

## Ecological, Morphological, and Genetic Diversity of Burbot (*Lota lota* L., 1758) in Large River Basins of Western Siberia

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**Abstract**—A study has been performed on the ecological, morphological, and genetic diversity of burbot (sample size  $n = 204$  and  $n = 134$ , respectively) from eight localities of the Ob–Irtysh and Taz river basins, Western Siberia. Fish differentiation in body size and weight depending on the dominant type of migration behavior, foraging conditions, and physiographic features of habitats has been revealed. A high genetic diversity (854-bp mtDNA control region) and a low level of between-sample differentiation is evidence for high intergroup gene flow.

**Keywords:** burbot, population structure, migration behavior, variation, growth rate, mtDNA control region, *Triaenophorus*

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The burbot (*Lota lota* L., 1758) is a circumpolar freshwater species with a wide spectrum of ethological and ecological adaptations to diverse habitat conditions. The world's largest group of burbot inhabits the Ob–Irtysh and Taz river basins, Western Siberia. According to data on commercial catches in 1991 to 2000, the burbot stocks of the middle and lower Ob and Taz accounted for 47–98% of Russia's total harvest and 23–48% of world's total harvest of this species [1, 2]. Western Siberia has an area of about 2.5 million km<sup>2</sup>, extending from the Ural Mountains to the Yenisei River and from the Arctic Ocean to the Kazakh Low Hills. The Ob River length (downstream of the source of the Irtysh) is 5410 km, its drainage basin has an area of 2990 thousand km<sup>2</sup>; the Taz river is 1401 km long, its drainage basin occupies about 150 thousand km<sup>2</sup>. Habitat conditions for local groups of burbot markedly differ within such a vast area, and this may have an impact on the formation of species population structure in the region and on its ecological, morphological, and genetic variation. Thus, studies on burbot migration and reproduction have shown that the Ob–Irtysh basin is inhabited by a large group of semianadromous burbot, while local settled groups of these fish are formed in large lakes and tributaries of different orders [3, 4].

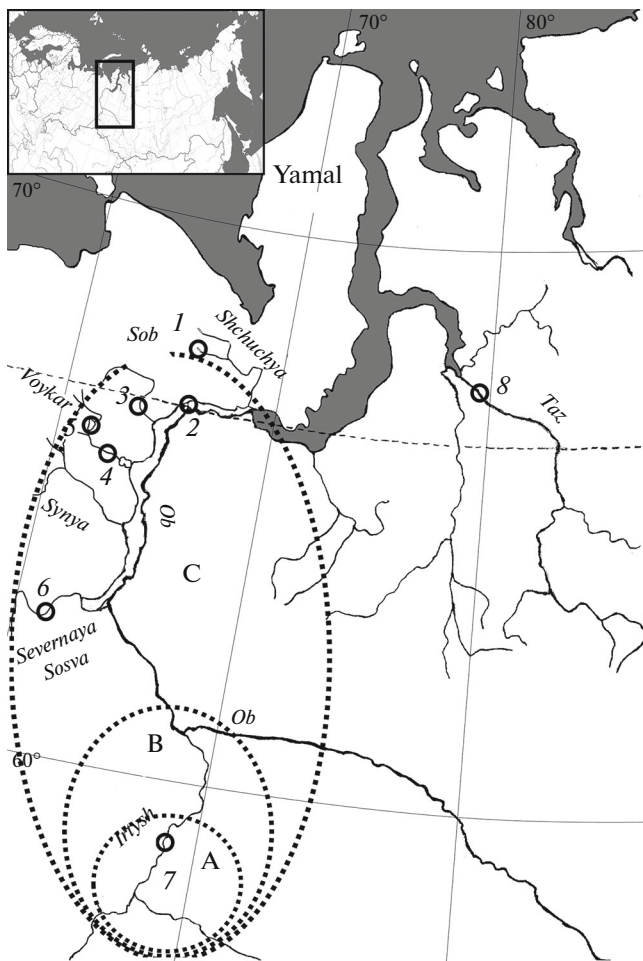
Analyzing polymorphism in the mtDNA control region of burbot, we have revealed high genetic diversity of this species in Western Siberia [5, 6], which is

comparable to that in large phylogroups distinguished in Eurasia [7], and identified several relatively high-differentiated haplotype groups, including those consisting of sequences described only from the study region. The results of our studies on the genetic diversity of burbot in Western Siberia are in line with the concept of highly abundant group occupying a large area with highly diverse habitat conditions. Therefore, it was of special interest to compare data on the ecological, morphological, and genetic variation of burbot from lakes and watercourses of different orders in the Ob–Irtysh and Taz basins.

### MATERIAL AND METHODS

The fish were collected with stake and drift gill nets, Kinaliev and ichthyoplankton nets, and hook-and-line gear in seven localities of the Ob–Irtysh basin and one locality of the Taz basin, in places of burbot foraging, migration, and spawning (2000–2013) (Fig. 1).

