haplotype, EB10, found in the Vyatka River, was previously described in Scandinavia [2] and is considered as basal



**Fig. 3.** Phylogenetic tree (BI) for the CR haplotypes (409 bp) of *L. lota*. The haplotypes found in the Volga–Kama river basin are shown in bold. Values over the branches indicate a posteriori probabilities (BI > 60/ML > 50/NJ > 50), dash is the low probability. The breaks in branches are reduction of the branch length (%).

for the North-European group, which also included one of the Vyatka haplotypes (crVK1) (Fig. 3).

The relatively high haplotype diversity (cyt  $b$ ,  $h = 0.605$ ; CR,  $h = 0.759$ ) at a low nucleotide diversity (cyt

$b$ ,  $\pi$  (%) = 0.108; CR,  $\pi$  (%) = 0.424) of the burbot from the Volga–Kama river basin points to its recent dispersal within the modern water area of the basin, which is consistent with the ideas on the formation of

the genetic differentiation of the Eurasian–Beringian lineage as a whole.

Thus, the analysis of the phylogenetic relationships of the burbot from the Volga–Kama river basin suggests that, similarly to the Danube basin, it played an important role in the formation of the genetic diversity of the species in Europe and in certain periods could serve as a peculiar corridor connecting river systems of the European and Asian parts of the species range.

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#### COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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