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Intraspecific Polymorphism of mtDNA in Sakhalin Taimen *Parahucho perryi*

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Abstract—The intraspecific polymorphism of Sakhalin taimen *Parahucho perryi* (Brevoort, 1856), Salmonidae, was assessed via the RFLP analysis of mitochondrial DNA fragments that contain *Cytb*, the control region (*D* loop), and the genes for the NADH dehydrogenase subunits 1–4, as well as the sequencing of the mitochondrial DNA *COI* gene. The statistically significant differentiation of the populations from the mainland coast of the Sea of Japan and the eastern and western coasts of Sakhalin Island was demonstrated. The higher level of haplotype diversity in Sakhalin populations compared to Primorye populations and the structure of haplotype genealogies suggested that all populations of *Parahucho perryi* originated from a common ancestor, which probably lived on the western coast of the Sakhalin Island.

INTRODUCTION

Sakhalin taimen *Parahucho perryi* (Brevoort, 1856) is a unique, relatively rare fish of the Salmonidae family. The only species of the *Parahucho* genus occupies narrow, focal, and steadily dwindling range, which includes the western coast of the Sea of Japan from the Tatar Strait to the Peter the Great Gulf, Sakhalin Island, Southern Kuril Islands, and Hokkaido Island [1, 2]. Sakhalin taimen can live up to 20–22 years, have the largest size compared to other salmonids, and are characterized by a semi-anadromous lifestyle [2–5]. Information on the morphological and biological properties of *Parahucho perryi*, as well as on its ecology and behavior is quite sufficient to form more or less clear idea on this and the range of intraspecific variation of the characters mentioned [4–16, etc.]. At the same time, genetic data that highlight the intraspecific structure of Sakhalin taimen are sporadic and mostly reflect the initial stage of these studies [17–22]. It has also been demonstrated that microsatellite loci were reliable markers for assessing the differentiation of the taimen intraspecific groups [19, 21, 22], while the intraspecific polymorphism of mitochondrial DNA (mtDNA) was not identified [20]. Taking into account the endangered status of Sakhalin taimen [23–25] and the current understanding of the need to conserve the intraspecific molecular genetic diversity of commercially exploited fish, in the present study, we continued to analyze the mtDNA of *Parahucho perryi*. The experimental material was supplemented with new specimens, including samples from the Sakhalin Island. The experiments were performed using RFLP analysis and sequencing. We sequenced a fragment of the mitochondrial gene for the first subunit of the cytochrome c oxidase complex, *COI*. This particular gene was chosen for analysis as one of the mitochondrial DNA genes suitable for phylogenetic reconstructions [26] and is most completely represented in the nucleotide databases. This *COI* gene fragment provides analysis of intra- and interspecific relationships and, because of this, it was proposed for use in DNA barcoding [27]. The Consortium for Barcodes of Life (www.barcoding.si.edu) had approved this gene fragment as a standard marker for the DNA barcoding of animal species.

MATERIALS AND METHODS

Samples and Methods Used for Obtaining Initial Data

Sampling sizes and locality of the populations sampled are represented in Table 1 and Fig. 1. Individual total DNA specimens were obtained from ethanol-fixed hearts and fins using a standard technique [28]. RFLP analysis of three mtDNA regions that encode the *Cytb*/D loop, ND1/ND2, and ND3/NDL/ND4 was described in detail in [20, 29]. Sequencing of the *COI* gene fragment and its amplification using the COI-FishF1 and COI-FishR1 primers [30] was described in detail in [31, 32].

Statistical Analysis

In order to perform a statistical analysis on the RFLP data, the key measures of genetic variations were calculated using the REAP [33] and ARLEQUIN version 3.5 [34] software packages. The level of the

Table 1. Main characteristics of samples of Sakhalin taimen *Parahucho perryi* and Siberian taimen *Hucho taimen*

Species	Population	Geographic position	Latitude/Longitude	Sample size
1. *Parahucho perryi*	Kievka River	Western coast of the Sea of Japan	43°06′ N/134°17′ E	6
2. *P. perryi*	Tumnin River, 1998	″	49°11′ N/140°21′ E	10
3. *P. perryi*	Tumnin River, 2005	″	49°11′ N/140°21′ E	4
4. *P. perryi*	Tumnin River, 2007	″	49°11′ N/140°21′ E	12
5. *P. perryi*	Maksimovka River	″	46°05′ N/137°54′ E	3
6. *P. perryi*	Ainskoe Lake	Sakhalin Island, western coast (basin of the Sea of Japan)	48°29′ N/142°04′ E	16
7. *P. perryi*	Nabil'skii Bay	Sakhalin Island, eastern coast (basin of the Sea of Okhotsk)	51°35′ N/143°17′ E	5
8. *Hucho taimen*	Manoma River	Basin of Amur River	49°21′ N/137°24′ E	2
9. *H. taimen*	Arichi River	″	49°27′ N/136°50′ E	5

within-population polymorphism was assessed using the values of nucleotide (π) and haplotype (h) diversity [35, 36]. The heterogeneity of haplotype frequencies between each pair of samples was assessed using the Monte Carlo test with 10000 pseudorandom replicates [37] and one-way AMOVA analysis with F_{ST} [38]. To assess the genetic variations in the population, the distribution of the differences among the haplotypes based on the number of nucleotide substitutions between all haplotype pairs (mismatch distributions) [39, 40] was analyzed. The quantitative analysis of the geographical partition of the mtDNA variation, which implies the decomposition of total molecular variance of haplotype frequencies into hierarchical levels and subsequent assessment of the degree of genetic differentiation according to the identified hierarchy, was performed using the AMOVA test [38]. To test the statistical significance of the hierarchical components of variance, the corresponding measures of F statistics were calculated [41, 42]. In phylogenetic analysis, the nucleotide divergence was assessed using maximum parsimony (MP) technique as implemented in the PAUP version 4.0b10 [43] and PHYLIP 3.67 [44] software packages (http://evolution.qs.washington.edu/phylip.html). The robustness of clusters obtained was estimated by means of bootstrap analysis with 1000 random permutations [45]. The graphical representation of the dendrograms was obtained in the TreeView software program (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). To reconstruct phylogenetic relationships between mtDNA haplotypes, minimum spanning trees (MST) were constructed using the median network method (MJ algorithm) [46] in the Network 4.5.02 software program (Fluxus Technology Ltd., www.fluxus-engineering.com).

