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ANIMAL GENETICS

The Genetic Diversity of Burbot (*Lota lota* L., 1758) of Western Siberia (the Analysis of the mtDNA Control Region Polymorphism)[1]

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Abstract—The genetic variability of burbot (*Lota lota* L., 1758) inhabiting the Ob-Irtysh and Taz river basins in Western Siberia has been studied based on the polymorphism of the hypervariable fragment of mtDNA control region (407 bp). The analysis of 134 fish samples revealed 30 haplotypes, 23 of which were new. Among haplotypes, previously detected in Eurasia and North America, EB30 was the most frequently found in Western Siberia (45.5% frequency). The results of our study are in agreement with previous research pointing to the genetic differentiation of two burbot subspecies, *L. l. lota* and *L. l. maculosa*, and indicate that burbot inhabiting the Ob-Irtysh and Taz river basins belong to the Eurasian-Beringian clade (nominative subspecies *L. l. lota*). However, a high genetic diversity of burbot in Western Siberia, along with a relatively high differentiation of burbot groups within studied territory, points to a regional specificity of burbot population.

Keywords: mitochondrial genome, polymorphism, phylogeography, genetic differentiation, freshwater ichtyofauna, circumpolar range

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INTRODUCTION

Burbot (*Lota lota* L., 1758) is a freshwater species of the Lotidae family with a circumpolar distribution and is a single representative of the *Lota* genus. *L. lota* inhabits rivers and lakes of Eurasia and North America across the Northern Hemisphere to about 40 degrees north latitude [1–3]. Being a top predator, it plays an important role in ecosystems function. In some regions this species is the object of amateur and commercial fishery. Due to its wide Holarctic distribution, a significant ecological and commercial role, burbot is one of the most studied species. However, some issues concerning burbot are still contradictory, including the ones, related to its intraspecies differentiation. Based on the morphological analysis of *L. lota*, which was earlier thought to be a monotypic species [4], three subspecies were described by the middle of the 20th century: *L. l. lota*, *L. l. maculosa* and *L. l. leptura* [5]. Further studies, however, pointed to the clinal variability of some morphological parameters, which threw a doubt on the existence of the latter subspecies [6–8].

During the last decades, genetic methods, including the mitochondrial DNA (mtDNA) polymorphism analysis, are widely used to resolve taxonomic problems, study genetic variability and conduct phylogeographic research. The results of such research can shed the light on the historical formation of species range and are used to evaluate the impact of global climatic changes and anthropogenic factors. Along with the increasing reliability of phylogeographic reconstructions and clarification of the history of modern intraspecies burbot structure formation, the data on mtDNA variability may play an important role in the species conservation [9–11].

The studies of intraspecies genetic structure of burbot employing two genetic markers, mtDNA cytochrome *b* and control region, pointed to the existence of two well differentiated phylogroups of *L. lota* [12, 13], which correspond to the two subspecies, previously distinguished on the basis of morphological criteria. One of the phylogroups, North American burbot subspecies *L. l. maculosa*, occupies the southern part of burbot North American habitat up to the Great Slave Lake (Canada). The other burbot phylogroup is represented by a nominative subspecies *L. l. lota* with a circumpolar distribution. The intraspecies genetic studies of *L. l. maculosa* was carried out extensively includ-

