

# Variation of the Mitochondrial DNA Control Region in the Populations of Southern Form of Dolly Varden (*Salvelinus malma krascheninnikovi*) from Sakhalin

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**Abstract**—Analysis of a 551-bp segment of the mitochondrial DNA control region in 23 individuals from nine populations of Dolly Varden from Sakhalin and three individuals from the Shikaribetsu Lake (Hokkaido) revealed the presence of seven haplotypes of southern form, along with one haplotype of northern form of Dolly Varden. All seven haplotypes of southern Dolly Varden were earlier described in the populations from Hokkaido. Hierarchical analysis of molecular variance (AMOVA) based on the haplotype frequencies, performed using literature data, suggested that, during the glacial epoch, there were three regional population groups of Dolly Varden (from eastern and western coasts of Sakhalin, and from Southern Primorye). Population groups from Sakhalin and Primorye were clearly separated. The differences between two Sakhalin population groups in the mtDNA haplotype frequencies were not statistically significant. However, relative to the earlier obtained data on microsatellite loci, these differences were statistically significant. For the populations of Sakhalin Dolly Varden, the data on mitochondrial and microsatellite DNA variation supplement each other.

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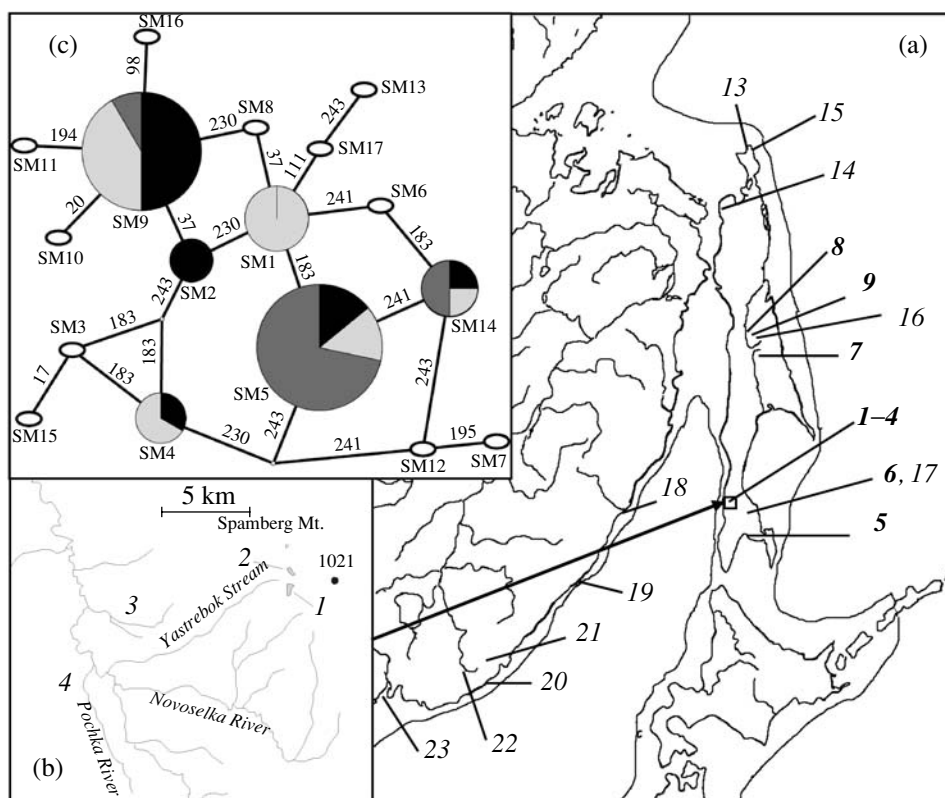
## INTRODUCTION

The Asian coast of the Pacific is inhabited by northern and southern forms of Dolly Varden, whose taxonomic status, similarly to other members of *Salvelinus alpinus*–*Salvelinus malma* complex, is the subject of intensive discussion. The forms have some morphological differences, and their modern ranges practically do not overlap [1–6]. The data on allozymes [7, 8], on the sequence of the first internal transcribed spacer of ribosomal DNA (ITS1 rDNA) [9], on restriction fragments of mitochondrial DNA (mtDNA), and the sequence of a segment of mtDNA control region [12, 13] pointed to substantial divergence of these forms. Earlier analysis of mtDNA control region variation in the populations of *Salvelinus alpinus* complex revealed no distinct differences between the southern and the northern forms of Asian Dolly Varden, albeit the first form was represented by only four fish from two river populations from the Paramushir Island [14]. This was caused by the fact that the fish mentioned had mtDNA of the northern, but not the southern Dolly Varden form. In further studies it was demonstrated that haplotypes typical of the northern form of Dolly Varden were present (including the cases of their high frequency) in a number of populations of the southern form of Dolly Varden. These data were explained in terms of earlier introgressive hybridization, which took place during the secondary contact of these forms [10, 13].