Sequences of the *COI* fragment were aligned using the MEGA5 software program according the ClustalW protocol [47]. The best-fit nucleotide substitution model was calculated in the jModelTest 0.1.1 [48] under the Akaike information criterion (AIC). Genetic distances between the sequences were calculated using the Tamura−Nei (TrN) model [49].

Phylogenetic trees were constructed using neighbor joining (NJ) [50], maximum likelihood (ML) [51], and maximum parsimony (MP) [52] approaches as implemented in the MEGA5 software program [51]. The robustness of the obtained clusters was estimated using bootstrap analysis with 1000 random permutations [45]. The NJ, MP, and ML trees were constructed to provide graphical representation of the deviation patterns between the Sakhalin taimen groups identified. The search for the best-fit phylogenetic tree topology was conducted through reconstruction of Bayesian tree according to the MrBayes 3.2.1 algorithm (http://mrbayes.sourseforge.netindex.php). Haplotype network was constructed using the SplitsTree4 version 4.12.6 (MJ) program [53].

In the present study, nucleotide sequences of Siberian taimen *Hucho taimen* were used in phylogenetic reconstructions as the outgroup. The sequences determined were deposed in the database of the National Center for Biotechnology Information (NCBI/GenBank).

RESULTS

Analysis of Restriction Fragment Length Polymorphism

The total length of the mtDNA fragments amplified constituted 7814 base pairs (bp). The total number of sites inferred from the analysis of the restriction
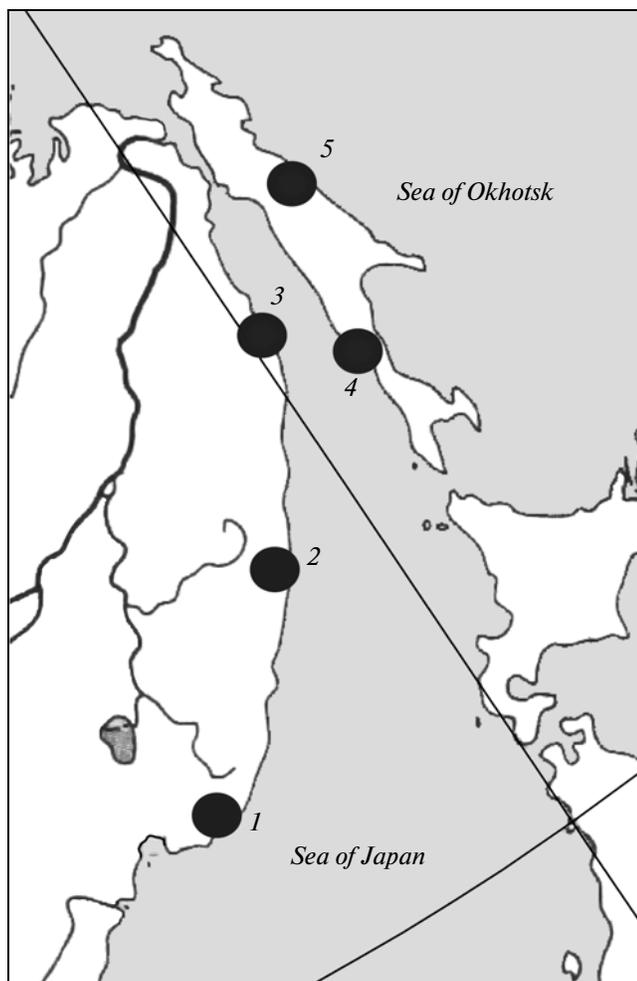
Fig. 1. Location of *Parahucho perryi* sampling sites: *1*, Kievka River; *2*, Maksimovka River; *3*, Tumnin River; *4*, Ainskoe Lake; *5*, Nabil'skii Bay.

fragment sizes and their distribution in the haplotypes was equal to 360, which constituted about 1700 nucleotides, or 10.2% of the mitochondrial genome. Among 56 *Parahucho perryi* individuals examined, a total of 12 haplotypes were identified. Eight of these haplotypes were discovered for the first time. Among seven *Hucho taimen* individuals, four haplotypes were identified. The two species examined had no common haplotypes. The distribution and absolute haplotype frequencies in the populations are demonstrated in Table 2.

Comparative characteristics of genetic diversity are demonstrated in Table 3. The mean values of haplotype and nucleotide diversity constituted 0.6589 ± 0.18985 and 0.0011986, respectively. In Sakhalin populations, the value of haplotype diversity (0.8250 ± 0.11927) was considerably higher than in the mainland populations (0.5482 ± 0.23691). The nucleotide diversity of Sakhalin populations was an order of magnitude higher (0.00251) compared to Primorye populations

(0.0003243). The highest h and π values were found in the sample from Nabil'skii Bay.