Bolshoye Khadata-Yugan-Lor and Maloye Khadata-Yugan-Lor lakes (locality 1) are actually a single lake formed after partial moraine damming of the Bolshaya Khadata River (the Shchuchya River basin) in its upper mountain reaches (210 m a.s.l.). The river flowing out of the lake has numerous violent rapids with steep drops (above 32 km from the mouth) [8], its gradient in the middle reaches is 4.6 m/km.



**Fig. 1.** Schematic map of the study region and localities of burbot sampling in Western Siberia: (1) Bolshoye and Maloye Khadata-Yugan-Lor lakes, (2) Ob River, (3) Sob River, (4) Vorkar River, (5) Varchato Lake, (6) Severnaya Sosva River, (7) Irtysh River, (8) Taz River. Dotted lines delimit zones of burbot migrations from the lower reaches of the Irtysh (sample 7): (A) resident fish, (B) “roaming” (migration distance  $\leq 700$  km), (C) semianadromous fish (migration distance  $\leq 2000$  km).

The Ob River in the middle reaches (locality 2) has a unique floodplain system with numerous channels and temporary pools formed by branches of the Bolshaya, Malaya, and Gornaya Ob, which is up to 65 km wide. The lower floodplain may remain flooded for a period of up to 130 days. The gradient of the Ob in the lower reaches averages about 0.2 m/km. Winter fish kills in the main channel are common in the middle and lower Ob.

The Sob, Vorkar, and Severnaya Sosva (localities 3, 4, and 6, respectively) are left-bank tributaries of the Ob. These spawning rivers originate in the Ural Mountains and have well-developed floodplain systems in the mouth reaches.

Varchato Lake (the Vorkar River basin, locality 5) lies in the Polar Urals foothills (43 m a.s.l.). This is a

relatively shallow flowing-water lake with a surface area of 55 km<sup>2</sup>. The spawning Tanyu River flows into the lake in the northeast, and the Varcha-Ty-Vis stream flows out of it in the southwest, with its gradient averaging 0.12 m/km.

In the lower reaches of the Irtysh (locality 7), the current speed decreases as the river mouth is approached, and the floodplain system in this zone consists of oxbow lakes and temporary pools that serve as foraging areas for juvenile and adult fish.

Such temporary floodplain water bodies are also numerous in the middle and lower reaches of the Taz River (locality 8). As in the Ob, winter fish kills are common in this river, spreading over most of its tributaries and throughout the main channel, from the source to the Taz Bay.

To study the ecological and morphological variability of Western Siberian burbot, 204 mature fish were collected from these localities. Parameters used for the morphological description of spawners included standard body length, total body weight, and carcass weight [9]. Body condition was estimated from the hepatosomatic index calculated as the percent ratio of liver weight to carcass weight [10]. Fish sex, food spectrum, and the numbers and species composition of parasites were determined. Age determination was based on ring counts in otoliths and vertebrae. Body length was measured with calipers to 1 mm accuracy; body and organ weights, with a Kern CH15K20 digital hanging scales and a Kern 442-51 balance (Kern & Sohn GmbH, Germany) to 1 g accuracy. To estimate typical foraging conditions for fish during their lifetime, average annual increments in body length and weight (with and without viscera) were calculated as the ratio of body length/weight to complete age in years. The results were processed statistically by methods of descriptive statistics, Epps–Pally and Mann–Whitney tests, and principal component analysis (PCA) based on correlation matrix using Statistica for Windows v. 6.0 and a package for statistical analysis of interval-censored observations on one-dimensional continuous random variables, v. 4.2.41.21.

Analysis of genetic diversity was based on data on the variability of mtDNA fragment containing the control region in 134 individuals. Total DNA was extracted from the samples of muscle tissue and fin clips fixed in 96% ethanol by a modified high-salt method [11]. An 854-bp fragment corresponding to the full-length mtDNA control region with segments of flanking genes (*tRNA-Pro* and *tRNA-Phe*) was amplified using primers LProF [12] and 12S5R [13]. PCR was conducted in 30  $\mu$ L final volume containing 10–100  $\mu$ g template DNA, 1 $\times$  PCR *Taq* buffer with potassium chloride (Fermentas – Thermo Fisher Scientific), 1.25 mM MgCl<sub>2</sub>, 50  $\mu$ M dNTP (SibEnzyme), 0.5  $\mu$ M of each primer and 0.5 U of *Taq* polymerase (SibEnzyme). Initial denaturation at 95°C for 1 min was followed by 45 cycles of denaturation at 95°C for

10 s, primer annealing at 58°C for 20 s, elongation at 72°C for 50 s, with final elongation at 72°C for 10 min. To purify PCR products before sequencing, they were subjected to 1% agarose gel electrophoresis, the corresponding fragments were cut out of the gel and dissolved in a saturated solution of chaotropic salt (6 M NaI); DNA was then adsorbed on silica beds and eluted into solution. Amplicons from each fish were sequenced with the ABI Prism 3130 Genetic Analyzer using Big-Dye v.3.1 kits (Applied Biosystems) and the same primers as above.