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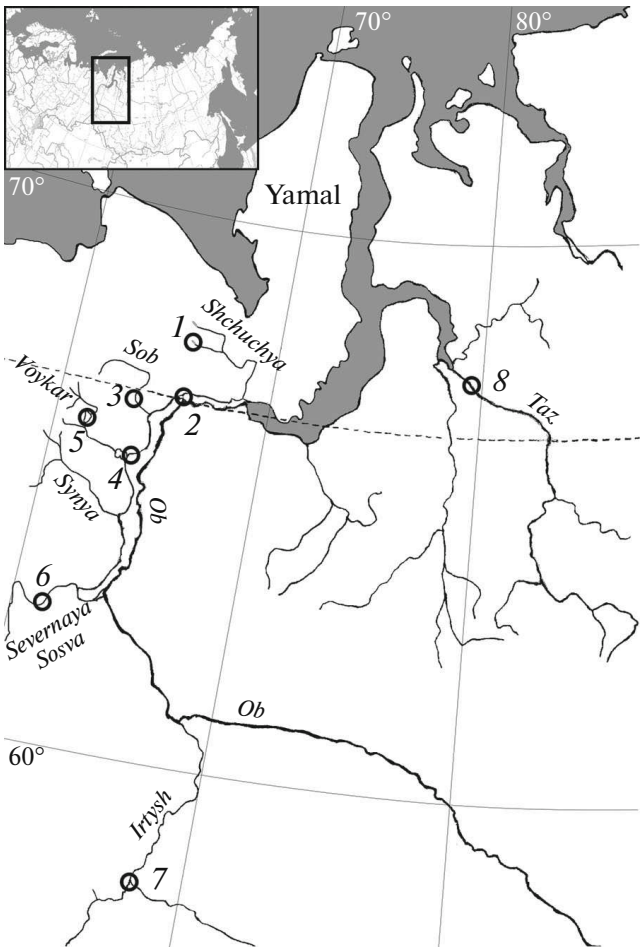

Fig. 1. The schematic representation of the localities across Western Siberia, where burbot samples (*L. lota*) were collected. The Ob-Irtysh River basin: *1*, "Khadata"—Bolshoe Khadata-Yugan-Lor Lake (mountain part of the Shchuchya River basin, Shchuchya River is the left-bank tributary of the Lower Ob); *2*, "Karantinskiy"—the Ob River (the Malaya Ob tributary), in the vicinity of Labytnangi town; *3*, "Sob"—the Sob River (the left-bank tributary of the Lower Ob); *4*, "Ust-Voykar"—the Ob River (the Gornaya Ob tributary); *5*, "Varchato"—Varchato Lake (the River Voykar basin, the left-bank tributary of the Ob River); *6*, "Severnaya Sosva"—the Severnaya Sosva River (the left-bank tributary of the Ob River); *7*, "Tobolsk"—a site of flowing of the Tobol River into the Irtysh River. The Taz River basin: *8*, "Taz"—the Taz River in the vicinity of Tazovskiy village.

ing the analysis of two subspecies submixture and the level of hybridization with *L. l. lota* [14], however, the five clades, described for nominative subspecies *L. l. lota* [13], were detected without taking into account the genetic diversity of burbot inhabiting some major river basins of Central and Eastern Eurasia. As was shown recently [15, 16], the data on genetic diversity of burbot from these territories can significantly change the concept of the intraspecies genetic structure of the nominative subspecies *L. l. lota*. The analysis of the population genetic structure of burbot, using two mtDNA markers, cytochrome *b* and control region, pointed to the existence of a distinguished clade of *L. lota* inhabiting the Amur River (China), which is suggested by the authors to represent another burbot subspecies along with *L. l. lota* and *L. l. maculosa* [15]. The preliminary data on the control region of mtDNA variability of burbot samples, taken from the two localities in the Ob-Irtysh River basin (Western Siberia) [16], also point to the necessity of further genetic studies of burbot inhabiting Central Eurasia in order to complete the reconstruction of *L. l. lota* phylogeographic structure. One should note, that the role of Western Siberia in the development of natural communities is often underestimated, although this region, along with Europe, Eastern Siberia, and North America, has a unique geological history and represents biogeographic crossroads affecting the formation of modern biomes of the Northern Hemisphere [17–19]. Moreover, the Ob-Irtysh River basin in Western Siberia, one of the major river basins in Eurasia, provides the highest amount of burbot in the world [20, 21].

Taking into account a possible significance of Western Siberian burbot populations in the formation of intraspecies *L. lota* structure, our study aimed to analyze the genetic variability of burbot inhabiting the Ob-Irtysh and Taz river basins employing the polymorphism of the mtDNA control region.