The results of testing for the statistical significance of genetic differences between the samples at the haplotype frequencies obtained using the χ^2 test and F_{ST} index are demonstrated in Table 4. Since testing for the heterogeneity of the three samples from the Tumnin River collected in different years did not reject the null hypothesis of homogeneity, all specimens were pooled into a single sample. Both tests showed statistically significant differences ($p < 0.01$) between all sample pairs, except the pairs of Kievka River–Maksimovka River, Maksimovka River–Ainskoe Lake, and Maksimovka River–Nabil'skii Bay (F_{ST} test). The level of differences was consistent with the data obtained for *Parahucho perryi* in the previous study [20]. Testing of the samples grouped in accordance with their assignment to different regions (mainland coast–Sakhalin Island) showed statistically significant differences revealed by the χ^2 test at $p < 0.05$.

To quantify the genetic differences between all sample pairs, the molecular variance was subdivided into the interregional (populations of the mainland coast and the populations of Sakhalin Island), interpopulation, and intrapopulation hierarchal levels (Table 5). The analysis performed showed (variants I, II) that the majority of the molecular variance of *Parahucho perryi* was associated with the genetic diversity within the samples. Furthermore, all variance components were statistically significant. Testing of the hypothesis on subdivision of the range into two parts (variants III, IV) showed its inconsistency. However, the assessment of the genetic variations in the population using the mismatch distribution analysis acquired a distinct bimodal mismatch distribution pattern based on the number mismatches between the haplotypes from mainland samples and those from Sakhalin Island (Fig. 2). The ambiguous results of the analyses can serve as evidence of either sampling errors or the complex pattern of the spatial population genetic differentiation of the given species.

The values of the intraspecific sequence divergence of the three mtDNA regions examined in the populations of *Parahucho perryi* are expressed in the percentage of nucleotide substitutions per restriction site and presented in Table 6. The divergence level among the populations Sakhalin taimen varies in the range of 0–0.11%. The divergence between Sakhalin and Siberian taimen is higher than 20%.

The MP phenogram was constructed based on the estimates obtained. The phenogram topology is demonstrated in Fig. 3. In this topology, only two clusters are highly supported. These clusters are represented by a basal cluster, which groups two haplotypes found in five taimen individuals from Ainskoe Lake (84%), and a cluster that contains the haplotypes found in the remaining 51 *Parahucho perryi* individuals examined (88%).

Table 2. Occurrence of mtDNA haplotypes in examined taimen populations (based on RFLP data)

Gaplo-type	Population									Number of haplotypes
	Kievka River	Tumnin River, 1998	Tumnin River, 2005	Tumnin River, 2007	Maksi-movka River	Ainskoe Lake	Nabil'skii Bay	Manoma River	Arichi River	
Par 1	4	1	0	0	2	0	0	0	0	7
Par 2	1	8	4	12	1	3	0	0	0	29
Par 3	1	0	0	0	0	0	0	0	0	1
Par 4	0	1	0	0	0	0	0	0	0	1
Par 5	0	0	0	0	0	4	0	0	0	4
Par 6	0	0	0	0	0	7	0	0	0	7
Par 7	0	0	0	0	0	1	0	0	0	1
Par 8	0	0	0	0	0	1	0	0	0	1
Par 9	0	0	0	0	0	0	1	0	0	1
Par 10	0	0	0	0	0	0	2	0	0	2
Par 11	0	0	0	0	0	0	1	0	0	1
Par 12	0	0	0	0	0	0	1	0	0	1
Huc 1	0	0	0	0	0	0	0	2	1	3
Huc 2	0	0	0	0	0	0	0	0	1	1
Huc 3	0	0	0	0	0	0	0	0	1	1
Huc 4	0	0	0	0	0	0	0	0	2	2

Table 3. Comparative measures of mtDNA variation in populations of Sakhalin taimen *Parahucho perryi* examined based on RFLP data

Populations	Number of individuals examined	Number of haplotypes	Number of polymorphic sites	Haplotype diversity (h)	Nucleotide diversity (π)
1. Kievka River	6	3	2	0.6000 ± 0.21517	0.000438
2. Tumnin River, 1998	10	3	2	0.3778 ± 0.18128	0.000201
3. Tumnin River, 2005	4	1	0	0.0000 ± 0.00000	0.000000
4. Tumnin River, 2007	12	1	0	0.0000 ± 0.00000	0.000000
5. Maksimovka River	3	2	1	0.6667 ± 0.31427	0.000334
6. Ainskoe Lake	16	5	11	0.7500 ± 0.07754	0.002087
7. Nabil'skii Bay	5	4	12	0.9000 ± 0.16100	0.002933

Table 4. Testing of level of differentiation in *Parahucho perryi* sample based on RFLP data

Population	1	2	3	4	5
1. Kievka River	–	20.45*	0.75	18.22*	11.00*
2. Tumnin River	0.59220*	–	11.46*	30.69*	31.00*
3. Maksimovka River	−0.27500	0.65266*	–	13.36*	8.00*
4. Ainskoe Lake	0.23921*	0.34545*	0.15009	–	21.00*
5. Nabil'skii Bay	0.42782*	0.71360*	0.30667	0.27726*	–

Above the diagonal, χ^2 [37]; below the diagonal, F_{ST} index [41] (asterisk, the differences are statistically significant at $p < 0.05$).