Forwards and reverse contigs were assembled manually using BioEdit 7.2.5 [14] and aligned with MEGA v. 5.1 [15]. Arlequin v. 3.5 [16] was used to calculate inter-group genetic differentiation (AMOVA, *Fst*), haplotype diversity (*h*), nucleotide diversity ( $\pi$ ), mean number of pairwise differences between haplotypes (*k*), and also Tajima's *D* and Fu's *Fs* tests of selective neutrality.

## RESULTS AND DISCUSSION

Data on the sex ratio and size–weight parameters of Western Siberian burbot are presented in Table 1.

The normality hypothesis for the distribution of certain parameters in samples 3, 4, 5, and 7 (the Sob and Voykar rivers, Varchato Lake, the lower reaches of the Irtysh) was rejected at 0.01 significance level. However, their distribution proved to be normal in samples from spawning tributaries of the Ob flowing from the Urals that have no large lakes in the upper reaches (the Severnaya Sosva) and are not inhabited by resident burbot groups. An analysis of the entire dataset from Western Siberia did not confirm the normality hypothesis, indicating that the studied samples belong to different general populations.

Parasitological studies in zoology and ichthyology are highly effective as supplementary means to elucidate the history of range formation and migrations of host species [17, 18]. In the burbot, cestodes *Triaenophorus* sp. (Pseudophyllidea) may serve as a marker of migration activity. Their first intermediate hosts are planktonic crustaceans of the subclass Copepoda. Studies on foraging conditions for semianadromous burbot have shown that juvenile fish prefer biotopes with a low current speed [3], where they feed on plankton and become directly infected by these cestodes. In adult burbot, a major proportion of the diet consists of planktivorous fish species [19], the second intermediate hosts of *Triaenophorus* sp., and in this case reinvasion by cestodes takes place. The ingested plerocercoids usually migrate from the gut to the liver without developing into the adult stage [20–22].

The intensity of infection by *Triaenophorus* sp. plerocercoids in burbot from the lower reaches of the Irtysh (sample 7) was found to differ among individuals, unlike in samples from the Ural tributaries, Ob channel, and Taz River where infection intensity was consistently high. Since the main habitats for *Triaeno-*

*phorus* sp. in the Ob–Irtysh basin are lakes, temporary floodplain water bodies, river segments with slow current, etc. [23, 24], infection of burbot takes place mainly in the lower Ob floodplain ad, to a lesser extent, in the mouth reaches of the Irtysh. Therefore, the absence of these cestodes in the liver of burbot from the lower Irtysh (sample 7) indicates that these fish have spent the greater part of their life in the river segment with a relatively high current speed (1 m/s or higher) near the inflow of the Tobol River (Fig. 1). We considered that fish with only one to three plerocercoids in the liver most probably foraged during the past few years in the mouth reaches of the Irtysh or in the adjacent segment of the Ob (up to 700 km downstream from the Tobol mouth); individuals with more than ten plerocercoids in the liver were classified as semi-anadromous, since prevalence of *Triaenophorus* sp. infection among them reached 100% [21]. Thus, based on the intensity and prevalence of plerocercoid infection, the burbot sample from the lower Irtysh was divided into three groups: resident fish, fish migrating for distances of up to 700 km (conditionally named “roaming”), and semianadromous fish migrating for up to 2000 km (Fig. 1, Table 2).

Unlike in the total sample from the lower Irtysh (Table 1), the size–weight parameters of fish in these three groups conformed to normal distribution, except for the total body weight of resident fish (Table 2). A likely explanation to this exception is that fish of this group were collected over the entire year, with the hepato- and gonadosomatic indices changing significantly by seasons; consequently, the total body weight also changed during the year [25]. The validity of this explanation is confirmed by the fact that the carcass weight in the resident group is a normally distributed parameter. Therefore, it is this parameter that should be used in calculations in order to reduce systematic observation error.

The resident, roaming, and semianadromous groups of burbot from the lower Irtysh were found to differ in size–weight parameters (Table 2). For example, the average annual increment in carcass weight (allowing differences in age and viscera weight between the samples to be leveled off) increases in the series resident–roaming–semianadromous fish, with differences between the groups being statistically significant (pairwise Mann–Whitney test: resident vs. semianadromous and roaming vs. semianadromous,  $U_{\text{emp}} = 0.0, p \leq 0.01$ ; resident vs. roaming,  $U_{\text{emp}} = 142, p \leq 0.01$ ).