Analysis of a 484-bp segment of the mtDNA control region in the populations of the southern form of Dolly Varden from the Kuril Islands, Sakhalin, and Southern Primorye revealed the presence of six haplotypes of the southern form along with four haplotypes of northern form of Dolly Varden. Moreover, in Sakhalin, four “southern” and three “northern” haplotypes were identified [13]. Analysis of 551-bp segment of the mtDNA control region in the populations of southern Dolly Varden from the Hokkaido Island identified 17 haplotypes of southern Dolly Varden and two haplotypes of white-spotted charr *Salvelinus leucomaenis* [12]. In the present study, new data on the mtDNA control region variation in Dolly Varden from Sakhalin are presented. Using the literature data, some issues concerning the Dolly Varden population structure at the present time and in the history, are discussed. The experiments were performed using resident southern Dolly Varden populations from Sakhalin, which were earlier investigated over the morphological characters [15] and microsatellite loci [16]. For this reason, the data on mitochondrial and microsatellite DNA were compared.

## MATERIALS AND METHODS

Nine population samples of southern form of Dolly Varden from Sakhalin were collected in August through September 2006 (for details on sampling localities, fishing time, and biological features of Dolly Varden



**Fig. 1.** Sampling localities for the Dolly Varden populations from Sakhalin and Primorye (a and b). 1, Mokhovoe Lake; 2, Osochnoe Lake; 3, Krasnaya River; 4, Pochka River; 5, Rogatka River; 6 and 17, Belaya River; 7, Longari River; 8 and 16, upper Tym River; 9, Krasnaya River; 13, Toi River; 14, Tym River; 15, Taliki River; 18, Samarga River; 19, Maksimovka River; 20, Miloradovka River; 21, Izvilinka River; 22, Ussuri River. Samples 13–23 were taken from [13]. The map also shows the coastline edge, where it was 15 thousand years ago (for explanations, see the text). (c) Phylogenetic network of the mtDNA control region haplotypes for southern Dolly Varden (SM1–SM17), constructed using the TCS algorithm. Nucleotide positions differing between the haplotypes are shown on the branches. Haplotypes of southern Dolly Varden from eastern and western coasts of Sakhalin, and from Primorye, are designated by gray, black, and dark-gray shaded circles, respectively. The sizes of the circles and sectors are proportional to the haplotype frequencies (Table 2). Haplotypes identified exclusively on Hokkaido (without taking into account the haplotype frequency) was designated by open ovals. Small open circles designate the “lost” haplotypes.

from different water bodies, see [15]). The samples were as follows: four samples from the Novoselka river basin, southwestern coast of Sakhalin (Mokhovoe and Osochnoe lakes, and Krasnaya and Pochka rivers), one sample from the south of Sakhalin (Rogatka River from the Susuya River basin), one sample from southeast of Sakhalin (Belaya River from the Naiba River basin (Bol’shoi Takoi), one sample from the Longari River (upper Poronai River basin), and two samples from the upper Tym River basin (one from the main riverbed and another from the Krasnaya River (Figs. 1a, 1b). In addition, three individual Dolly Varden fish from Shikaribetsu Lake, Hokkaido Island, kindly provided by S. Yamamoto, were examined.

DNA was isolated from the ethanol-fixed fin pieces using salt-extraction technique [17]. Amplification of a segment of mtDNA control region was performed using polymerase chain reaction with primers HN20 (5'-GTG TTA TGC TTT AGT TAA GC-3') and Tpro2 (5'-ACC CTT AAC TCC CAA AGC-3'). Sequencing was performed using primer Tpro2, and for some indi-

viduals (haplotypes) primer HN20 was used. Sequences were aligned in the CLUSTAL X program [18]. Phylogenetic relationships between haplotypes were evaluated using the method of statistical parsimony [19], realized in the TCS program [20]. Analysis of genetic differentiation over the haplotype frequencies (an analog of the exact Fisher’s test), as well as hierarchical analysis of molecular variance AMOVA [21] based on the haplotype frequencies was performed using the ARLEQUIN 3.1 program [22].

