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GENETICS

Genetic Variability in Anadromous Fishes, Chum Salmon *Oncorhynchus keta* (Walbaum, 1792), and Sakhalin Taimen *Parahucho perryi* (Brevoort, 1856) from the Northwestern Pacific as a Reflection of Paleoclimate Oscilations

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Abstract—The genetic variability distribution of two mtDNA segments of chum salmon (*Oncorhynchus keta*) (Walbaum, 1792) and Sakhalin taimen (*Parahucho perryi*) (Brevoort, 1856) was examined in populations of the Sea of Japan and the Sea of Okhotsk. The values of haplotype and nucleotide variability in these species are, in general, of the same level. The dating of the divergence time of species haplotypes revealed four evolutionary periods in Sakhalin taimen and three in chum salmon. In the taimen, the first divergence time occurred approximately 430 thousand years (kyr) ago, the second 220 kyr ago, and the third 70 kyr ago. In the chum salmon, the first divergence time corresponds to 220 kyr; the second is approximately 100 kyr ago. In both species, the main portion of presently revealed haplotypes evolved over the past 50–10 kyr. Certain glacioeustatic sea level fluctuations influenced each stage of evolution history of species, contributing to their geographic isolation. Demographic population history research found that the initial stage of population growth in the taimen occurred at the time period of approximately 12 kyr ago and was apparently associated with the end of the Last Glacial Maximum. In the chum salmon, this period began somewhat earlier, 30–35 kyr ago; it has accelerated in the past 10–15 kyr. The last glaciation to a lesser extent impacted the demographics of chum salmon, probably due to the greater eurythermity and to the larger range of this species.

Keywords: phylogeography, chum salmon, Sakhalin taimen, coalescence, mitochondrial DNA, Northwest Pacific

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The significance of historical and ecological factors in the formation of intraspecific units (population genetic structure) and the formation of new species is one of the most important problems in evolutionary biology [12, 31]. Defining the boundaries of populations is of great practical importance, since the populations are objects of human exploitation.

In the North Pacific, climate oscillations over the past several million years significantly influenced the evolution of species and the formation of the intraspecific structure not only in terrestrial animals, but also in freshwater and seawater fishes [12]. Periods of cooling alternated with warming at intervals of approximately 100000 years [24]. Glacial sheets that formed in periods of cooling decreased the sea level significantly; most of the species were forced out into the southern regions (refugia), from which the species spread to the north when the glaciers retreated and sea level rose again. The formation of two or more refugia led to isolation of populations, and to a decrease or complete breaking of gene flow between populations. In the case of several isolated refugia the probability of the divergence of isolated forms and formation of two or more species increased. Historical factors (e.g., fragmentation, expansion, recolonization, and hybridization), accompanied by changes in the ranges of species under the influence of cyclical climate fluctuations, also significantly affected the distribution of genetic variation within species [39].

Comparative analysis of genetic variation within closely related species and within same geographic area enables one to determine the time period of the divergence of basic breeding lines and to define the most significant factors that affected in the past or currently affect population genetic structure of the species. The mitochondrial DNA (mtDNA) is a more suitable molecule for evolutionary and population analysis due to its maternal inheritance, rapid evolution and lack of recombinations [14]. The phylogeographic approach based on analysis of mtDNA varia-

Table 1. The basic characteristics of samples of chum salmon *Oncorhynchus keta* and Sakhalin taimen *Parahucho perryi*

Sample number	Population, sampling year	Number of individuals	Geographical position	Number		Diversity	
				haplotypes	polymorphic sites	haplotype	nucleotyde
			Oncorhynchus keta				
1	Narva River, 2009	10	Primorye, southwest coast, the Sea of Japan	10	14	1.000 ± 0.045	0.00226
2	Kievka River, 2009	10	Primorye, west coast, the Sea of Japan	3	3	0.689 ± 0.104	0.00078
3	Naiba River, 2009	10	Sakhalin Island,the west coast, the Sea of Okhotsk	4	6	0.644 ± 0.152	0.00083
			Parahucho perryi				
4	Kievka River, 1997	6	Primorye, west coast, the Sea of Japan	2	1	0.333 ± 0.215	0.00028
5	Maksimovka River, 1997	3	The same	2	1	0.667 ± 0.314	0.00043
6	Tumnin River, 1998, 2005, 2007	26	"	2	5	0.471 ± 0.063	0.00150
7	Nabil'sky Bay, 2005, 2006, 2007	5	Sakhalin Island, the east coast, the Sea of Okhotsk	3	14	0.700 ± 0.218	0.00447
8	Leonidovka 2014	4	Sakhalin Island, the south-east coast, the Sea of Okhotsk	1	0	0.000 ± 0.000	0.00000
9	Ainskoe Lake 2008, 2008	16	Sakhalin Island, the west coast, the Sea of Okhotsk	8	10	0.758 ± 0.110	0.00211

tion was helpful to understand the ways and mechanisms of the formation of species and intraspecific units [12, 39]. The breaking of genetic exchange between populations leads to differences in the mtDNA of individuals from different populations as a result of the accumulation of mutations and stochastic processes. With the long-term independent existence of populations they form distinct mtDNA phylogroups. The phylogeographic approach based on an analysis of molecular variability enables one to reconstruct the events of divergent evolution in species and groups of species and to refer events of divergent evolution to a particular historical period, as the molecular clock hypothesis suggests a uniform accumulation of nucleotide substitutions in mtDNA in time [12].