A PCA analysis of morphological variability of burbot from the Ob–Irtysh and Taz basins showed that 94% of the total variance is explained by the first two principal components, PC1 and PC2: 82 and 12%, respectively (Table 3). All the test parameters (factors) contribute to PC1, with their contributions having the same (negative) sign. The main factor contributing to

**Table 1.** Sex ratio and size–weight parameters of burbot from the studied localities of Western Siberia

Sample no.	N	Male : female ratio	Completed age, years	Standard body length, mm	Total body weight, g	Carcass weight, g	Hepatosomatic index	Average annual increment in standard body length, mm	Average annual increment in body weight, g	
									total	without viscera
1	15	1 : 1.5	$\frac{11.3 \pm 2.1}{8-15}$	$\frac{435 \pm 53 (0.05)}{340-520}$	$\frac{516 \pm 172 (0.02)}{200-800}$	$\frac{410 \pm 137 (0.02)}{159-636}$	$\frac{2.5 \pm 0.6 (0.07)}{1.5-3.7}$	$\frac{39 \pm 7 (0.08)}{29-56}$	$\frac{46 \pm 14 (0.26)}{25-65}$	$\frac{36 \pm 11 (0.27)}{20-52}$
2	18	1 : 2	$\frac{8.1 \pm 1.2}{6-11}$	$\frac{729 \pm 50 (0.20)}{645-790}$	$\frac{3046 \pm 614 (0.12)}{1820-4560}$	$\frac{2413 \pm 452 (0.06)}{1520-3540}$	$\frac{12.6 \pm 3.5 (0.12)}{6.3-18.5}$	$\frac{91 \pm 9 (0.10)}{72-108}$	$\frac{374 \pm 41 (0.18)}{303-453}$	$\frac{297 \pm 28 (0.06)}{250-350}$
3	38	2 : 1	$\frac{9.0 \pm 2.2}{4-13}$	$\frac{684 \pm 93 (0.18)}{510-860}$	$\frac{2759 \pm 1102 (0.37)}{1180-5280}$	$\frac{2001 \pm 784 (0.36)}{880-3640}$	$\frac{6.9 \pm 2.2 (0.23)}{3.3-12.2}$	$\frac{79 \pm 15 (0.96)}{54-128}$	$\frac{300 \pm 69 (0.33)}{183-440}$	$\frac{218 \pm 48 (0.28)}{138-305}$
4	21	2.4 : 1	$\frac{7.2 \pm 2.4}{4-12}$	$\frac{686 \pm 120 (1.08)}{583-886}$	$\frac{2903 \pm 1724 (1.08)}{1600-6500}$	$\frac{2017 \pm 1174 (1.10)}{1100-4500}$	$\frac{8.8 \pm 3.0 (0.07)}{3.9-14.6}$	$\frac{100 \pm 18 (0.13)}{69-151}$	$\frac{380 \pm 116 (0.42)}{263-633}$	$\frac{264 \pm 78 (0.57)}{190-444}$
5	17	1.1 : 1	$\frac{4.8 \pm 0.7}{3-6}$	$\frac{577 \pm 58 (1.02)}{453-638}$	$\frac{1568 \pm 432 (0.36)}{820-2300}$	$\frac{1127 \pm 318 (0.62)}{440-1450}$	$\frac{10.8 \pm 3.5 (0.10)}{5.2-20.0}$	$\frac{123 \pm 17 (0.14)}{91-160}$	$\frac{328 \pm 76 (0.30)}{164-460}$	$\frac{235 \pm 57 (0.47)}{120-300}$
6	9	1 : 2	$\frac{9.4 \pm 2.5}{6-13}$	$\frac{767 \pm 97 (0.12)}{595-910}$	$\frac{4524 \pm 1877 (0.24)}{1420-6400}$	$\frac{3039 \pm 1184 (0.25)}{1100-4150}$	$\frac{10.1 \pm 3.6 (0.10)}{4.9-14.7}$	$\frac{84 \pm 14 (0.14)}{69-104}$	$\frac{466 \pm 137 (0.01)}{237-656}$	$\frac{314 \pm 82 (0.02)}{183-439}$
7	65	1.3 : 1	$\frac{4.7 \pm 1.6}{2-10}$	$\frac{394 \pm 104 (2.61)}{249-672}$	$\frac{607 \pm 582 (5.64)}{140-2420}$	$\frac{494 \pm 472 (5.69)}{116-1940}$	$\frac{9.1 \pm 3.5 (0.04)}{2.5-18.3}$	$\frac{88 \pm 17 (0.08)}{53-135}$	$\frac{116 \pm 71 (4.91)}{35-362}$	$\frac{95 \pm 58 (4.87)}{29-318}$
8	21	1.1 : 1	$\frac{8.21 \pm 1.7}{7-12}$	$\frac{678 \pm 67 (0.11)}{570-810}$	$\frac{3022 \pm 803 (0.24)}{1910-5000}$	$\frac{2110 \pm 562 (0.11)}{1350-3520}$	$\frac{9.3 \pm 2.6 (0.11)}{4.3-15.5}$	$\frac{85 \pm 13 (0.02)}{63-111}$	$\frac{369 \pm 63 (0.17)}{273-517}$	$\frac{259 \pm 49 (0.42)}{193-377}$
1–8	204	1.2 : 1	$\frac{7.1 \pm 2.8}{2-15}$	$\frac{571 \pm 168 (1.36)}{249-910}$	$\frac{1954 \pm 1533 (2.25)}{140-6500}$	$\frac{1426 \pm 1068 (2.10)}{116-4500}$	$\frac{8.7 \pm 3.8 (0.19)}{1.5-20.0}$	$\frac{86 \pm 23 (0.35)}{29-160}$	$\frac{254 \pm 148 (1.28)}{25-656}$	$\frac{187 \pm 103 (1.44)}{20-444}$

Here and elsewhere, the numbers of samples correspond to those of localities in Fig. 1. Figures above and below the line show mean value with standard deviation and range of values, respectively. Figures in parentheses show Epps–Pally test values, with boldface indicating that the normality hypothesis was rejected at 0.01 significance level.