Table 5. Analysis of haplotype differences between populations of *Parahucho perryi* using AMOVA [38]

Diversity level	*d.f.*	Absolute estimates	Percentage of variance	Probability (*p*)
Variant I (all populations studied)				
Among the populations	6	0.39061 Va	29.07	0.00035
Within the populations	49	0.95323 Vb	70.93	0.00000
Variant II (Sakhalin populations—mainland populations)				
Among the regions	1	0.32812 Va	22.13	0.04594
Among the populations within the regions	5	0.20145 Vb	13.59	0.00391
Within the populations	49	0.95323 Vc	64.29	0.00098
Variant III (population from Ainskoe Lake—all other populations)				
Among the groups	1	0.04325 Va	2.98	0.41349
Among the populations within the groups	3	0.49128 Vb	33.82	0.00000
Within the populations	51	0.91826 Vc	63.21	0.00000
Variant IV (population from Nabil'skii Bay—all other populations)				
Among the groups	1	0.73603 Va	36.88	0.19746
Among the populations within the groups	3	0.34132 Vb	17.10	0.00000
Within the populations	51	0.91826 Vc	46.01	0.00000

Table 6. Values of intra- and interspecific mtDNA divergence (in percentage of nucleotide substitutions) in *Parahucho perryi* and *Hucho taimen* taimen inferred from RFLP data [35, 36]

Taxon (population)	1	2	3	4	5	6	7	8
1. *P. perryi* (Kievka River)	—							
2. *P. perryi* (Tumnin River, 1998)	0.0133668	—						
3. *P. perryi* (Tumnin River, 2005)	0.0200254	−0.0000038	—					
4. *P. perryi* (Tumnin River, 2007)	0.0200254	−0.0000038	0.0000000	—				
5. *P. perryi* (Maksimovka River)	−0.0078039	0.0100464	0.0167122	0.0167122	—			
6. *P. perryi* (Ainskoe Lake)	0.0620087	0.0414723	0.0414035	0.0414035	0.0583577	—		
7. *P. perryi* (Nabil'skii Bay)	0.1079464	0.0905977	0.0904740	0.0904740	0.1076113	0.08223256	—	
8. *H. taimen* (basin of Amur River)	20.7197876	20.7660659	20.7878665	20.7878665	20.7319227	20.5597919	20.6818296	—