The Northwest Pacific is worthy of interest because in periods of major climate changes, the basic physical parameters of the interior Sea of Okhotsk and Sea of Japan varied significantly. In periods of declining sea level, the flow of water into the sea through straits was sharply reduced or completely stopped; their temperature and salinity changed respectively.

The objective of this work was to study the distribution of genetic variation in mtDNA segments from two anadromous salmons, the chum salmon *Oncorhynchus keta* (Walbaum, 1792), and Sakhalin taimen *Parahucho perryi* (Brevoort, 1856). The choice of the objects of study is based on the fact that they have similar biological characteristics (anadromous), but chum salmon was found throughout the North Pacific while the Sakhalin taimen has a limited distribution area.

MATERIALS AND METHODS

To analyze the nucleotide sequences of mtDNA segments we used 30 chum salmon individuals, viz., 20 from the populations of the mainland coast (the rivers Narva and Kievka) and 10 from the population of Sakhalin Isl. (Naiba River), as well as 60 individuals of Sakhalin taimen, 35 individuals from the populations of the mainland coast (the rivers Kievka, Maksimovka and Tumnin) and 25 from the populations of the Sakhalin Isl. (Nabil'sky Bay, Leonidovka River, Ainskoe Lake.) (Table 1; Fig. 1). The DNA samples were obtained from muscle tissue of the heart and fins, which were fixed in 96% ethanol, by a standard technique using chloroform and proteinase K [34].

As investigated molecular genetic markers, we chose fragments of the mitochondrial genes *COI* and *cytb,* amplified with the COI-FishF1/COI-FishR1 [41] and Fishcytb-F/Truccytb-R primers [35] under appropriate condition of the polymerase chain reaction. After further isolation of the reaction products the samples were sequenced using a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, v. 3.1) with an ABI Prism 3130 genetic analyzer (Applied Biosystems, United States).

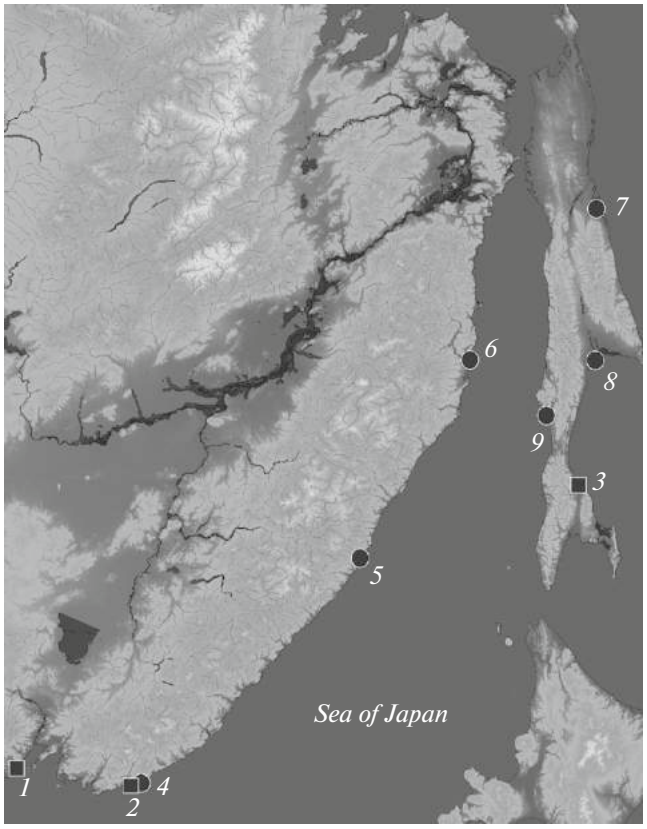

Fig. 1. The location of the investigated samples of chum salmon *Oncorhynchus keta* (black squares) and Sakhalin taimen *Parahucho perryi* (black circles): *1*, Narva River; *2*, Kievka River; *3*, Naiba River; *4*, Kievka River; *5*, Maksimovka River; *6*, Tumnin River; *7*, Nabil'sky Bay; *8*, Leonidovka River; *9*, Ainskoe Lake.

Multiple alignment and analysis of the nucleotide sequences of the studied gene fragments were carried out using MEGA 5.05 software [38] based on the ClustalW algorithm [40]. Aligned sequences of genes for each individual of the studied species were pooled into combined sequences. Selection of an optimal model of nucleotide substitution for each data section was performed in jModelTest 2.1.7 [17] using the Bayesian Information Criterion (BIC).

To visualize the different phylogroups of mtDNA, the median nets of haplotypes were built in the SplitsTree4 4.12.3 environment [26] by the MJ (Median-Joining) algorithm [13]. Additional sequence databases of GenBank from Canada, Vancouver Isl. (JX960914, JX960807), and of Japan, Hokkaido Isl. (JX960915, JX960808) [16] were included into the comparative analysis of chum salmon haplotypes. The values of haplotype and nucleotide diversity for each population and the distribution of the differences between haplotypes (mismatch distribution) were calculated using the DnaSP v. 5.10 program [29].

The range of evolution rates was specified in the BEAUti (BEAST 1.8.0) module. The lower limit of the evolution rate in the chum salmon was 0.42%/My and 0.63%/My for the *COI* and *cytb* segments, respectively. For the Sakhalin taimen, this boundary is defined at the level of 0.31%/My, corresponding to the average rate of evolution for all described species of the Salmonidae family derived from combinations of molecular markers, paleontological data, and statistical methods [16]. The upper rate limit in the analysis was 1.7% for 1 million years [36]. When calculating the Markov chains (MCMC, Markov Chain Monte Carlo), all values of the *ucld.mean* parameter used for secondary calibration data were normally distributed [25].