**Table 2.** Sex ratio, size–weight parameters, and values of Epps–Pally test for their deviation from normal distribution (in parentheses) in resident, “roaming,” and semianadromous burbot from the lower reaches of the Irtysh River

Parameter	Group		
	(A) resident	(B) roaming	(C) semianadromous
Sample size, ind.	43	12	10
Male : female ratio	1.5 : 1	1 : 1.5	1 : 1
Completed age, years	$4.1 \pm 1.2$ 2–8	$5.1 \pm 1.4$ 3–7	$6.8 \pm 1.7$ 5–10
Number of <i>Trienophorus</i> sp. plerocercoids in the liver	$\frac{0}{0}$	$\frac{1.4 \pm 0.7}{1-3}$	$\frac{21.9 \pm 9.0}{10-35}$
Standard body length, mm	$344 \pm 46 (0.06)$ 249–490	$404 \pm 56 (0.23)$ 340–527	$596 \pm 75 (0.39)$ 495–672
Total body weight, g	$355 \pm 148 (0.61)$ 140–994	$509 \pm 201 (0.44)$ 310–900	$1811 \pm 574 (0.3)$ 834–2420
Carcass weight, g	$288 \pm 110 (0.33)$ 116–685	$420 \pm 156 (0.37)$ 268–791	$1468 \pm 482 (0.29)$ 717–1940
Hepatosomatic index	$10 \pm 3.5 (0.01)$ 2.7–18.3	$8.2 \pm 3.0 (0.25)$ 2.5–12.3	$6.3 \pm 2.1 (0.06)$ 3.3–10.5
Average annual increment in standard body length, mm	$88 \pm 18 (0.06)$ 53–135	$83 \pm 18 (0.17)$ 62–114	$90 \pm 15 (0.08)$ 63–111
Average annual increment in total body weight, g	$85 \pm 18 (0.23)$ 35–124	$101 \pm 25 (0.02)$ 52–143	$265 \pm 64 (0.08)$ 167–362
Average annual increment in carcass weight, g	$70 \pm 14 (0.31)$ 29–88	$84 \pm 19 (0.07)$ 47–113	$215 \pm 57 (0.13)$ 143–318

Figures above and below the line show mean value with standard deviation and range of values, respectively. Boldfaced Epps–Pally test values indicate that the normality hypothesis was rejected at 0.01 significance level.

**Table 3.** Results of principal component analysis for morphological variability of Western Siberian burbot

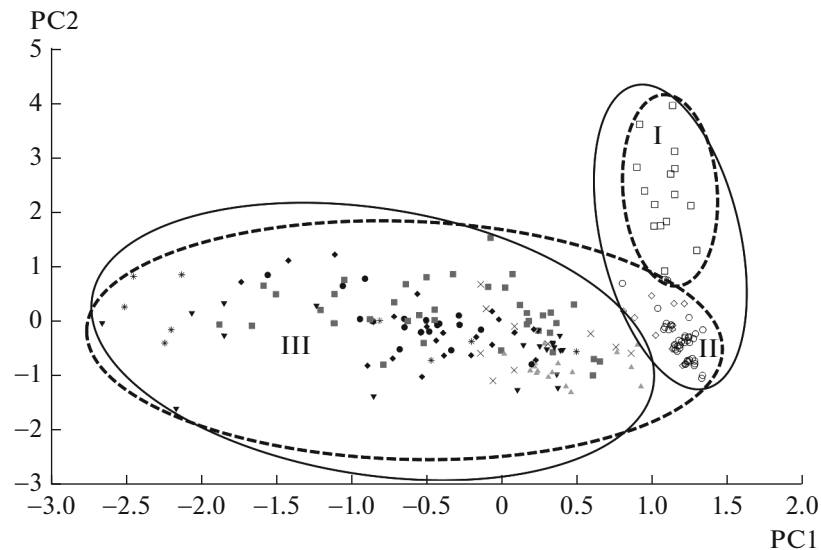
Parameter	Factor loadings				
	PC1	PC2	PC3	PC4	PC5
Age	–0.717	0.681	0.150	–0.006	0.0001
Standard body length	–0.954	–0.012	–0.239	–0.178	0.008
Total body weight	–0.981	–0.087	–0.084	0.139	0.054
Carcass weight	–0.986	–0.085	–0.093	0.093	–0.063
Liver weight	–0.869	–0.354	0.339	–0.063	0.002
Eigenvalue	4.1	0.60	0.21	0.06	0.01
Explained variance, %	82	12	4	1	1

PC2 is the age of fish, and its contribution is positive, while those of other factors have the negative sign.