The haplotype genealogies of the examined *Parahucho perryi* samples is reflected in the MTS tree in Fig. 4. The analysis performed revealed no alternative connections, arising as a result of either reverse or parallel mutations. Haplotypes included into the genealogical structures can be conditionally subdivided into three phylogeagraphic groups (A, B, C). The major haplotype of group A is **Par 2**, which is found in all populations, excluding the populations from Nabil'skii Bay (eastern coast of the Sakhalin Island). The major haplotype of group B is **Par 6**, which is only found in the populations from Ainskoe Lake. Although haplotypes **Par 2** and **Par 6** differ from one another in one nucleotide substitution, the first group is mixed and unites the members of Primorye populations (Kievka River, Tumnin River, Maksimovka River) and eight Sakhalin taimen from the populations of Ainskoe Lake (Table 2). Group B unites the
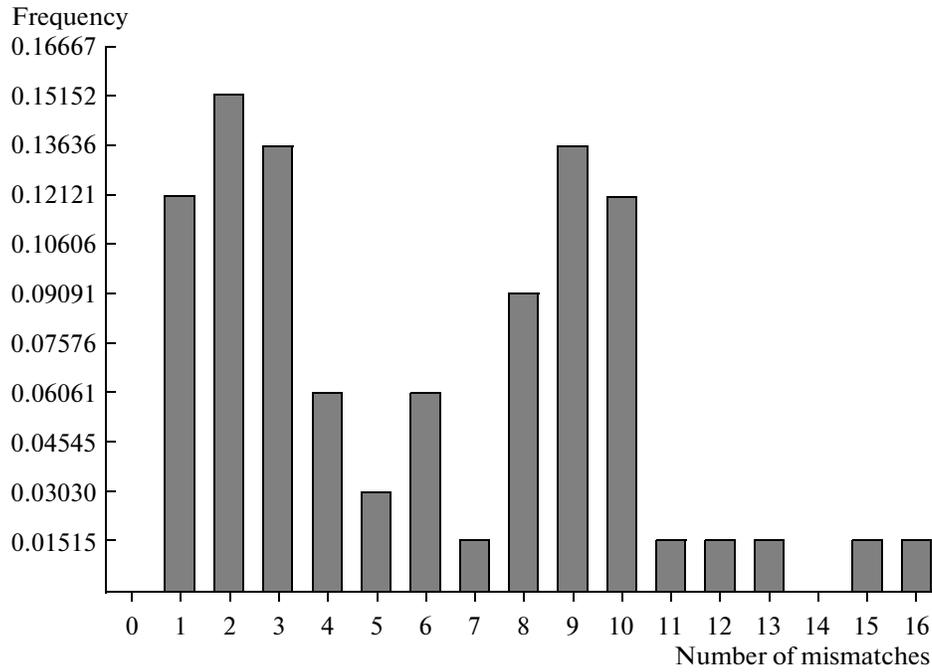
Fig. 2. Variation distribution patterns among populations of *Parahucho perryi* inferred from RFLP data.
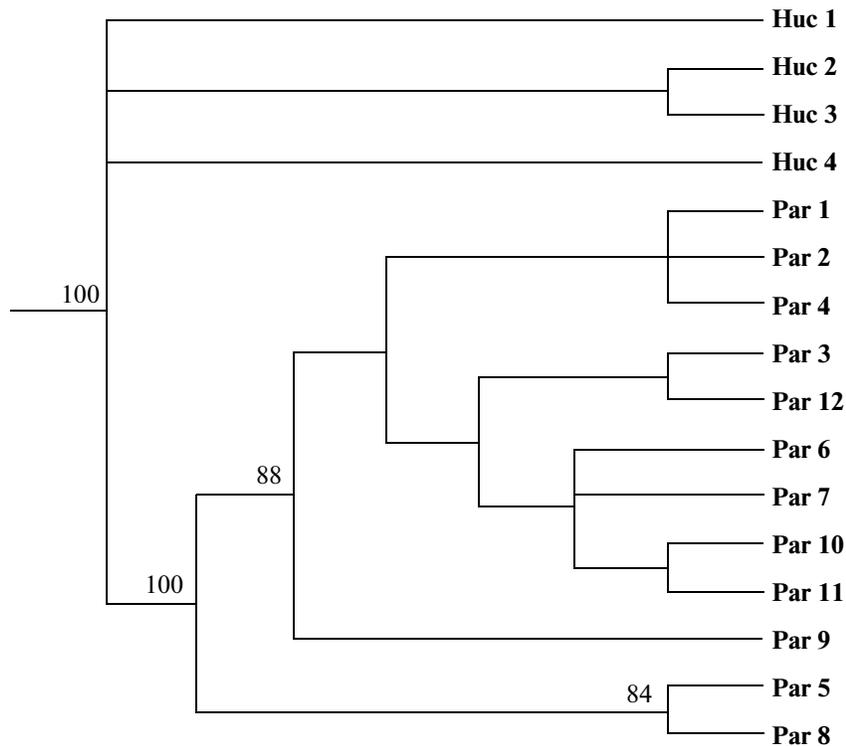
Fig. 3. Intraspecific phylogenetic relationships (MP) of the *Parahucho perryi* mtDNA haplotypes inferred from the RFLP data. Figures at branching nodes are bootstrap support values (% of 1000 replicates). *Hucho taimen* (haplotypes **Huc 1** to **Huc 4**) was used as outgroup. The haplotype designations, their distribution among the populations examined, and absolute frequencies are demonstrated in Table 2.
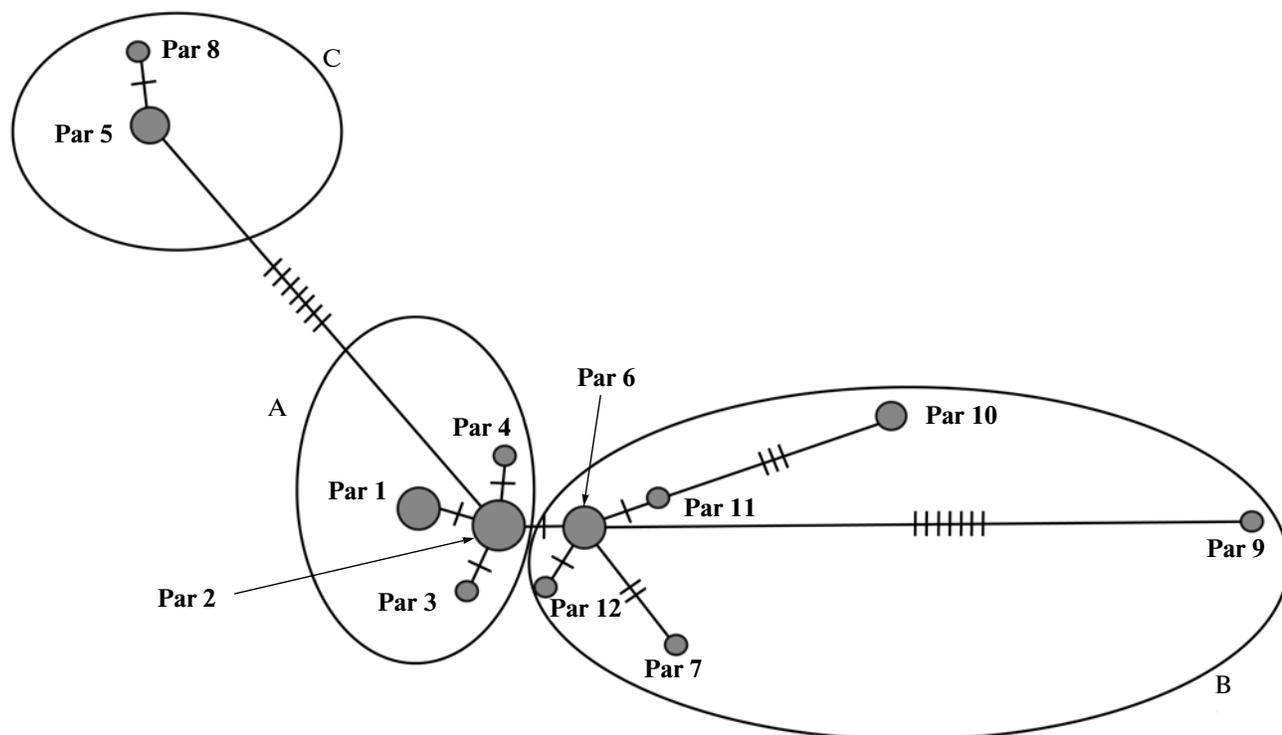
Fig. 4. Genealogical network (MST tree) of *Parahucho perryi* mtDNA haplotypes constructed based on RFLP data. Haplotype designations and their distribution among the examined populations are demonstrated in Table 2. Circle sizes are proportional to absolute haplotype frequencies.

representatives of only Sakhalin samples. Haplotypes that belong to the population from Nabil'skii Bay (eastern coast of the Sakhalin Island) are separated from **Par 6** by the highest number of mutational steps (three to seven). In addition, haplotypes **Par 5** and **Par 8**, found in five taimen individuals from Ainskoe Lake (western coast of Sakhalin Island, group C), are separated from **Par 2** by seven nucleotide substitutions.