The selection of the best model of the molecular clock of the three main hypotheses (strict, relaxed, and without a molecular-clock analysis) was performed using pairwise comparisons of the Bayesian factor (BF). Positive reliable values for the test data sets (>1) [27] were obtained using a relaxed clock.

The reconstruction of the demographic history of the populations of the species based on the molecular data consisted of two basic phases. In the first phase, as a result of phylogenetic analysis, viz., the intraspecific genealogy, dating divergence events were defined using a Bayesian interface (BI) in the BEAST 1.8.0 environment [20] under the following conditions: the model of nucleotide substitution for the chum salmon *COI* sequences was HKY + I, *cytb*–TrN; for gene fragments of Sakhalin taimen, HKY + I, model molecular clock–relaxed clock with an uncorrelated lognormal distribution of the evolution rates relative to the branches of trees (the uncorrelated lognormal relaxed clock) [18]; the analyzed time period was 30 million generations, with selection of each thousandth state and 10% of the burn-in value. The phylogenetic trees derived from the calculations were summarized in the TreeAnnotator program of the BEAST 1.8.0 package using the algorithm of maximum clade credibility (MCC).The tree topology was considered valid if the values of the Bayesian posterior probability (BPP) in the nodes were 0.95 or higher.

The second phase included an analysis of the history of populations based on data on intraspecific genealogies. In accordance with the known technique [23] using the BEAST 1.8.0 program and conditions described above, the Bayesian skylines were plotted [19] for the populations of the studied species. The number of groups that determine the degree of optimization of the demographic functions of Bayesian skyline plots (BSP) were also selected on the basis of BF [37]. For the BSP, a piecewise-linear model that allows linear variation in the effective population size at different coalescence intervals was used. Calculation based on the Bayesian interface was carried out on the basis of the CIPRES high-performance cluster [33]. Values of the ESS (effective sample size) parameter thus amounted to more than 200. The calculations were processed in Tracer 1.6.0.

The sequences of *COI* and *cytb* gene fragments of chum salmon and Sakhalin taimen are deposited in GenBank under accession numbers KR607578-

Table 2. The occurrence of mtDNA haplotypes in the studied populations of chum salmon *Oncorhynchus keta*

Haplotype	Population			The number of individuals
	Narva River	Kievka River	Naiba River	
Keta1	1(16.7)	5(83.3)	0	6
Keta2	1(33.3)	2(66.7)	0	3
Keta3	1(14.3)	0	6(85.7)	7
Keta4	0	0	2(100)	2
Keta5	0	0	1(100)	1
Keta6	1(100)	0	0	1
Keta7	0	0	1(100)	1
Keta8	1(100)	0	0	1
Keta9	1(100)	0	0	1
Keta10	1(100)	0	0	1
Keta11	1(100)	0	0	1
Keta12	1(100)	0	0	1
Keta13	1(16.7)	0	0	1
Keta14	0	3(100)	0	3

The relative number of individuals of each haplotypes, %, is in parentheses.

KR607600, KR607518-KR607577; KR778850-KR778879, and KR778820-KR778849.

RESULTS

Chum salmon Oncorhynchus keta

The analysis of the nucleotide sequences of the studied chum salmon individuals revealed 14 different mtDNA haplotypes (Table 2). The total length of the combined mtDNA *COI* and *cytb* sequences was 1643 base pairs (bp). A total of 12 of the 20 variable sites were phylogenetically informative.

The chum salmon population of the Narva River had the highest number of haplotypes (ten). In the chum salmon sample from the Kievka River we found three haplotypes at a relatively high frequency; two of them were in common with haplotypes of the chum salmon from the Narva River. The population of Sakhalin chum salmon (Naiba River) revealed four haplotypes. The most common one was the Keta3 haplotype, the rest of the haplotypes were characteristic only for this population (Naiba River, Sakhalin Island). Haplotypes found in populations of chum salmon from the mainland Narva River and Kievka River virtually did not overlap with the haplotypes of the Naiba River chum salmon, excepting the Keta3 haplotype, which was revealed in the chum salmon sample from the Naiba River and in some individuals from the Narva River. Three individuals from the Kievka River had the unique haplotype Keta14, which was recorded only in that population. The highest values of haplotype (1.000 ± 0.045) and nucleotide (0.00226) variability were revealed in the chum salmon sample from the Narva River. The lowest values were determined in chum salmon samples from the Kievka River, 0.689 ± 0.104 and 0.00078, as well as in the Sakhalin population from the Naiba River, 0.644 ± 0.152 and 0.00083, respectively (Table 1).

All of the identified chum salmon haplotypes form two main phylogroups with the central haplotypes Keta1 and Keta3; the remaining haplotypes differ from them by one to four nucleotide substitutions (Fig. 2). All the haplotypes of phylogroup B are specific only to individuals of the populations of the Narva River and Kievka River. Phylogroup A is separated by five nucleotide substitutions from the closest haplotype of the mainland group B, and includes almost all individuals in the chum salmon population from the Naiba River (Sakhalin Isl.). This phylogroup also includes haplotypes of chum salmon from North America (Vancouver Isl.) and Japan (Hokkaido Isl.). The haplotypes of the A and B groups form a star-like structure (Fig. 2).

Bayesian analysis corroborates two distinct phylogroups in chum salmon with a reliable distribution respective to geographical regions. The calculated mean value difference between phylogroups indicates that the divergence began approximately 220 kyr ago with a 95% highest posterior density (HPD) interval from 70 to 395 kyr ago. Two clusters formed within each phylogroup. The posterior probabilities in the nodes are close to being significant (0.90 and 0.93) (Fig. 1). The divergence time of within the clusters was 90000–110000 years.