MATERIALS AND METHODS

The genetic analysis of burbot inhabiting Western Siberia was conducted based on the sequencing of mtDNA control region of 134 individuals, caught in 2013–2014 at eight localities (Fig. 1): seven sites are located in the Ob-Irtysh River basin from the mouth of the Tobol River up to the Gulf of Ob including the left bank uralian tributaries of the Ob, while one locality is represented by the lower course of the Taz River in the vicinity of Tazovskiy village.

Genomic DNA was extracted from the samples of muscle tissue and fin clips, stored in 96% ethanol, using a modified high salt method [22, 23]. 854 bp long PCR product corresponding to the burbot full length mtDNA control region along with the flanking segments of tRNA-Pro and tRNA-Phe was amplified employing primers LProF [24] and 12S5R [25]. Amplifications were carried out in 30 μL final volume containing 10 to 100 μg template DNA, 1× PCR *Taq*-buffer containing potassium chloride (Fermentas (Thermo Fisher Scientific)), 1.25 mM $MgCl_2$, 50 μM dNTP (SibEnzyme), 0.5 μM of each primer and 0.5 U *Taq*-polymerase (SibEnzyme). The reactions were submitted to an initial 1-min denaturation at 95°C and then 45 cycles with denaturation at 95°C for 10 s, primer annealing at 58°C for 20 s, and extension at 72°C for 50 s, followed by a final 10-min extension at 72°C. The fragments, excised from 1% agarose gel, were purified employing high concentration (6 M) of chaotropic salt (NaI), which was followed by the sorption to silica and

Table 1. The characteristics of the genetic polymorphism of the burbot mtDNA sequences (854 bp) including the full length CR sequence (based on the analysis of burbot samples from Western Siberia)

Region, basin, samples dataset	Number of						
	samples	haplotypes	polymorphic sites	substitutions	transitions	transversions	insertions / deletions
Western Siberia	134	45	33	32	19	13	7
Taz River Basin, Taz	11	5	7	7	4	3	0
Ob-Irtysh River Basin:	123	44	33	32	19	13	7
Khadata	8	2	2	2	2	0	0
Karantinskiy	18	10	11	10	7	3	1
Sob	38	18	17	17	9	8	4
Ust-Voykar	21	13	16	17	11	6	0
Varchato	8	4	4	4	4	0	0
Severnaya Sosva	3	3	3	2	2	0	1
Tobolsk	27	13	16	15	9	6	1

the elution of DNA into ddH_2O [26]. Sequencing of purified PCR products was carried out using ABI3130 Genetic Analyzer (Applied Biosystems) employing the same primer pair.

Forward and reverse contigs were assembled via BioEdit 7.2.5 [27] and aligned using MEGA v. 5.1 [28]. Interpopulation genetic differentiation (AMOVA, F_{st}), haplotype diversity (h), nucleotide diversity (π) and mean number of pairwise differences between haplotypes (k) were estimated using ARLEQUIN v. 3.5 [29]. Bayesian phylogenetic inference was performed using MrBAYES v. 3.2.2 [30, 31] following the model of Hasegava–Kishino–Yano of nucleotide substitution with gamma-distributed rate variation among sites and a proportion of invariant sites (HKY + I + G) [32]. The evolutionary model was selected after running ModelGenerator v. 0.85 [33] according to the Akaike Information Criterions (AIC1 and AIC2) and the Bayesian Information Criterion (BIC). The Markov Chain Monte Carlo (MCMC) simulation was run for 10000000 generations saving trees every 1000 generations. The consensus tree was calculated after discarding the initial 25% of trees as burn-in. Node stability was evaluated using posterior probabilities. To visualize a phylogenetic tree the FigTree v. 1.4.2 program [34] was applied. In comparative analysis along with our data sequences of 39 haplotypes of the mtDNA control region of *L. lota*, described in papers [13, 15] and presented in the GenBank (NCBI), were also included.