### RESULTS AND DISCUSSION

The sequences of 551-bp control region segments of 23 individuals from nine Sakhalin populations and of three individual fish from Hokkaido were represented by haplotypes of southern Dolly Varden and haplotypes of northern Dolly Varden (Table 1). Haplotype of northern Dolly Varden appeared to be identical to the earlier identified BER3 [14]. All seven haplotypes of southern Dolly Varden from Sakhalin were earlier distinguished

**Table 1.** Variable positions of 551-bp segment of the mtDNA control region in the southern Dolly Varden population samples from Sakhalin and Shikaribetsu Lake, Hokkaido

Haplo-type	1 3 3 5 7 1 1 1 1 2 2 2 2 2 2 2 5
	7 6 7 3 2 0 8 9 9 3 3 4 4 4 5 5 0
	3 3 1 4 0 5 1 3 4 0 5 1
SM1	c g c t a t t a t t c c g t t g c
SM2	. . . . . c . . . . .
SM4	. . . . . c . . c . . a . . . .
SM5	. . . . . c . . . . .
SM9	. . t . . . . . c . . . . .
SM11	. . t . . . . . c c . . . . .
SM14	. . . . . c . . . . t . . . . .
BER3	t a . c g c . - . c t . a c c c t

Note: For common 481-bp segment, haplotypes OKH1=SM14, OKH2=SM5, OKH3=SM4, OKH5=SM9, OKH6=SM1, and BER21=BER3. For explanations, see the text. GenBank accession numbers: AB206973 (SM1) and AF298020 (BER3).

in Japanese populations of Dolly Varden [12], and because of this, they got the corresponding designations. Similarly to the earlier reported [12], in three individual fish from Shikaribetsu Lake, a single haplotype SM11 was identified. Over the common 481-bp segment of the control region, haplotypes earlier described by Shedko et al. [13] were identified. The differences among seven haplotypes of southern Dolly Varden constituted from one to four, and between them and the haplotypes of northern Dolly Varden, from ten to twelve nucleotide substitutions. At control region position 191 of northern Dolly Varden there is a deletion (Table 1).

Phylogenetic relationships between haplotypes identified in resident Dolly Varden from Sakhalin are presented in Fig. 1c. The data on prevalence of different haplotypes in the samples from populations of southern Dolly Varden from Sakhalin and Primorye are presented in Table 2. Analyzing the prevalence of different haplotypes in the samples examined, possible sample errors should be considered, since the sample sizes were not higher than two to three individuals per population. For instance, analysis of 11 microsatellite loci in the samples from the neighboring rivers Krasnaya and

**Table 2.** Prevalence of different haplotypes of the mtDNA control region in southern Dolly Varden samples from Sakhalin and Primorye

Population (basin)	SM1	SM2	SM4	SM5	SM9	SM14	BER3	BER20	BER22
<u>Sakhalin</u>									
1. Mokhovoe Lake (Novoselka)					2				
2. Osochnoe Lake (Novoselka)					3				
3. Pochka River (Novoselka)		1			1	1			
4. Krasnaya River (Novoselka)		1		1					
5. Rogatka River (Susuya)			1	1					
6. Belaya River (Naiba)	1				1	1			
<b>17. Belaya River (Naiba)</b>	1				1				1
7. Longari River (Poronai)	1				1				
8. Krasnaya River (Tym)	1				2				
9. Tym River	1						2		
<b>16. Tym River</b>			1	1			1		
<b>15. Taliki River</b>			1				1		
<b>14. Tum River</b>				1				1	
<b>13. Toi River</b>							1		
<u>Primorye</u>									
<b>18. Samarga River</b>				2					
<b>19. Maksimovka River</b>				1	1				
<b>20. Miloradovka River</b>				2					
<b>21. Izvilinka River</b>				1					
<b>22. Ussuri River</b>				2					
<b>23. Ryazanovka River</b>				2		2			

Note: Haplotype designations are as in Table 1.

Pochka, Novoselka river basin, showed that these samples were indifferent relative to the allele frequencies [16], and they had one shared haplotype out of four mtDNA haplotypes identified (Table 2). The samples from the Belaya River (Naiba River basin) and the upper Tym River, analyzed in the present study and by Shedko et al. [13], differed in the haplotype composition. The differences in haplotype frequencies between all sample pairs of Dolly Varden from Sakhalin were not statistically significant ( $P > 0.05$ ). Comparison of the samples from Sakhalin and Primorye revealed statistically significant differences only between the samples from Osochnoe Lake and the Ryazanovka River ( $P < 0.05$ ). These data indicate that analysis of the small samples can result in substantial underestimation of the level of haplotype diversity in some Sakhalin populations. On the other hand, it cannot be excluded that the differences between some populations do exist, and with an increase of the sample size these differences will become statistically significant.