The assessment of the genetic variability of chum salmon individuals by analyzing the distribution of the differences between all haplotypes showed a bimodal distribution curve (Fig. 4a). The estimated haplotype distribution, which was calculated for a population of a constant size, was different from the observed one.

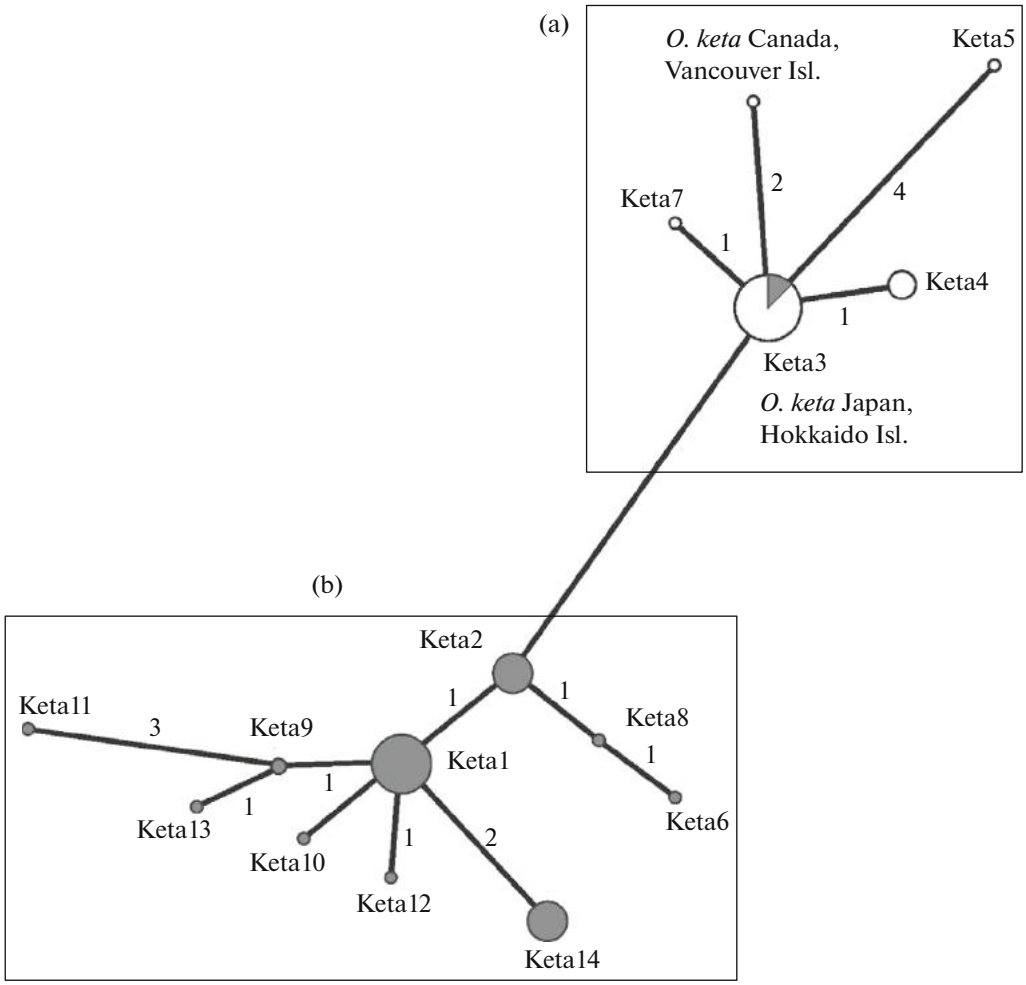

Fig. 2. The genealogical network of haplotypes based on data analysis of the nucleotide sequences of *COI* and *cytb* genes of mtDNA of *Oncorhynchus keta*. (a, b) Phylogroups. The proportion of individuals of chum salmon from the mainland coast of the Sea of Japan is marked in gray. The designations of haplotypes are the same as in Table 2; the size of the circles is proportional to the number of studied individuals.

Two distribution peaks occur on the plot, i.e., those linked to two different chum salmon phylogroups. BSP analysis (Fig. 5a) displayed a relatively constant size of chum salmon populations since the divergence of the main phylogroups (220 kyr ago) to the point of 75000 years. Further there is a gradual increase in the abundance of this species, which is still continuing.

Sakhalin Taimen Parahucho perryi

The analysis of the variability in the nucleotide sequences of the studied individuals of Sakhalin taimen from different samples revealed 13 mtDNA haplotypes (Table 1). The length of the combined sequences was 1565 bp. A total of 17 of the 21 variable sites were identified as being phylogenetically informative. In all the taimen samples, excepting the sample from the Nabil'sky Bay, the common PP1 haplotype was found. Another common haplotype, PP2, was revealed in the mainland population from the Tumnin River and in the Ainskoe Lake (the west coast of Sakhalin Isl.). All other haplotypes were unique to a particular sample. The sample from Ainskoe Lake had the highest number of haplotypes (8), including six unique ones. The highest value of haplotype (but not nucleotide) diversity recorded for this population was 0.758 ± 0.110 and 0.00211, respectively (Table 1). The sample of Sakhalin taimen from the Nabil'sky Bay contained three unique haplotypes (PP9, PP10, and PP11), and was characterized with high values of haplotype diversity (0.700 ± 0.218) and the highest value of the nucleotide diversity (0.00447). In the sample from the Tumnin river, 17 individuals formed a common (PP2) phylogroup with the sample from the Ainskoe Lake. It should be recorded that in continental taimen samples from the Kievka, Maksimovka, and other rivers, one unique haplotype (haplogroup PP12 and PP13, respectively) was found.