RESULTS

A fragment of mtDNA of 854 bp length from 134 samples of burbot, obtained from eight localities in Western Siberia, was sequenced. It included 6 nucleotides of the upstream tRNA-Pro gene, full sequence of the control region (CR) and 3' 44 bp-long part of tRNA-Phe gene. The alignment of analyzed 854 bp long fragments demonstrated 33 polymorphic sites including 7 indels (insertions/deletions) and 29 sites with substitutions, 16 of which were parsimony informative (Fig. 2, Table 1). For comparative analysis of obtained CR sequences with the data from the GenBank (NCBI) we employed a shorter CR sequence (herein short CR sequence) of 407 bp length from the 5' end, which included 20 polymorphic sites. This fragment is homologous to the maximum number of burbot CR sequences, uploaded in the GenBank, which comprise a hypervariable segment of *L. lota* CR (the first 400 bp) representing 90% of the CR variability [13].

The analysis of 134 CR sequences revealed 30 haplotypes (Table 2), seven of which were described earlier for the Eurasia territories (EB21, EB30, EB33, EB35, EB41, EB43, EB44), and three were found in North America (EB30, EB35, EB44) [13, 14]. 31.2% of all the analyzed short CR sequences from Western Siberia was represented by 23 previously never detected haplotypes, named WS1–WS23 (Western Siberian 1–23). The obtained new sequences (WS1–WS23) were uploaded in the GenBank (NCBI) under the nos. KX017626–KX017648. Among all the haplotypes, detected in the studied region, the percentage of WS haplotypes accounted for 76.7% and in analyzed localities it varied from 0 to 66.7% (Table 2). The WS1 was shown to be the most wide-spread haplotype and it was recorded in the half of analyzed localities. Among the earlier described haplotypes, two haplotypes were shown to be the most frequent and widespread: EB30 (with a relatively high frequency it was present in all the localities except for the "Severnaya Sosva," which was presented by a low number of samples, $N = 3$) and EB41 (detected in the all analyzed localities) (Table 2). All four burbot haplotypes, found