The data on mtDNA and microsatellite DNA [16] for the Dolly Varden populations from Sakhalin supplement each other and give consistent results. For instance, analysis of Dolly Varden from the Novoselka river basin over 11 microsatellite loci showed that genetic differentiation of two river and two lake populations was low, while it was high between river and lake populations. Moreover, the level of genetic variation in two lake populations was extremely low, while it was high in two river populations, respectively. The differences between these two population groups were statistically significant at all indices of genetic variation. The data obtained indicated that the river and lake populations were isolated from each other (probably, due to the existence of physical barriers). Furthermore, the lake populations have lost a substantial part of genetic variation as a result of a bottleneck effect [16]. In two river populations of Dolly Varden from the Novoselka River basin, a total of four mtDNA haplotypes were identified. At the same time, in two lake populations only one common haplotype was detected (Table 2). These data confirmed the earlier suggestion. Earlier, based on the analysis of morphological characters [15] and microsatellite loci [16], it was concluded that there could be a gene exchange between the populations from the upper tributaries of Tym (Krasnaya River) and Poronai basins (Longari River) during their short-timed joining by a branch during the spring flood. The samples from the Krasnaya River (Tym) and the Longari River possess identical haplotype sets, which favors the suggestion made.

Despite of the small sample sizes, and the number of populations examined ([13] and present study), variation of the mtDNA control region was examined in the Dolly Varden populations from northern, southern, eastern, and western coasts of Sakhalin. The numbers of haplotypes identified in the two studies were only slightly different. In the present study, one more haplotype of southern Dolly Varden, SM4, was identified.

However, we did not detect BER22 and BER20 haplotypes of northern Dolly Varden. The latter haplotype was identified only in one population from the north of the island, from which we had no samples. Sakhalin populations of Dolly Varden may also have some other haplotypes. However, it seems likely that these haplotypes are characterized by low frequencies and local distribution. In 271 Dolly Varden individuals from 22 populations inhabiting different regions of Hokkaido Island, a total of 17 haplotypes of southern Dolly Varden and two haplotypes of white-spotted charr were identified. The appearance of the latter two haplotypes is explained in terms of introgressive hybridization [12]<sup>1</sup>. Some haplotypes (SM8, SM10, SM11, SM12, SM13, and SM16) were detected only in one population, i.e., were private. Haplotypes SM10, SM11, SM13, and SM16 occupy peripheral position in the phylogenetic network, and are suggested to be relatively young. For instance, haplotype SM11 was identified exclusively in an isolated population of Shikaribetsu Lake, which is usually treated as a subspecies *Salvelinus malma miyabei* [23]. Taking into consideration our data, this haplotype is the only (or, at least, extremely highly frequent) in this population. It cannot be excluded, that SM11 appeared in this very population. Haplotype SM9, phylogenetically close to SM11 (the differences in one nucleotide substitution), is found not only in the basin of the river, to which the lake is attributed, but it is also widely distributed in the Dolly Varden populations from Sakhalin and Japan. In general, populations of southern Dolly Varden from Sakhalin are characterized by lower haplotype diversity (not registering the haplotypes of northern Dolly Varden and white-spotted charr) than the populations from Hokkaido, and by substantially higher haplotype diversity than the populations from Primorye. Individual populations from Hokkaido and Sakhalin were characterized by low genetic variation, which probably resulted from their passing through a bottleneck and isolation from other populations, most likely by physical barriers.

Analysis of nine resident populations of Dolly Varden from Sakhalin over 11 microsatellite loci revealed the high level of their genetic differentiation (global values of  $F_{ST} = 0.182$  and  $R_{ST} = 0.325$ , the values are statistically significant,  $P < 0.001$ ). On NJ trees, constructed based on the  $(\delta\mu)^2$  distances [24] and chord distances ( $D_c$ , [25]) two main clusters are reliably isolated. One of the clusters includes Dolly Varden populations from the Novoselka River basin (western coast), while the second cluster consists of all other populations (from the south and eastern coast) [16]. However, according to the hierarchical analysis of molecular variance (AMOVA) based on the microsatellite data, division of all populations into only two groups, one of

<sup>1</sup> Unfortunately, the authors did not present the frequencies of individual haplotypes in the samples, which make impossible using their data for quantitative analysis.