According to the haplotype network based on the MJ algorithm, the mtDNA haplotypes of Sakhalin taimen form three distinct phylogroups (Fig. 1). The central position was occupied by the PP1 haplotype in

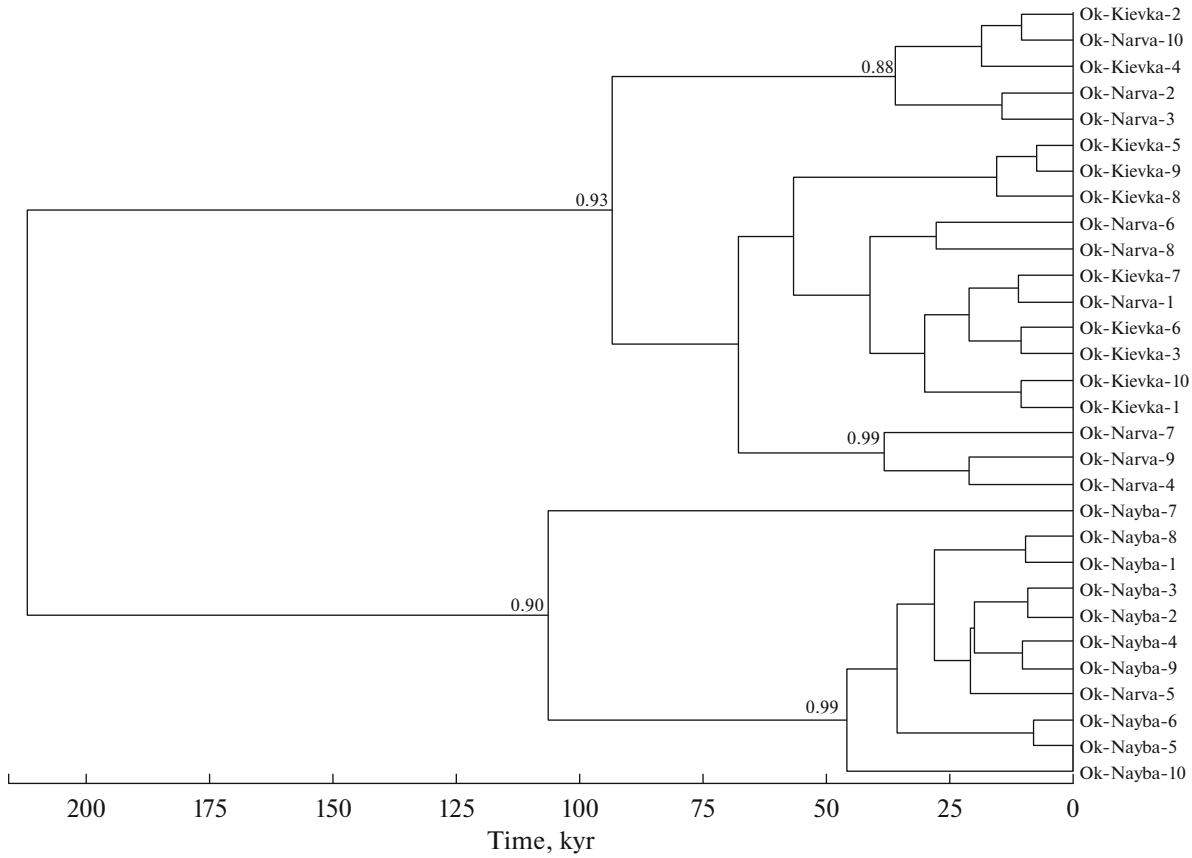

Fig. 3. A Bayesian consensus phylogenetic tree that reflects the genetic diversity of populations and the time of divergence of the main genealogical lines of *Oncorhynchus keta,* thousand years, according to the combined sequences of the two segments of mtDNA *(COI* and *cytb)*. To the right of the tree nodes, the values of the posterior probabilities (confidence ≥ 0.95).

the A phylogroup and in the B phylogroup by the PP2 haplotype, which is characterized by five nucleotide substitutions. Both haplotypes often occurred in mainland populations, but also were recorded in the island ones. The B phylogroup included only a single haplotype, PP10, which was identified in individuals from the Nabil'sky Bay; it differs from the closest haplotype by eight nucleotide substitutions.

The Bayesian tree topology reflects an isolation of the PP10 haplotypes of three taimen individuals sampled in the Nabil'sky Bay from other haplotypes of this species. The divergence time from the rest of the phylogroups was approximately 430 kyr (the 95% HPD interval is 55 kyr to 1 million years.) (Fig. 7). Among the nodes that have significant posterior probabilities, we should note two subdivided clusters of haplotypes in the mainland and island samples. The splitting of these phylogroups occurred approximately 80 kyr ago. The assessment of the genetic variability of taimen individuals by analyzing the distribution of the differences between all haplotypes resulted in a multimodal distribution curve (Fig. 4b), which is related to the presence of several phylogroups.

The BSP reflects the sustained reduction of the effective population number of Sakhalin taimen throughout the demographic history of the species from the time of the divergence of the genealogical lineage (430 kyr ago) to 12–15 kyr ago. After this period, the taimen population began to increase sharply, although it did not reach the initial effective size (Fig. 5b).