In the plane of the first two principal components (Fig. 2), samples from Bolshoye and Maloye Khadata-Yugan-Lor lakes and resident and roaming groups from the lower Irtysh (samples 1, 7A, and 7B, respectively) are clearly differentiated from other samples along the PC1 axis (reflecting the variability of

size–weight parameters), and sample 1 is also differentiated along PC2 (reflecting age-related variability). The body length and weight and growth rate of fish in these three samples are lower than in samples from other localities (Table 1), with the differences being highly significant (Table 4).

The increment of body length and weight in burbot depends on foraging conditions and food spectrum [4,



**Fig. 2.** Distribution of burbot individuals from the Ob-Irtysh and Taz basins (Western Siberia) in the plane of the first two PCs: (I) sample 1; (II) samples 7A and 7B; (III) samples 2–6, 7C, and 8. Contours delimited by solid and dashed lines indicate differentiation along PC1 and PC2, respectively.

25, 26]. A resident way of life usually does not allow successful foraging throughout the year because of limited amount of high-energy food resources (with invertebrates prevailing in the diet), ambient conditions for males and females are the same, and their ratio in different physiological periods of life remains almost equal [27, 28]. This results in the stunted growth of fish, as is the case with burbot from Bolshoye and Maloye Khadata-Yugan-Lor lakes and resident and roaming groups from the lower Irtysh. This is especially true of fish from the above mountain lake system, where food resources are relatively poor [29]: they have the lowest size–weight parameters at a given age, which is reflected in their differentiation from other samples along the PC2 axis.

Unlike in Bolshoye and Maloye Khadata-Yugan-Lor lakes, food supply to fish in the shallow, well-warmed Varchato Lake is fairly abundant, which apparently provided for a higher rate of their growth (Table 1). It should also be noted that the corresponding sample possibly included a certain proportion of semianadromous fish intermixing with resident fish during the spawning migration, and this could contribute to the variability of size–weight parameters, particularly in the deviation from normal distribution and the absence of statistically significant differences in samples 4 and 5 (Tables 1, 4).

Semianadromous burbot migrate for long distances to optimal foraging areas (spring–summer upstream foraging migration), and their main food is fish, which is more nutritious than invertebrates [4, 19]. These fish (especially females) often remain physiologically unprepared for spawning in due time and fail to spawn [28]. The energy saved in this way accounts for an

increased growth rate observed in semianadromous burbot from the Ob and Taz river basins (samples 2–4, 6, 7C, and 8).

Since the growth rate in semianadromous burbot is higher in females than in males [19], differences in the sex ratio between the samples (Table 1) may contribute to the variability of size–weight parameters. An example is provided by sample 2 from the lower reaches of the Ob and sample 3 from its spawning tributary, the Sob. During the spring–summer anadromous migration, spawners in the Ob are represented mainly by females, which are on average a year younger than spawners from the Sob, where males dominate in numbers. The absolute values of size–weight parameters are greater in fish from the lower reaches of the Ob, and the average annual increments of body length and weight (with and without viscera) differ at a statistically significant level between the two samples (Table 4).

Thus, the analysis of ecological and morphological diversity of burbot from the Ob-Irtysh and Taz basins has revealed differences between the resident and semianadromous groups. These differences are especially distinct when comparing semianadromous fish from rivers and resident fish from isolated mountain lakes with poor food resources. Since the migrational status is not a genetically fixed trait, burbot in the Ob-Irtysh and Taz basins may display both variants of stereotyped migration behavior, depending in habitat conditions. It should also be noted that the average annual increment in carcass weight is the most adequate index for distinguishing between resident and semianadromous fish, since the systematic observation error is reduced in this case.

**Table 4.** Results of the Mann–Whitney test ( $U_{emp}$ ) used to compare average annual increments in the total body weight (above the diagonal) and carcass weight (below the diagonal) in burbot from the studied localities of Western Siberia