**Table 3.** Hierarchical analysis of molecular variance (AMOVA) based on the haplotype frequencies of mtDNA control region in the populations of southern Dolly Varden from Sakhalin and Primorye

Groups (G1–3): populations (no. 1–23)	Variance components, %			$F_{CT}$	$F_{SC}$	$F_{ST}$
	among the groups (CT)	among the populations within the groups (SC)	within the populations			
Sakhalin						
G1: 1–9	–	7.02	92.98	–	–	0.072
G1: 1–5, 6 + 17, 7–8, 9 + 16, 14–15	–	8.58	91.42	–	–	0.086
G1: 1–4; G2: 5–9	5.94	3.47	90.59	0.059	0.037	0.094
G1: 1–4, 14–15; G2: 5, 6 + 17, 7–8, 9 + 16	2.41	7.15	90.44	0.024	0.073	0.096
Sakhalin and Primorye						
G1: 1–4, 5, 6 + 17, 7–8, 9 + 16, 14–15, 18–23	–	18.98	81.02	–	–	0.190*
G1: 1–4, 14–15, 18–23; G2: 5, 6 + 17, 7–8, 9 + 16	9.11	13.53	77.36	0.091	0.149	0.226*
G1: 1–5, 6 + 17, 7–8, 9 + 16, 14–15; G2: 18–23	26.00	4.11	69.89	0.260**	0.055	0.301*
G1: 1–4, 14–15; G2: 18–23; G3: 5, 6 + 17, 7–8, 9 + 16	20.87	2.95	76.18	0.209**	0.037	0.238*

Note: Haplotypes of northern Dolly Varden were excluded from the analysis. Designations (numbers) of the populations of southern Dolly Varden from Sakhalin and Primorye are given in accordance with Table 2. Samples from Belaya river (nos. 6 and 17) and from Tym river (no. 9 and 16) are pooled. The values of  $F$  statistics are statistically significant, \*  $P < 0.05$  and \*\* $P < 0.01$ .

which includes the populations from the western coast, while the second group consists of all other populations, does not look like preferable relative to other variants of the population groups isolation [16]. Analysis of molecular variance based on the mtDNA haplotype frequencies (Table 3), performed for nine (our data) or 11 (together with the data from [13]) Sakhalin populations of Dolly Varden, revealed a low level of population genetic differentiation (the global  $F_{ST}$  values were 0.072 and 0.086, respectively). In case of isolation of two population groups, one of which is formed by the western-coast populations, while another one consists of all other populations, the  $F_{ST}$  values are somewhat higher. However, only in case of the analysis of nine populations, the variance component falling on the differences between the groups is higher than the variance component falling on the differences within the groups. Furthermore, all  $F$  statistics values obtained at the analysis of Sakhalin Dolly Varden populations are not statistically significant ( $P > 0.05$ ).

Comparison of Dolly Varden samples from Sakhalin and Primorye showed that the differences between the populations from these two regions were more distinct and were associated with the prevalence of different haplotype sets in these populations (Table 2, Fig. 1c) and with the level of haplotype diversity. In Primorye only three haplotypes were identified. Moreover, two of these haplotypes were rare, and only haplotype SM5

was highly frequent in all populations. This haplotype was identified in two populations from northeastern coast of Hokkaido. Haplotype SM5 was detected in southwestern and southern Sakhalin populations, and in the Tym River basin. Haplotype SM14, which is phylogenetically close to SM5, was detected in one population from Primorye, as well as in one population from the northeastern coast of Hokkaido [12, 13]. In Sakhalin, haplotype SM9, as well as haplotypes SM1 and SM2, occupying central position in the phylogenetic network, were most frequent (Fig. 1c). Haplotype SM2 was identified on the northeastern and western coasts of Hokkaido, as well as in the southwest of Sakhalin. The global value of  $F_{ST}$  for all Sakhalin and Primorye populations was equal to 0.190 and was statistically significant ( $P < 0.05$ , Table 3). Haplotypes of northern form of Dolly Varden were absent from Primorye and Hokkaido. In Sakhalin, they were detected on the northern and the eastern coasts. These haplotypes were also found in the populations of Kuril ridge with high frequencies in northern islands [13, 14].

It is known that, as a result of 100- to 130-m regression of the sea level having occurred during the last two glacial periods (60000–40000 and 22000–11000 years ago), the northwest of Sakhalin merged with the mainland (Fig. 1a), while its southern part joined to Hokkaido and Kunashir [26, 27]. It can be suggested that, since Sakhalin experienced no continental glaciation,

many Dolly Varden populations, probably excluding the northernmost ones, could exist during the glacial period. Furthermore, there could be gene exchange between the populations from western coasts of Sakhalin and Hokkaido, as well as between the populations of the eastern coasts of these islands. On the contrary, the gene flow between the eastern and western coastal populations, even if it was, was extremely limited. It seems likely that only during the post-glacial period, with the opening of the straits between Sakhalin and Hokkaido (12000–11000 years ago), and between Sakhalin and the mainland (about 7000 years ago) [26], there appeared the next possibility for gene exchange between the Dolly Varden populations from the western and the eastern coasts of Sakhalin. It is suggested that especially during the post-glacial period, a new stage of southern and northern Dolly Varden dispersal occurred and their secondary contacts were renewed.