DISCUSSION

The two salmon species studied here, viz., the chum salmon *Oncorhynchus keta* and the Sakhalin taimen *Parahucho perryi,* have different origins, biological parameters, areas of distribution, and abundance. The taimen samples were taken over a significant part of the habitat of this species. For the chum salmon the studied area is only a small part of its range. Nevertheless, similar patterns were revealed in both species.

The values of haplotype and nucleotide variability in these species differ (0.9011 ± 0.034 and 0.00266 in the chum salmon, and 0.8215 ± 0.03 and 0.00259 in the Sakhalin taimen), but generally remain at the same level. Obviously, the relatively high level of haplotype variation in the taimen was caused by the relatively long time of the existence of the species. According to various estimates, the origin of this species occurred within the range of 23 [9] to 30 million years ago [11].

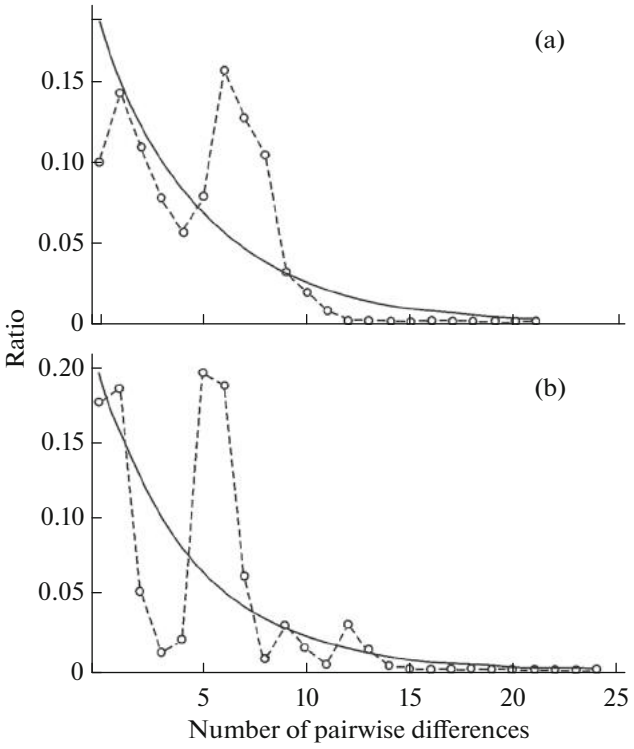

Fig. 4. The distribution diagram of the differences between haplotypes *(mismatch* distribution) *Oncorhynchus keta* (a) and *Parahucho perryi* (b).

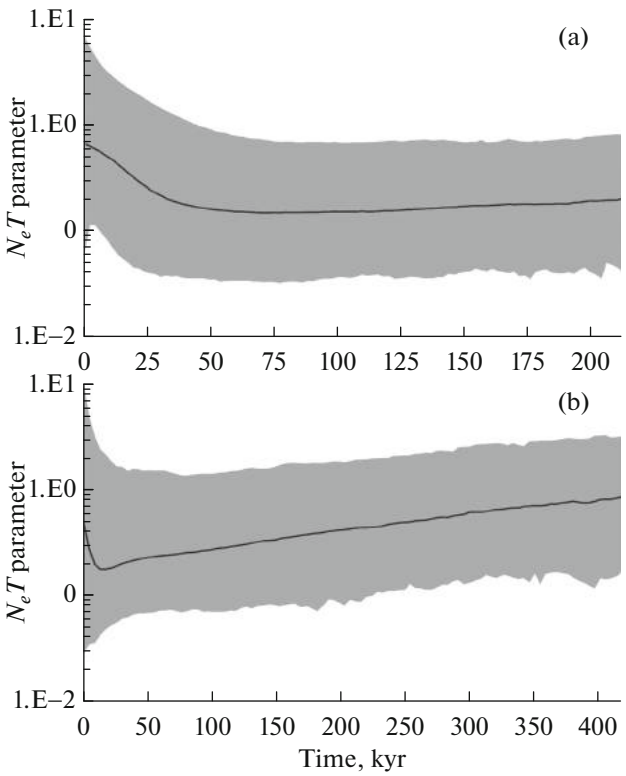

Fig. 5. A Bayesian skyline plot, built for the combined sequences of mtDNA of *Oncorhynchus keta* (a), and *Parahucho perryi* (b). The graph shows the variation in the N_eT values (the product of the effective population size and length of one generation) over time. Solid line, the median of the N_eT parameter values, the darkened area is the 95% interval of the highest posterior density of distribution estimates.

The chum salmon has existed as a species for a much shorter time (4–6 million years) [11, 16], but its high abundance and intraspecific subdivision, as a consequence, lead to high values of variability.

Similarity is also revealed upon the collation of haplotypic networks and haplotype phylograms of these species. Both species are characterized by the presence at least two large haplotype phylogroups (Figs. 2 and 6). Modeling reveals that a stochastic formation of two phylogroups in panmixed population is a possible, but extremely unlikely event [30]. A more likely cause for the existence of the two phylogroups is the presence in the past history of two populations with limited genetic exchange. The long independent existence of such populations leads to the accumulation of mostly neutral mutations and, consequently, to phyloproup divergence. It follows from our data that chum salmon phylogroups are separated in space to a great extent. All haplotypes (1, 2, 6, 8, and 9–14) of the B group were found in the Sea of Japan in mainland populations, and most haplotypes (4, 5, and 7) of the A group were found in the Okhotsk sample. This fact conforms with the idea that the formation of phylogroups was determined by the existence of isolated populations in the past history of the species. The closeness of the haplotypes of individuals from North America and Japan, as well as phylogroups of the studied chum salmon, is consistent with the data presented by Yoon et al. [42]. Their revealed haplotype frequencies of the control region of mtDNA reflect the chum salmon distribution along the coast of the Northwest Pacific from the probable place the origin of the chum salmon in the Sea of Japan and adjacent areas to North America. The authors noted that this process in chum salmon might be caused by climate changes during the Pleistocene [41].