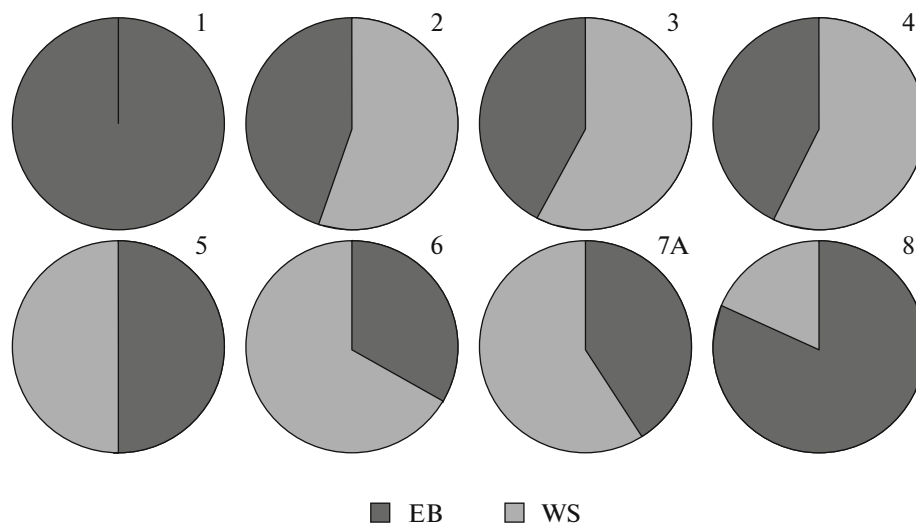
Sample no.		1	2	3	4	5	6	7			8
								A	B	C	
1			<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>26</b>	<b>6</b>	<b>0</b>	<b>0</b>
2		<b>0</b>		<b>146</b>	167	103	38	<b>0</b>	<b>0</b>	<b>13</b>	166
3		<b>0</b>	<b>59</b>		<b>221</b>	237	<b>51</b>	<b>0</b>	<b>0</b>	139	<b>189</b>
4		<b>0</b>	117	254		148	59	<b>0</b>	<b>0</b>	<b>39</b>	203
5		<b>0</b>	<b>49</b>	258	160		<b>28</b>	<b>0</b>	<b>0</b>	41	137.5
6		<b>0</b>	72	<b>54</b>	60	32		<b>0</b>	<b>0</b>	<b>8</b>	47
7	A	<b>22</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>		153	<b>0</b>	<b>0</b>
	B	<b>4</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>142</b>		<b>0</b>	<b>0</b>
	C	<b>0</b>	<b>20</b>	179	68	68	<b>14</b>	<b>0</b>	<b>0</b>		<b>26</b>
8		0	<b>83</b>	<b>232</b>	197	155	52	<b>0</b>	<b>0</b>	56	

Boldface indicates that differences between the corresponding samples are significant at  $p \leq 0.01$ .

The genetic variability of burbot was studied using samples from the same localities, except that only resident fish from the lower Irtysh (sample 7A) were included in analysis. The sequenced fragment of the mtDNA genome (854 bp) contained 6 nucleotides of the *tRNA-Pro* gene, full sequence of the mtDNA control region, and 44-bp part of the *tRNA-Phe* gene. Sequence alignment revealed 33 polymorphic sites, including 7 insertions/deletions (indels) and 29 sites with substitutions, with 16 sites being parsimony informative. A total of 45 haplotypes were identified: 29 haplotypes proved to be unique for Western Siberia (designated WS) and 16 haplotypes contained the

hypervariable fragment of mtDNA control region (the first 400 bp) homologous to sequences previously described from Eurasia and North America (designated Eurasian–Beringian, EB) [7, 30]).

An analysis of haplotype distribution in burbot samples based on a fragment of mtDNA control region (407 bp from the 5' end, 20 variable sites) showed that the ratio of WS and EB haplotypes varied widely, from complete dominance of the latter to their proportion not exceeding one-third of the sample (Fig. 3). As the length of the test fragment was reduced from 854 to 407 bp, the total number of identified haplotypes decreased to 30 (23 WS and 7 EB) [6].



**Fig. 3.** The ratios of haplotypes of the mtDNA control region sequence (407 bp) in samples of burbot from the Ob–Irtysh and Taz basins: EB, Eurasian–Beringian haplotypes previously described in Eurasia and North America; WS, unique haplotypes described in Western Siberia.



**Table 5.** Indices of genetic diversity in the 854-bp mtDNA fragment (including the full-length control region sequence) of Western Siberian burbot and the results of Fu's  $F_s$  test for selective neutrality

Sample no.	$N$	$Nh$	$S$	$h \pm SD$	$\pi(\times 100) \pm SD$	$k \pm SD$	Fu's $F_s$
1–8	134	45	33	0.822 $\pm$ 0.032	0.285 $\pm$ 0.172	2.438 $\pm$ 1.329	–26.864**
1	8	2	2	0.250 $\pm$ 0.180	0.059 $\pm$ 0.063	0.500 $\pm$ 0.472	0.762
2	18	10	11	0.876 $\pm$ 0.063	0.297 $\pm$ 0.182	2.529 $\pm$ 1.427	–3.950*
3	38	18	18	0.821 $\pm$ 0.062	0.287 $\pm$ 0.177	2.445 $\pm$ 1.354	–11.379**
4	21	13	16	0.829 $\pm$ 0.085	0.328 $\pm$ 0.202	2.800 $\pm$ 1.540	–6.819**
5	8	4	4	0.857 $\pm$ 0.082	0.201 $\pm$ 0.149	1.714 $\pm$ 1.114	–0.155
6	3	3	3	1.000 $\pm$ 0.272	0.235 $\pm$ 0.221	2.000 $\pm$ 1.512	–0.693
7A	27	13	16	0.883 $\pm$ 0.044	0.354 $\pm$ 0.212	3.020 $\pm$ 1.625	–4.731*
8	11	5	7	0.709 $\pm$ 0.137	0.188 $\pm$ 0.136	1.600 $\pm$ 1.025	–0.918

Designations:  $N$ , sample size;  $S$ , number of polymorphic sites;  $Nh$ , number of haplotypes;  $h$ , haplotype diversity;  $\pi$ , nucleotide diversity;  $k$ , mean number of pairwise differences between haplotypes; SD, standard deviation; \*  $p < 0.5$ , \*\*  $p < 0.001$ .