Hierarchical analysis of molecular variance was performed using three variants of the isolation of regional population groups. Two of these variants assumed the presence of two population groups during glacial period. In the third variant, the existence of three population groups during the time of interest was assumed. In terms of the first variant, we suggested that during glacial period, populations from eastern and western coasts of Sakhalin were isolated from each other. At the same time, there was gene flow between the populations from western coast of Sakhalin and those from Primorye, and because of this, these populations were united in one group. In the second variant, we suggested that still there was gene flow between the populations from eastern and western coasts of Sakhalin (for example, as a result of up-river interceptions), and these populations were united in one group, while the Primorye populations were isolated from them, and correspondingly, formed another group. The third variant assumed that during the glaciation period, populations from eastern and western coasts of Sakhalin, and those from Primorye were substantially isolated from each other; in other words, there were three regional populations of southern Dolly Varden. The results of the three variants of hierarchical analysis showed that the latest two variants looked like preferred. According to these variants, the variance component, evaluating the differences among the populations within the groups accounted for 3–4% of the total variation, while most part of the variation was explained by the differences between the groups (21 and 26%, respectively). The  $F_{ST}$  and  $F_{CT}$  values were statistically significant (Table 3). Thus, the data obtained pointed to the existence of minimum two regional populations (Sakhalin and Primorye), which were long isolated from each other. It seems likely that during glacial period in Primorye there was a small refugium. The existence of two groups representing the populations from eastern and western coasts of Sakhalin remains questionable. It seems quite probable that in the glacial period, populations from the two coasts of Sakhalin could be isolated

from each other. In this case, the absence of clear differences between them can be explained by the leveling action of the gene flow between these populations, which renewed in the post-glacial period. The idea on the gene flow between the populations of the eastern and data the western Sakhalin coasts is supported by microsatellite DNA [16]. Low level of haplotype variation characteristic of the Dolly Varden populations from Primorye can be explained by either a bottleneck effect in the small refugium during the glacial period, or by a transgression in the post-glacial period. During large transgressions, in small rivers the areas essential for spawning and development of Dolly Varden larvae and fry were greatly reduced, which could result in a dramatic reduction of the population size and even extinction of some populations.

The specific distribution patterns of certain haplotypes in the range of southern Dolly Varden can be at least partly associated with the isolation of some regional populations in the glacial period along with specific dispersal and genetic contacts in the post-glacial period. Based on the available data [12, 13, and the present study], it can be suggested that Dolly Varden populations from northeastern and eastern coasts of Hokkaido are close to the populations of Kunashir and the south of Sakhalin. For these populations, possible dispersal route from Hokkaido along the eastern coast of Sakhalin up to Tym, and possibly northwards, can be followed. By the haplotype composition, four populations from the western coast of Hokkaido differ from the Dolly Varden populations from southern Primorye. This finding can be considered as the evidence in favor of the isolation of these populations during the glacial and the post-glacial periods. In earlier studies, in the rivers of Primorye flowing into the Sea of Japan, populations of blunt-snouted lenok *Brachymystax lenok*, unique relative to the allele composition of allozyme loci, were described, suggesting the existence of a small glacial refugium on this territory [28]. In Sakhalin, blunt-snouted lenok was detected only in the northwest, in the region of Amur estuary, and it was completely absent from Japan.

These results suggest, the existence of past gene flow from the western-coast populations, through the northern populations, to the eastern ones till Tym. Judging by the northern Dolly Varden haplotype distribution patterns, migrants of this form might come to Sakhalin from the north, along the coast of the Sea of Okhotsk. In this context, the presence of another charr, reproductively isolated from southern Dolly Varden, and treated as a novel species *Salvelinus vasiljevae* [29], in some rivers of northwestern Sakhalin, can be explained by secondary contacts either between northern and southern Dolly Varden, or between the southern Dolly Varden populations from different glacial refugia. However, resolution of this issue requires reliable genetic data. The data obtained for other fish species also point to fragmentation of their ranges in the glacial period, as well as on dispersal and secondary contacts

between different populations during interstadials and in the post-glacial period [30, 31].