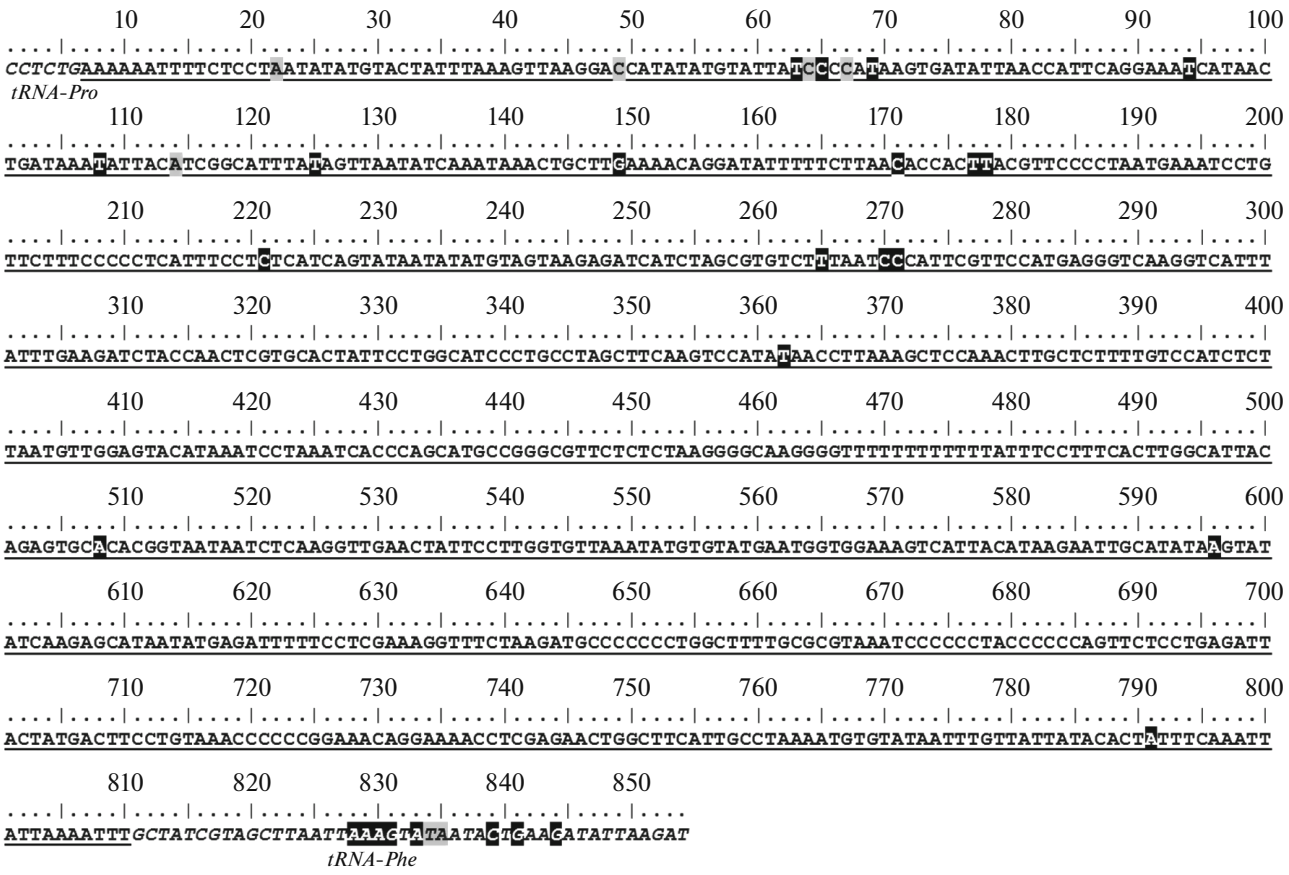

Fig. 2. Polymorphic sites (white font on the black background highlights substitutions, black font on the gray background demonstrates indels (at positions 64, 67 and 835 are also present substitutions)) of the 854 bp long burbot mtDNA CR sequences, detected in samples from Western Siberia; the full length CR sequence is underlined.

in the Taz River basin, were also shown within burbot inhabiting the Ob-Irtysh River basin localities.

In Table 3, it is demonstrated that the values of haplotype and nucleotide diversity of burbot, caught in the Taz River basin and in the middle and lower courses of the Ob-Irtysh River basin (Western Siberian Plain) were significantly higher compared to the ones from the upper course of the Ob-Irtysh River basin (the Black Irtysh River, China): $h = 0.380 \pm 0.067$, π ($\times 100$) = 0.116 ± 0.112, however, these values were comparable to the values of *L. lota* from the upper course of the Amur River basin ($h = 0.702 \pm 0.050$, π ($\times 100$) = 0.239 ± 0.180) [15]. The genetic diversity of burbot inhabiting Western Siberia was in general of the same level as in the whole Eurasian-Beringian clade of the Eurasian group ($h = 0.85$, π ($\times 100$) = 0.47) [13], to which the haplotypes found in Western Siberia belong. The variability of h, π and k indices among the Ob-Irtysh River basin localities was insignificant except for the "Khadata" locality, where these indices were 3.5 times lower compared to the rest of the localities.

Interpopulation genetic differentiation was evaluated based on the variance of frequencies of the mtDNA haplotypes (AMOVA, F_{st}). The analysis of molecular variance (AMOVA) showed insignificant levels of interpopulation differentiation, which accounted for only 1.78% ($P = 0.145$) of variance, while the rest of dispersion accounted for the intrapopulation variability. A significant interpopulation differentiation was recorded only when comparing the following burbot populations: Khadata–Varchato ($P = 0.043$), Ust-Voykar–Varchato ($P = 0.029$), and Ust-Voykar–Tobolsk ($P = 0.016$).