Sequencing of COI Gene

The mtDNA *COI* gene fragment was sequenced in 37 Sakhalin taimen specimens and seven Siberian taimen specimens. The size of the *COI* fragment

examined constituted 655 bp; 77 positions were phylogenetically informative. Variable sites identified in the mtDNA *COI* sequence in the populations of *Parahucho perryi* are demonstrated in Table 7. In total, ten substitutions were identified. Among these, three were single nucleotide substitutions and seven substitutions were phylogenetically informative. A total of six haplotypes were identified, which differed in one to six nucleotide substitutions. Most of the mutations identified were of transition type. There were eight transitions and two transversions; the transition to transversion ratio constituted 4 to 1. All substitutions were synonymous. Based on the data obtained, the consensus NJ, MP, ML, and Bayesian (BA) trees were con-

Table 7. Variable sites in the mtDNA *COI* sequence identified in samples of *Parahucho perryi*

| Haplo-type	49	160	175	287	352	427	475	505	532	575
A	A	T	T	C	A	G	C	T	C	C
B	.	C	C	.	.	.	.	.	A	T
C	.	C	C	.	.	.	.	.	.	T
D	.	C	C	.	G	.	.	C	.	T
E	.	C	C	T	.	.	.	.	A	T
F	G	C	C	.	.	A	A	.	.	T

Identical positions are designated by dots.

Table 8. Values of intra- and interspecific mtDNA divergence (in percentage of nucleotide substitutions) in *Parahucho perryi* and *Hucho taimen* taimens inferred from the *COI* sequencing data [49]

Taxon (haplotype)	1	2	3	4	5
1. *P. perryi* (**A**)	–				
2. *P. perryi* (**F**)	0.9232961	–			
3. *P. perryi* (**C** and **D**)	0.5388406	0.5383027	–		
4. *P. perryi* (**B** and **E**)	0.6345854	0.6339913	0.2487346	–	
5. *H. taimen*	12.2394259	12.6057586	12.5275955	12.6298427	–

structed. The Bayesian tree topology was congruent with those of NJ, MP, and ML trees. In accordance to the dendrogram, all taimen individuals examined grouped into four compact clusters. The largest cluster (haplotype **A**) united ten taimen individuals from Ainskoe Lake, 11 individuals from Tumnin River, and one individual from Nabil'skii Bay. Three taimen with haplotype **F**, collected in different years in Nabil'skii Bay, formed a separate cluster. Four individuals from Ainskoe Lake were grouped into the third cluster (haplotypes **C** and **D**). The last cluster (haplotypes **B** and **E**), like the first one, was mixed. This means that
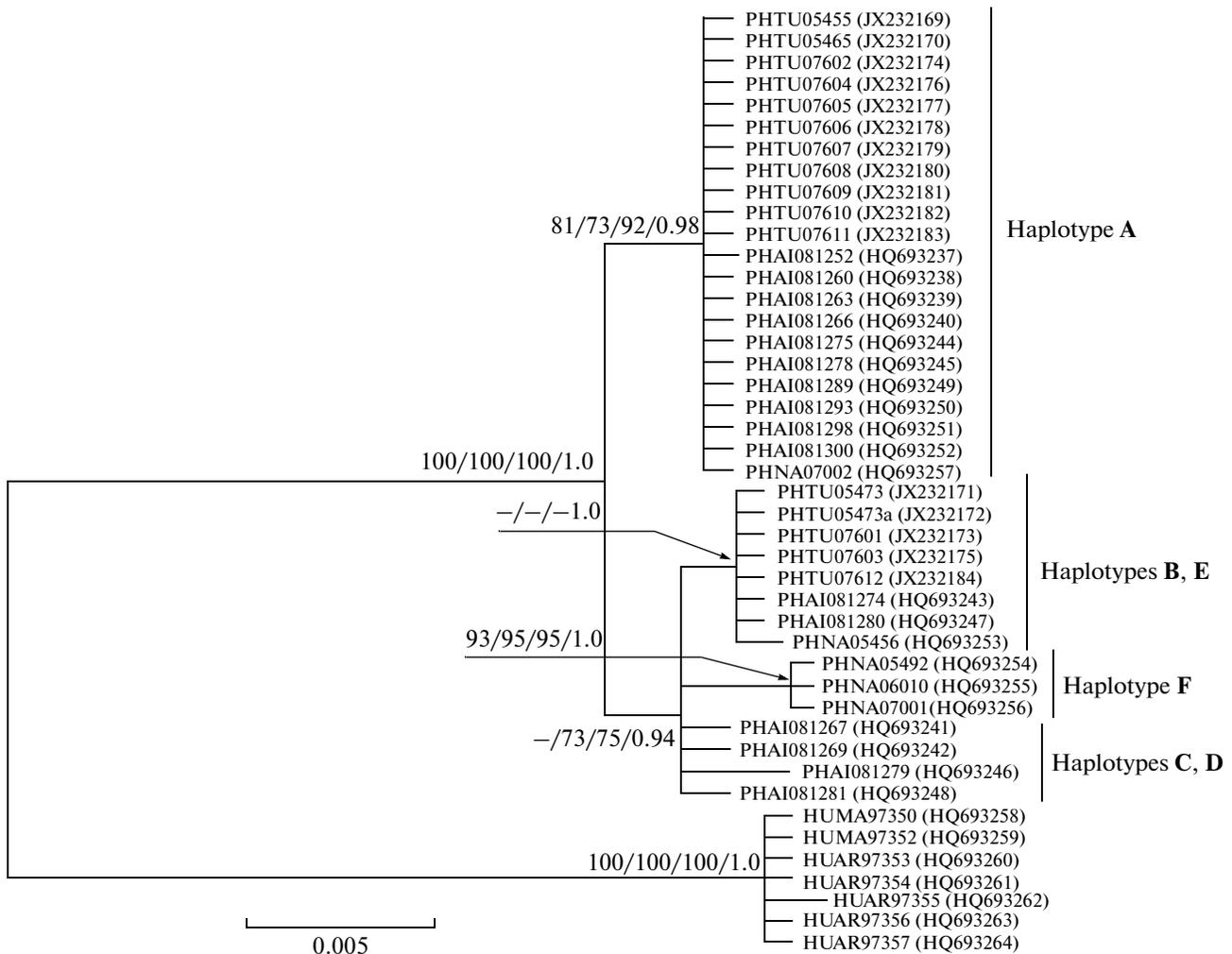
Fig. 5. Phylogram reflecting intraspecific phylogenetic relationships of the haplotypes, inferred from the 655-bp mtDNA *COI* gene fragment dataset of *Parahucho perryi* (based on the Bayesian tree). Figures at branching nodes are bootstrap support values (% of 1000 replicates) for NJ/MP/ML/ and Bayesian posterior probabilities. *Hucho taimen* was used as outgroup.
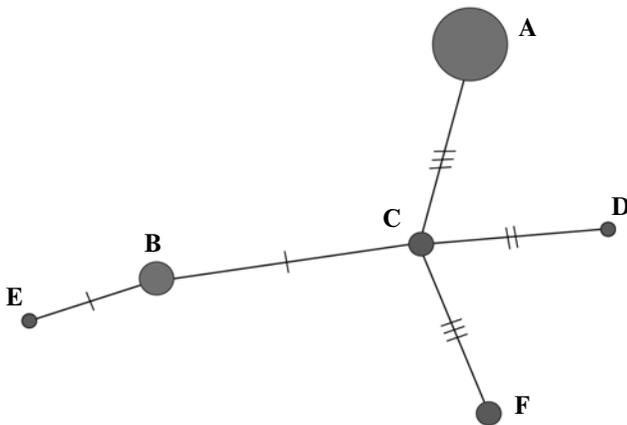
Fig. 6. Genealogical network of haplotypes obtained based on sequence data for mtDNA *COI* gene of *Parahucho perryi*. Circle sizes are proportional to number of individuals examined.