The pattern of formation in the region of two or more phylogroups in freshwater and marine fish is corroborated by the existence of two phylogroups in two endemic species of the *Tribolodon* redfins [3], sturgeon [10], Okhotsk Sea pollock [5] and masu salmon [43].

The distribution of nucleotide differences among all haplotypes in both species, as expected, has a bimodal character (Fig. 4). The first peak is in differences at one nucleotide; the second peak is at five to six nucleotides. The common distribution points to the similarity of the factors that determine the formation of phylogroups in the species. In the taimen, additional peaks occur in the area of 9 and 12 nucleotides. These peaks are caused by the significantly different haplotypes that were revealed at the North of Sakhalin Island, which combined with high support in a sepa-

Table 3. The occurrence of mtDNA haplotypes in the studied populations of taimen *Parahucho perryi*

Haplotype	Population						Number of individuals
	Kievka River	Tumnin River	Maksimovka River	Ainskoe Lake	Nabil'sky Bay	Leonidovka River	
PP1	1(6.25)	9(56.25)	1(6.25)	1(6.25)	0	4(25)	16
PP2	0	17(94.4)	0	1(5.6)	0	0	18
PP3	0	0	0	8(100)	0	0	8
PP4	0	0	0	2(100)	0	0	2
PP5	0	0	0	1(100)	0	0	1
PP6	0	0	0	1(100)	0	0	1
PP7	0	0	0	1(100)	0	0	1
PP8	0	0	0	1(100)	0	0	1
PP9	0	0	0	0	1(100)	0	1
PP10	0	0	0	0	3(100)	0	3
PP11	0	0	0	0	1(100)	0	1
PP12	5(100)	0	0	0	0	0	5
PP13	0	0	2(100)	0	0	0	2

The relative number of individuals of each haplotypes, %, is in parentheses.

rate clade (Fig. 7). The peak with the average difference of 1 nucleotide corresponds to the star-like distribution of the haplotypes in each phylogroup around the central haplotype. The peak of the differences of five to six nucleotides reflects the average differences between haplotypes in the phylogroups. The star-like genealogy of haplotypes around the central, most common haplotype of each phylogroup suggests a recent sharp increase in the species abundance [42]. An alternative relationship (Fig. 6) between the haplotypes of Sakhalin taimen may result from homoplasy, namely, repeated and reverse mutations in mtDNA [15].

The analysis of Bayesian individual phylograms revealed four phases of divergent evolution in populations of Sakhalin taimen (Fig. 7) and three in chum salmon (Fig. 3). The first phase of divergence in taimen occurred 430 kyr ago, the second occurred 220 kyr ago, and the third 70 kyr ago. It is noteworthy that the period of 170 kyr, recorded at phylogram, has relatively low posterior probabilities and is not considered in the research as a certain phase of divergent evolution. In the first phase the chum salmon divergence corresponds to 220 kyr years ago, in the second its approximately 100 kyr ago. The main part of currently defined haplotypes in both species was formed 50–

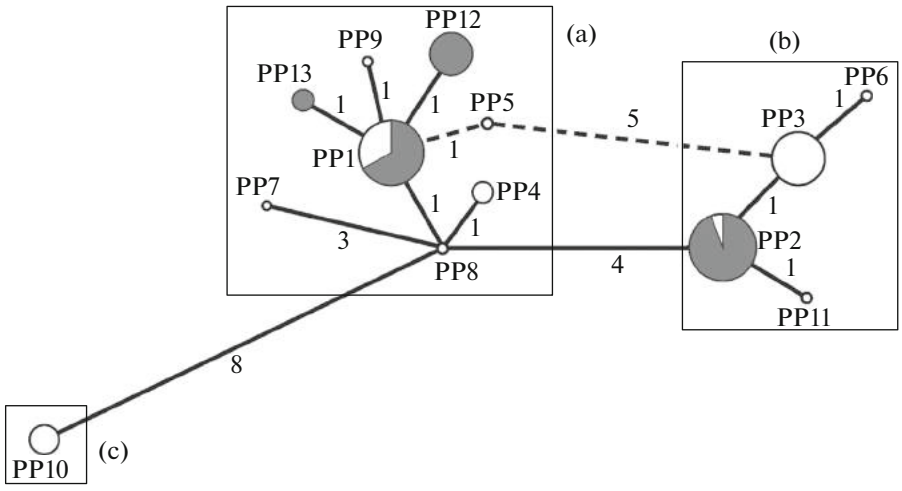

Fig. 6. The genealogical network of haplotypes, based on data analysis of the nucleotide sequences of the *COI* and *cytb* genes of mtDNA of Sakhalin taimen *Parahucho perryi*. (a, b, c) Phylogroups. The proportion of taimen individuals from mainland coast of the Sea of Japan is marked in grey. Designations of haplotypes are the same as in Table 3; the size of the circles is proportional to the number of individuals studied.