The phylogenetic tree, constructed on the basis of Bayesian phylogenetic inference of burbot short CR sequences, demonstrated the presence of the two major clades with a high support (Fig. 3), which correspond to the earlier described two lineages of burbot, considered to be two burbot subspecies [13]: Eurasian-Beringian clade (*L. l. lota* subspecies), comprising the haplotypes from Eurasia and the north-western part of North America and North-American clade (*L. l. maculosa* subspecies), which is represented by the haplotypes from the south-eastern part of the species range in North America. The specific differentiation is also observed within the two clades. One should note, that within the Eurasian-Beringian clade, which also includes the haplotypes from Western Siberia, a group

Table 2. The distribution of short mtDNA CR (407 bp) sequences in analyzed data sets of burbot from Western Siberia

Haplotype	Names of localities							
	Khadata	Karantinskiy	Sob	Ust-Voykar	Varchato	Severnaya Sosva	Tobolsk	Taz
EB21		1						
EB30	7	9	17	11	2		8	7
EB33			1					
EB35			2	1		1	1	
EB41	1	3	2	2	2	1	4	1
EB43							1	
EB44			2		2		1	2
WS1			3		2		4	1
WS2							1	
WS3							1	
WS4							2	
WS5			1					
WS6		1	1					
WS7			2					
WS8							1	
WS9			1					
WS10				1				
WS11				1				
WS12		1						
WS13			3	2				
WS14			1					
WS15				1				
WS16			1					
WS17				1				
WS18		1					1	
WS19						1		
WS20		1						
WS21		1	1	1				
WS22							1	
WS23							1	
Number of haplotypes	2	8	14	9	4	3	13	4
The number of unique haplotypes per locality, %	0.0	62.5	64.2	66.7	25.0	33.3	61.5	25.0

of haplotypes from the Amur River has a strict differentiation. Besides, two WS groups are also distinguished with a high support: one group was represented by two haplotypes, detected in the Irtysh River ("Tobolsk"—WS4, WS23) and another one is consisted of four burbot haplotypes from the Ob-Irtysh River basin (WS1, WS2, WS3, and WS21 within "Karantinskiy," "Sob," "Ust-Voykar," "Varchato," and "Tobolsk" localities). A number of earlier described Eurasian-Beringian haplotypes [13] form separate groups: EB15–EB17 were detected in lakes in the north of Finland and Kola Peninsula, while EB20 and EB22 were found in the central Europe. The rest of Eurasian-Beringian clade consists of either groups with a low support or some haplotypes were not grouped at all (Fig. 3).

DISCUSSION

Based on the genetic diversity of 134 burbot samples from the Ob-Irtysh and Taz River basins of West-

Table 3. Genetic diversity indices of burbot inhabiting Western Siberia, based on the results of mtDNA CR (407 bp) sequences

Region, basin, samples dataset (number of samples)	$h \pm SD$	π (×100) $\pm$ SD	$k \pm SD$
Western Siberia (134)	0.771 ± 0.035	0.471 ± 0.293	1.869 ± 1.077
Taz River Basin, Taz (11)	0.600 ± 0.154	0.262 ± 0.211	1.065 ± 0.760
Ob-Irtysh River Basin (123):	0.786 ± 0.036	0.477 ± 0.302	1.941 ± 1.110
Khadata (8)	0.250 ± 0.180	0.123 ± 0.132	0.500 ± 0.472
Karantinskiy(18)	0.745 ± 0.102	0.481 ± 0.320	1.954 ± 1.161
Sob (38)	0.792 ± 0.065	0.462 ± 0.300	1.877 ± 1.097
Ust-Voykar (21)	0.729 ± 0.102	0.464 ± 0.311	1.886 ± 1.121
Varchato (8)	0.857 ± 0.082	0.422 ± 0.313	1.714 ± 1.114
Severnaya Sosva (3)	1.000 ± 0.272	0.493 ± 0.464	2.000 ± 1.612
Tobolsk (27)	0.883 ± 0.044	0.638 ± 0.393	2.598 ± 1.435

h—haplotype diversity; π—nucleotide diversity; *k*—mean number of pairwise differences between haplotypes; SD—standard deviation.

ern Siberia employing 407 bp long mtDNA CR fragment as a marker we revealed 30 haplotypes, 23 of which were not detected earlier neither in Eurasia, nor in North America. Over a half (68.7%) of all the CR sequences were represented by seven haplotypes, described earlier for the territory of Eurasia [13], moreover, 45.5% of all the CR sequences belong to the Eurasian haplotype EB30, which is dispersed within European (Isar, Visla, Elba) and Asian (Lena) rivers, and was also detected in the territory of North America [14].