The indices of haplotype and nucleotide diversity calculated for 134 individuals (Table 5) provide evidence that the genetic diversity of burbot in Western Siberia is high and comparable to that in Northern, Western, Central, and Eastern Europe, Eastern Siberia, Eastern Asia, and Alaska:  $h = 0.57$ ,  $\pi(\times 100) = 0.16$ ;  $h = 0.54$ ,  $\pi(\times 100) = 0.15$ ;  $h = 0.85$ ,  $\pi(\times 100) = 0.47$ ;  $h = 0.63$ ,  $\pi(\times 100) = 0.22$ ;  $h = 0.70$ ,  $\pi(\times 100) = 0.24$ ;  $h = 0.43$ ,  $\pi(\times 100) = 0.40$ , respectively [7, 31].

Th genetic diversity of burbot from different localities within Western Siberia varied insignificantly (Table 5). Analysis of molecular variance (AMOVA), revealed low levels of differentiation between samples: the proportion of variance explained by this factor was only 1.44% ( $p = 0.15$ ), with the rest being within-sample variance. It should be noted, however, that the indices of genetic diversity in burbot from the Bolshoye and Maloye Khadata-Yugan-Lor lake system (sample 1) were on average 3.5 times lower than in other samples (Table 5), and the spectrum of haplotypes consisted of two most widespread ones, EB30 (87.5%) and EB41 (12.5%). Tajima's  $D$  values [32] for all samples lacked statistical significance, whereas Fu's  $F_s$  [33], a more efficient test, had negative values in the majority of localities. Moreover, its values were statistically significant for samples 2, 3, 4, and 7A (the lower Ob, Sob. Voykar, and the lower Irtysh), indicating that the number of rare haplotypes in these samples was higher than expected from the neutral model of evolution. As an exception, Fu's  $F_s$  for the sample from Bolshoye Khadata-Yugan-Lor Lake had the positive sign (Table 5). Such a result and the low nucleotide diversity in this sample suggest a role for the founder effect in the formation of its genetic structure. However, the statistical significance of  $F_s$  value in this case was not confirmed because of insufficient sample size.

In contrast to other localities, the sample from Bolshoye Khadata-Yugan-Lor Lake consisted only of resident fish. The ingress of semianadromous fish to the lake is prevented due to its hydrological regime formed

under specific physiographic and climatic conditions of the mountain lake–river system (a steep river gradient, numerous rapids, solid freezing of the Bolshaya Khadata river channel in winter, etc.). Spring downstream migration of juveniles from the Bolshoye Khadata-Yugan-Lor Lake–Bolshaya Khadata River system to the Shchuchya River is possible, whereas the reverse (upstream) migration of burbot is hardly probable. The low levels of genetic diversity observed in fish from the Bolshoye and Maloye Khadata-Yugan-Lor lake system appear to be explained by relative isolation of this group from the main Ob population of burbot. Similar results were obtained for the close North American burbot subspecies *L. l. maculosa* [34, 35]. Experiments with nuclear and mitochondrial DNA markers showed that local populations from water bodies where fish migration in both directions is absent or restricted by natural (topographic) or human-made barriers (dams) not only have low genetic diversity but also show considerable genetic differentiation, being relatively isolated from each other.

## CONCLUSIONS

Comparative analysis of data on ecological, morphological, and genetic diversity of burbot from water bodies and watercourses of different orders in the Ob–Irtysh and Taz river basins has revealed differentiation by size–weight parameters between fish from different habitats, which depends primarily on whether these fish are resident or semianadromous. Body length, weight, and their average annual increments are lower in the resident than in the semianadromous form. Foraging conditions in a water body and the degree of its isolation play a considerable role in these differences.

The high genetic diversity of Western Siberian burbot estimated from data on polymorphism in the mtDNA control region and the low level of between-sample differentiation provide evidence for considerable intergroup gene flow taking place both in the past



and present. Analysis of genetic diversity in local groups has shown that its development is influenced by habitat physiographic characteristics conditioning the possibility of fish migration and also by ecoethological features of fish, namely, the dominant type of migration behavior.

Identification of factors having an effect on the formation of morphological and genetic diversity of burbot in large river basins in the present period is necessary for evaluating the contributions of autogenetic and historical-geographic factors while interpreting phylogeographic reconstructions based on morphological and genetic data.

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