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#### REFERENCES

1. Taranets, A.Ya., Freshwater Fishes of the North-Western Part of the Sea of Japan Basin, *Tr. Zool. Inst. Akad. Nauk SSSR*, 1936, vol. 4, no. 2, pp. 483–537.
2. Berg, L.S., *Ryby presnykh vod SSSR i sopredel'nykh stran* (Freshwater Fishes of the USSR and Adjacent Countries), Moscow: Izd. Akad. Nauk SSSR, 1948, part 1, 4th ed.
3. Behnke, R.J., A Systematic Review of the Genus *Salvelinus*, *Charrs: Salmonid Fishes of the Genus Salvelinus*, Balon, E.K., Ed., The Hague: Dr. W. Junk, 1984, pp. 441–480.
4. Behnke, R.J., Organizing the Diversity of the Arctic Charr Complex, *Biology of the Arctic Charr*, Proc. Int. Symposium on Arctic Charr, Johnson, L. and Burns, B.L., Eds., Winnipeg: Univ. of Manitoba Press, 1984, pp. 3–21.
5. Gritsenko, O.F., Savvaitova, K.A., Gruzdeva, M.A., and Kuzishchin, K.V., On the Taxonomic Position of Charr *Salvelinus* of the Northern Kuril Islands, *Vopr. Ikhtiol.*, 1998, vol. 38, no. 2, pp. 189–198.
6. Savvaitova, K.A., Gruzdeva, M.A., Kuzishchin, K.V., et al., Riverine Charrs of the Genus *Salvelinus* of the North Kuril Islands, *Vopr. Ikhtiol.*, 2004, vol. 44, no. 1, pp. 89–101.
7. Osinov, A.G. and Pavlov, S.D., Allozyme Variability and Genetic Divergence in Populations of Arctic Charr and Dolly Varden (*Salvelinus alpinus*–*S. malma* Complex), *Vopr. Ikhtiol.*, 1998, vol. 38, no. 1, pp. 47–61.
8. Osinov, A.G., Evolution Relationships between the Main Taxa of the *Salvelinus alpinus*–*S. malma* Complex: Results of Comparative Analysis of Allozyme Data of Different Authors, *Vopr. Ikhtiol.*, 2001, vol. 41, no. 2, pp. 167–183.
9. Phillips, R.B., Gudex, L.I., Westrich, K.M., et al., Combined Phylogenetic Analysis of Ribosomal ITS1 Sequences and New Chromosome Data Supports Three Subgroups of Dolly Varden Char (*Salvelinus malma*), *Can. J. Fish. Aquat. Sci.*, 1999, vol. 56, pp. 1504–1511.
10. Oleinik, A.G., Skurikhina, L.A., and Brykov, V.I.A., The Divergence of the Dolly Varden Char *Salvelinus malma* in the Asian Northern Pacific Populations Inferred from the RSR-RFLR Analysis of Mitochondrial DNA, *Russ. J. Genet.*, 2002, vol. 38, no. 10, pp. 1181–1188.
11. Radchenko, O.A., Genetic Differentiation Inferred from Data on Restriction Analysis of Mitochondrial DNA in the Northern and Southern forms of the Dolly Varden Char, *Russ. J. Genet.*, 2002, vol. 38, no. 4, pp. 421–428.
12. Yamamoto, S., Kitano, S., Maekawa, K., et al., Introgressive Hybridization between Dolly Varden *Salvelinus malma* and White-Spotted Charr *Salvelinus leucomaenis* on Hokkaido Island, Japan, *J. Fish. Biol.*, 2006, vol. 68, suppl. A, pp. 68–85.
13. Shed'ko, S.V., Ginatulina, L.K., Miroshnichenko, I.L., and Nemkova, G.A., Phylogeography of Mitochondrial DNA in South Asian Dolly Varden Char *Salvelinus curilus* Pallas, 1814 (Salmoniformes, Salmonidae): Mediated Gene Introgression?, *Russ. J. Genet.*, 2007, vol. 43, no. 2, pp. 165–176.
14. Brunner, P.C., Douglas, M.R., Osinov, A., et al., Holarctic Phylogeography of Arctic Charr (*Salvelinus alpinus* L.) Inferred from Mitochondrial DNA Sequences, *Evolution*, 2001, vol. 55, pp. 573–586.
15. Pichugin, M.Yu., Gritsenko, O.F., and Osinov, A.G., On the Morphological Diversity of the Southern Dolly Varden Trout *Salvelinus malma krashennikovii* from the Waterbodies of Sakhalin, *Vopr. Ikhtiol.*, 2008, vol. 48, no. 5, pp. 367–390.
16. Osinov, A.G. and Gordeeva, N.V., Microsatellite DNA Variability and the Genetic Differentiation in *Salvelinus malma* Resident Population *krashennikovii* from Sakhalin, *Vopr. Ikhtiol.*, 2008, vol. 48, no. 9, pp. 691–706.
17. Aljanabi, S.M. and Martinez, I., Universal and Rapid Salt-Extraction of High Quality Genomic DNA for PCR-Based Techniques, *Nucleic Acids Res.*, 1997, vol. 25, pp. 4692–4693.
18. Thompson, J.D., Gibson, T.J., Plewnia, F., et al., The ClustalX Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools, *Nucleic Acids Res.*, 1997, vol. 24, pp. 4876–4882.
19. Templeton, A.R., Crandall, K.A., and Sing, C.F., A Cladistic Analysis of Phenotypic Associations with Haplotypes Inferred from Restriction Endonuclease Mapping and DNA Sequence Data: III. Cladogram Estimation, *Genetics*, 1992, vol. 132, pp. 619–633.
20. Clement, M., Posada, D., and Crandall, K.A., TCS: A Computer Program to Estimate Gene Genealogies, *Mol. Ecol.*, 2000, vol. 9, pp. 1657–1659.
21. Excoffier, L., Smouse, P., and Quattro, J., Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data, *Genetics*, 1992, vol. 131, pp. 479–491.
22. Excoffier, L., Laval, G., and Schneider, S., Arlequin Ver.3.0: An Integrated Software Package for Population Genetics Data Analysis, *Evol. Bioinform. Online*, 2005, vol. 1, pp. 47–50.
23. Maekawa, K., Life History Patterns of the Miyabe Charr in Shikaribetsu Lake, Japan, *Biology of the Arctic Charr*, Proc. Int. Symposium on Arctic Charr, Johnson, L. and Burns, B.L., Eds., Winnipeg: Univ. of Manitoba Press, 1984, pp. 233–250.
24. Goldstein, D.B., Ruiz Linares, A., Cavalli-Sforza, L.L., and Feldman, M., Genetic Absolute Dating Based on