it was formed by the individuals from different populations from both regions, including two fish from Ainskoe Lake, five fish from Tumnin River (haplotype **B**), and one fish from Nabil'skii Bay (haplotype **E**). The composition of the cluster in all dendrograms was identical. The estimates of the *COI* sequence divergence between the Sakhalin taimen groups that form different clusters on the dendrograms (Table 8) constituted about 1%. The divergence between *Parahuch perryi* and *Hucho taimen* was higher than 12%.

Figure 6 demonstrates the haplotype network identified in the analysis of the *COI* sequences. The network has a radiated structure, and no alternative connections were detected. The central network position is occupied by haplotype **C**, and was found in three individuals from the population of Ainskoe Lake. Haplotypes identified in taimen from Nabil'skii Bay (**E** and **F**) are located at the periphery of the network and are separated from the central haplotype by two to three mutational steps. Haplotype **A**, which was detected in many individuals from Ainskoe Lake, the Tumnin River, and in one individual from Nabil'skii Bay, is also separated from the central haplotype by three nucleotide substitutions.

DISCUSSION

An analysis of *Parahucho perryi* performed using two different techniques enabled the identification of the intraspecific polymorphism of the mitochondrial *Cytb*, *COI* gene fragments, the mtDNA control region (D loop), and the genes for the NADH dehydrogenase subunits 1–4. The RFLP analysis identified four haplotypes in the mainland populations and nine haplotypes in the Sakhalin populations (Table 2). Only haplotype **Par 2** was common to all populations studied, except the one from Nabil'skii Bay. The frequency of haplotype **Par 2** constituted 51.78%. The eight newly

discovered haplotypes were distributed equally among the two Sakhalin populations. Moreover, the population from Nabil'skii Bay had no haplotypes in common with other populations, including the one from Ainskoe Lake. Correspondingly, the estimates of the level of the *Parahucho perryi* haplotype diversity (Table 3) in the Sakhalin populations are substantially higher than the Primorye populations. The population from the Tumnin River is the closest to the population from Ainskoe Lake with respect to haplotype composition (Table 2). The level of nucleotide diversity of Sakhalin populations is an order of magnitude higher than that of the mainland populations. Genetic differences between the populations from these two regions are confirmed by the bimodal distribution pattern of the number of mismatches between the haplotypes from mainland and Sakhalin populations (Fig. 3) typical of the conspecific representatives of spatially isolated populations [54]. The sequencing of the *COI* gene showed that the population from Nabil'skii Bay was also characterized by a unique haplotype set. Specifically, four out of five individuals examined had haplotypes (**E** and **F**) that were not detected in the samples from other populations (Table 7, Fig. 5). The population from Ainskoe Lake, where four out of six haplotypes (**A**, **B**, **C**, **D**) were detected, was the most variable. At the same time, in the population from the Tumnin River, only two haplotypes (**A**, **B**) were identified.

According to the theoretical calculations [55, 56], which are supported by empirical data [57−59], the highest species diversity is observed in regions located near or at the places of presumptive ancestral refugia. Moreover, there is a tendency for genetic diversity to decrease with increasing distance from the refugium, which probably reflects the action of genetic drift (founder effect and bottleneck) during the expansion of the range. Thus, both analyses conducted in the present study showed greater haplotype variations in Sakhalin populations compared to Primorye populations. These findings suggest that Sakhalin populations of *Parahucho perryi* are more ancient. An analysis of microsatellite loci of *Parahucho perryi* showed that the indices of genetic diversity were the highest in the smallest sample from the population of the western coast of Sakhalin Island (Agnevo River), compared to the three other populations inhabiting eastern coast of Sakhalin Island (Dagi, Nabil, and Poronai rivers) [22].