The results of phylogenetic analysis, which was conducted employing our burbot samples along with the CR sequences (GenBank), described for burbot inhabiting other regions of Eurasia (Europe, Eastern Siberia, China) and North America, do not contradict the earlier described concepts on the *L. lota* differentiation into the two major clades: Eurasian-Beringian and North American, which also correspond to the two subspecies, *L. l. lota* and *L. l. maculosa*, however, the genetic structure of Eurasian-Beringian clade differs in comparison to the schemes, offered earlier [12–15]. A high differentiation of burbot haplotypes from the Amur River basin within the Eurasian-Beringian clade was revealed in the work, dedicated to the studies of the population genetic structure of burbot populations from China [15]. Earlier within the nominative subspecies *L. l. lota* five groups of haplotypes were distinguished (except for the Amur group, the haplotypes of which were published later) with a relatively strict geographic differentiation, also supported by a geologic history of these regions: Western European group including burbot haplotypes from Denmark, France, Italy, the Netherlands, and Switzerland; Northern European group comprising burbot haplotypes, detected for Norway, Sweden, Finland, and Russia (Kola Peninsula); Beringian group of Russian burbot haplotypes (Central and Eastern Siberia, the Far East) along with North American burbot haplotypes; Alaskan group; and finally the most widespread Eurasian group consisting of burbot haplotypes from Finland, Germany, Poland, Sweden, and Russia (Buryatia) [13]. Van Houdt et al. [13] considered the formation of burbot modern genetic structure as a result of the post-Pleistocene recolonization from three European refugia and proposed a hypothesis that the territory from the Danube River up to Baikal Lake served as a transition zone of European haplotypes burbot expansion across Eastern and Western Siberia to Beringia and further to North America. The data from Eurasian territory between Eastern Europe and Eastern Siberia, which is covered by major river systems with a complicated formation history was missing in the previous works [13], which could hinder the above-described conclusions. The CR sequences of burbot inhabiting the Ob-Irtysh and Taz river basins, included into our analyses, are a significant addition to the data on burbot CR haplotypes [13], which also provide new insights into the model of genetic differentiation of *L. l. lota* [13], indeed, within the Eurasian-Beringian clade we have obtained four highly differentiated haplotype groups, two of which were completely formed exclusively by WS-haplotypes (Fig. 2).

Based on our analyses we also revealed a high genetic diversity of burbot inhabiting Western Siberia (Table 3), which was comparable to the described earlier Eurasian group of the Eurasian-Beringian clade [13]. The major part of all the CR sequences from the studied territory belongs to the haplotypes, recorded earlier for the territories of Eurasia and North America [13, 14], which points to the joint history of the formation of genetic structure of burbot from Northern Eurasia and Alaska. A relatively high share of unique *L. l. lota* haplotypes across Western Siberia and the presence of highly differentiated haplotype groups within this