- Microsatellites and the Origin of Modern Humans, *Proc. Natl. Acad. Sci. USA*, 1995, vol. 92, pp. 6723–6727.
25. Nei, M., Tajima, F., and Tateno, Y., Accuracy of Estimated Phylogenetic Trees from Molecular Data: II. Gene Frequency Data, *J. Mol. Evol.*, 1983, vol. 19, pp. 153–170.
  26. Bezverkhii, V.L., Pletnev, S.P., and Nabiulin, A.A., Essay of Geologic Structure and Development of the Kuril Island Arc System and Adjacent Territories, in *Rastitel'nyi i zhivotnyi mir Kuril'skikh ostrovov* (Plant and Animal World of the Kuril Islands), Proc. Int. Kuril Project, Vladivostok: Dal'nauka, 2002, pp. 9–23.
  27. Pletnev, S.P., Historical and Geological Development of the Sakhalin Island, in *Rastitel'nyi i zhivotnyi mir Kuril'skikh ostrovov* (Plant and Animal World of the Kuril Islands), Proc. Int. Kuril Project, Vladivostok: Dal'nauka, 2002, pp. 11–22.
  28. Osinov, A.G., Countercurrent Dispersal, Secondary Contacts, and Speciation in Lenoks of the Genus *Brachymystax* (Salmonidae, Salmoniformes), *Genetika* (Moscow), 1993, vol. 29, no. 4, pp. 654–669.
  29. Safronov, S.N. and Zvezdov, T.V., *Salvelinus vasiljevae* sp. nova - a New Species of Freshwater Charr (Salmonidae, Salmoniformes) from Northwestern Sakhalin, *Vopr. Ikhtiol.*, 2005, vol. 45, no. 6, pp. 737–748.
  30. Takahashi, H., Takata, K., and Goto, A., Phylogeography of Lateral Plate Dimorphism in the Freshwater Type of Ninespine Sticklebacks, Genus *Pungitius*, *Ichthyol. Res.*, 2001, vol. 48, pp. 143–154.
  31. Yamamoto, S., Morita, K., Kitano, S., et al., Phylogeography of White-Spotted Charr (*Salvelinus leucomaenis*) Inferred from Mitochondrial DNA Sequences, *Zool. Sci.*, 2004, vol. 21, pp. 229–240.