The assessment of the genetic differences among the population samples with respect to the haplotype frequencies using the F_{ST} and χ^2 tests (Table 2) produced statistically significant differences between the samples from different regions and between the Sakhalin samples, which represent the western (Ainskoe Lake) and eastern (Nabil'skii Bay) coasts of the Sakhalin Island. Moreover, the MP and BA tree topologies (Figs. 3, 5), as well as the haplotype genealogy structures (Figs. 4, 6) suggest that all populations of *Parahucho perryi* originated from one common ances-

tor, which most likely, inhabited the western coast of Sakhalin Island.

In accordance with the literature data, populations of *Parahucho perryi* also differ in their morphobiological characters [8, 12, 60]. Furthermore, V.P. Bushuev [60] revealed statistically significant differences between the populations of the southern Primorye (Kievka River) and the populations of western Sakhalin (Bogataya River).

Based on craniological and karyological data, M.K. Glubokovsky [61] suggested that the salmonid fish family appeared at the Pacific coast of Palearctic not later than Middle Eocene about 45 million years ago. The morphological and ecological features of these fish were close to those of contemporary *Parahucho*. For instance, these fish were characterized by an equally close association with both fresh and sea water pools. In the middle Miocene, about 40 million years ago, salmonid fish diverged from the common ancestor into three stems. The first, most archaic stem, which preserves the ancestral morphological and ecological characteristics, was retained in the native range and gave rise to the Parahuchoninae subfamily. More recent molecular genetic studies [62–64] showed the most basal position of the genera *Hucho* and *Brachymystax* compared to *Parahucho*. These studies also shifted the dating of the *Parahucho* divergence to the Middle Miocene (approximately 19–16 million years ago) [64]. In accordance with the fossil records from the Agnevo suite on the western coast of Sakhalin Island (the suite deposits are dated back to the end of Middle Miocene with the age of 11.6 to 12.25 million years), the Miocene *Parahucho* sp., as follows from its osteological characteristics, was not yet identical to the contemporary species [65]. However, the fact that Sakhalin taimen as a species appeared in the Paleo-Sea of Japans when it was a large brackish water lagoon is not disputed by anyone.

Throughout the Pleistocene, under the influence of tectonic movements and global fluctuations of the sea level, the Sea of Japan repeatedly changed its shape and size, becoming completely closed (during the period of regressions), half-closed (with the sea-level rise), and the modern type of water body [66]. In the Pleistocene, the Sea of Japan was partly desalinated, and there was periodically a land connection between Sakhalin and northern Primorye, Hokkaido Island, and the southern Kuril Islands [67–69]. Modern geomorphological shape and contours of Sakhalin was formed during the Sakhalin period of seismic activity (three to one million years ago) [69]. Furthermore, unlike the mainland coasts, Sakhalin is characterized by a straighter coast with pits and large lagoons. The last land connection between Sakhalin and the mainland was disrupted at the end of the Pleistocene–Holocene as a result of the continuing depression warping of the Gulf of Sakhalin, Amur River estuary, Nevelskoi Strait, and Tatar Strait [67]. The end of

Wurm, about 15000–13000 years ago, is characterized by the start of warming and the rise of the sea level. As a result of these events, Sakhalin first separated from Hokkaido (12000–11000 years ago), then from the mainland (about 7000 years ago) [70]. As for the formation of the largest water body west of the Sakhalin lagoon, Ainskoe Lake, its start was associated with the climatic optimum of the Holocene [71]. The final formation of this lake was the result of a small sea regression (about 1500 years ago). The basis of the lake fish fauna is currently represented by near-shore and brackish water species. Some saltwater fish periodically enter into the lake.

The Sakhalin fish fauna was formed over a long period of time and includes fish of both freshwater and saltwater genesis [72]. The probability of the appearance of taimen is higher in large water pools with low relief topology and large floodplains [16]. However, the formation of fish fauna in the rivers of the northern Primorye and western Sakhalin followed different patterns [73, 74], which is evidenced by the presence of some freshwater fish in the water pools of these regions and by specific features of the anadromous fish populations [75–77].

Based on the data obtained and taking into consideration the specific features of the Sea of Japan and fish fauna of the Sakhalin Island formation, the following conclusions can be drawn. First, there are statistically significant genetic differences between *Parahucho perryi* populations from Sakhalin Island and Primorye; second, Sakhalin populations are more ancient than Primorye populations, and this allows the suggestion that the species evolution began at the lagoons of the gently sloping coasts of Sakhalin Island; third, it seems likely that the dispersal of the population along the Primorye coasts occurred from north to south; fourth, the dispersal of Sakhalin taimen along western and eastern coasts is determined by the paleogeological history of the island and requires additional clarification.

In recent years, the examination of Sakhalin taimen is mostly focused on analyzing its behavior, ecology [4, 5, 14–16, 78–80], and genetics [19, 21, 22]. Despite that, due to the endangered status of the species, most of the studies are performed using limited material, and interesting and valuable results are often obtained. These data can substantiate the strategy for conservation and restoration measures. There are two opinions on realization of these measures. The first opinion implies that the main activities of population restoration should be focused on the river basins that provide better habitation conditions [16]. In accordance with the second opinion, the program on the restoration of small populations should be urgently adopted because the cloning of these populations from other regions can produce no positive results [12]. The second opinion is supported by the conservatism of the genetic adaptation of local fish stocks to certain envi-

ronmental conditions formed over thousands of generations [76, 81, 82] and seems to be more substantiated.

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