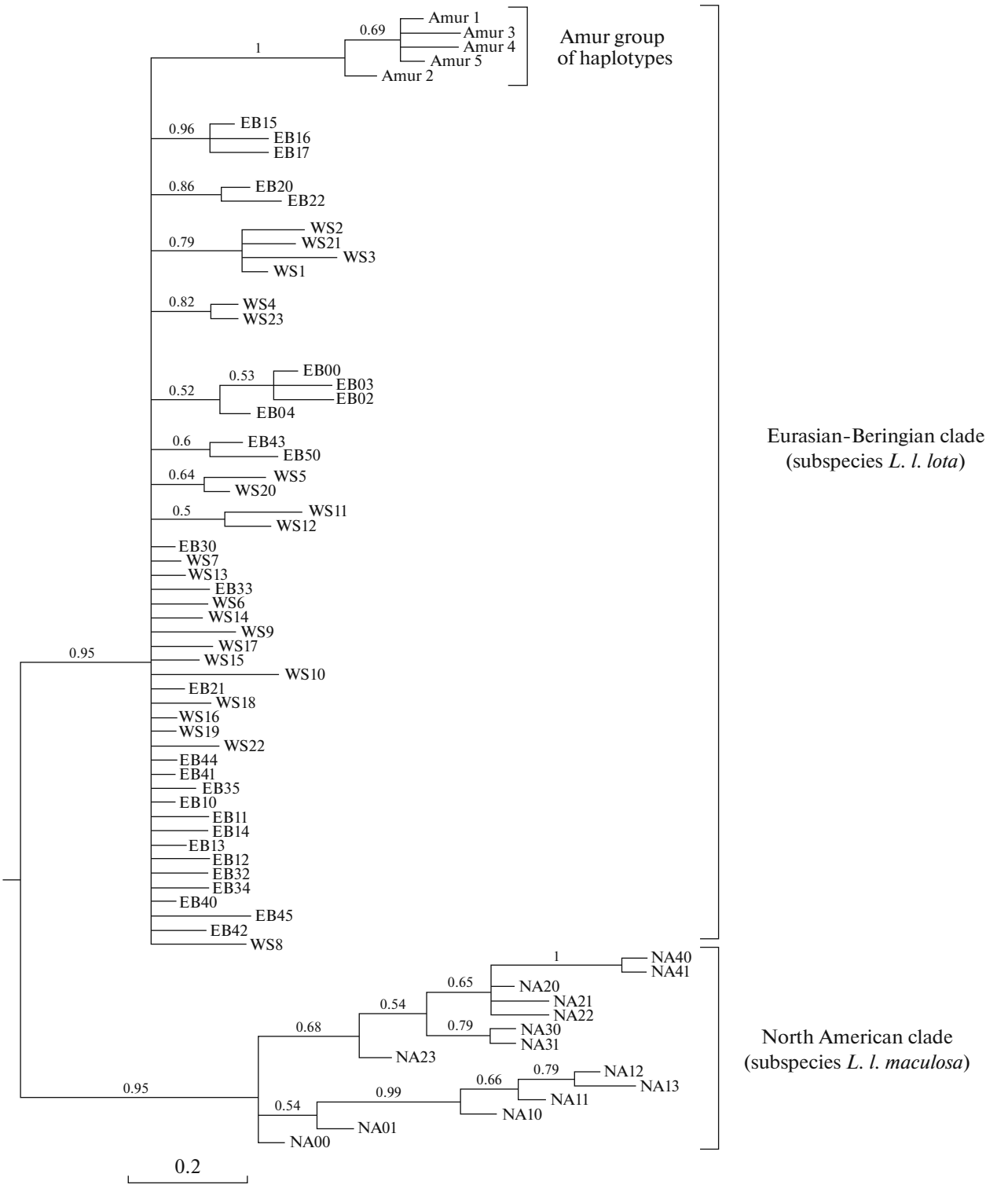

Fig. 3. Phylogenetic tree inferred using Bayesian approach employing 69 CR sequences (407 bp) of *L. lota* mtDNA. Numbers on branches indicate posterior probabilities. The scale interval corresponds to the number of nucleotide substitutions per site. Haplotypes are marked as following: Amur—unique for the Amur River basin haplotypes [15], WS—unique Western Siberian haplotypes, EB—haplotypes of the Eurasian-Beringian clade, which were described earlier (*L. l. lota*) [13], NA—North-American clade haplotypes (*L. l. maculosa*) [13].

area, however, is a sign of a regional specificity of burbot population, which was caused by both geologic history and autogenetic processes.

Therefore, the results of the study of Western Siberian burbot genetic diversity points to the glitches in the reconstruction of the evolutionary history of the species without taking into account the data from major regions. Obviously, to obtain a reasonably truthful reconstruction of the genetic structure formation of the Eurasian-Beringian clade it is crucial to include data from the other major basins (in particular, Caspian and Yenisei river basins), the geological history of which could affect the genetic variability of burbot and shed the light on the formation of genetic structure of Eurasian and Beringian burbot populations.

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