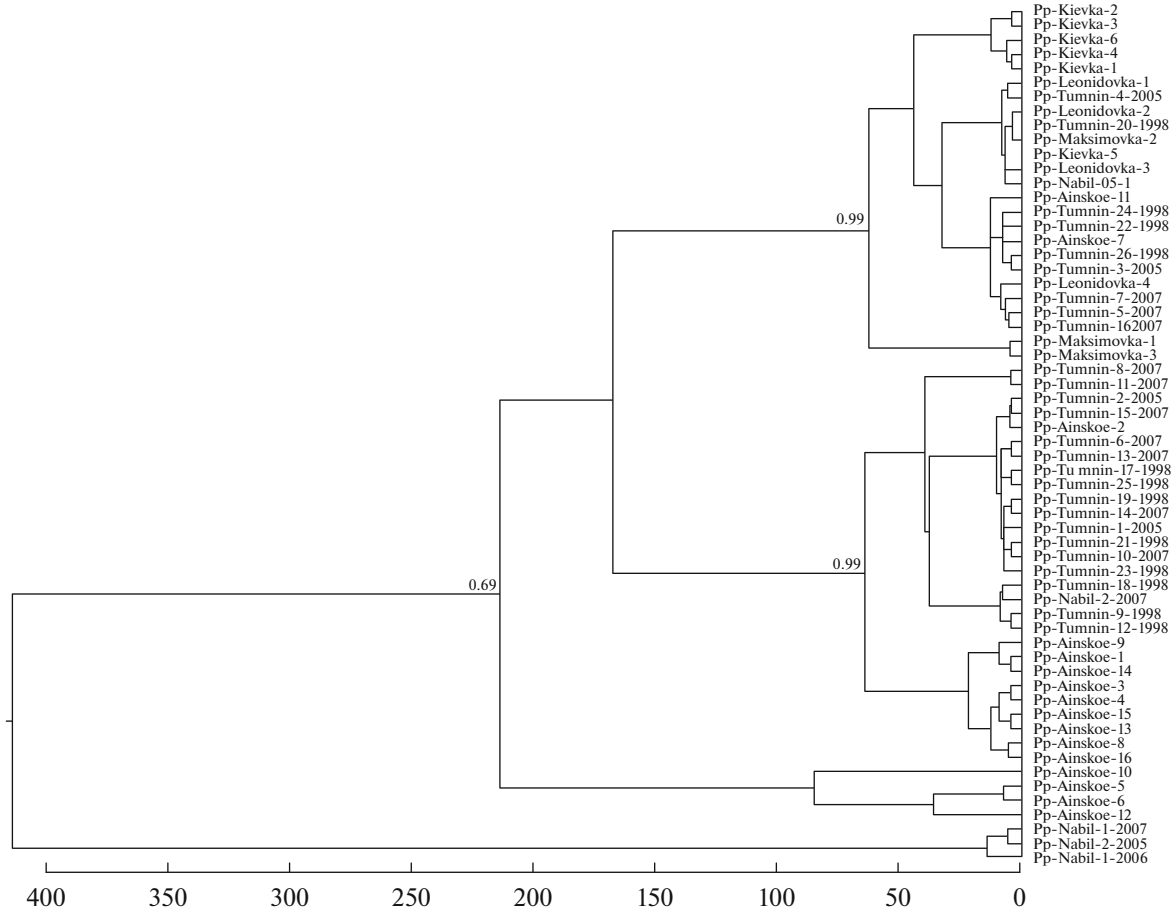

Fig. 7. A Bayesian consensus phylogenetic tree that reflects the genetic diversity of populations and the time of divergence of the main genealogical lines of *Parahucho perryi,* thousand years, according to the combined sequences of the two segments of mtDNA (*COI* and *cytb*). To the right of the tree nodes are the values of posterior probabilities (confidence ≥ 0.95).

10 kyr ago (fourth and third phases of divergence, respectively).

What can determine the phases of divergent evolution? The most significant event in the history of the North Pacific was climate oscillation with a period of approximately 100 kyr [32]. Cooling and advance of glaciers, decline of the ocean level and partial or complete isolation from the ocean with freshening of internal Japan and Okhotsk seas could be the main factors that determine the phases of divergence [1, 2, 8, 22]. Climate change in the Pleistocene resulted in changes on the Earth's surface, such as the redistribution of water between the oceans and glaciers, flooding or draining of riverine basins, great restructuring of the river systems, and the formation of bays and inlets [6].

The first stage of the formation of the individual phylogroups of taimen occurred in the Mindel (Oka) glacial period (478–424 kyr ago). The level of the Sea of Japan off the coast of the Sakhalin Isl. dropped to the level of approximately 100 m below the present level as a result of the Mindel regression [28]. Another period of mtDNA divergence in both studied species occurred in the Riss (Dnieper) glacial period (347–130 kyr ago). The Riss glacier caused a regression that formed the Sea of Japan at a level around 140–150 m below the present one. The two periods of the divergence of the studied species coincide with Wurm (Valdai) glaciation (110–12 kyr ago). Significant regressions in the region were recorded approximately 50 and 25 kyr ago [28].

The last phase of the "explosive" formation of haplotypes (50–10 kyr ago) is apparently associated with the retreat of glaciers and expansion of the areas of both species during the Holocene. The most significant events at the boundary of the Pleistocene and Holocene were the formation of the La Perouse Strait 13–12 kyr ago and the dramatic expansion of the Straits of Tsushima and Tsugaru. These processes had a great impact on the environment, as defined by new temperature conditions, water salinity in the Sea of Japan, and new possibilities for the migration of ichthyofauna [4].

The BSP method of study of the demographic history of populations enabled us to calculate that the initial stage of taimen population growth occurred approximately 12 kyr ago (Fig. 5b). In the chum

salmon this phase began 30–35 kyr ago and intensified in the past 10–15 kyr (Fig. 5a). In taimen, the population growth period is consistent with the end of the last glacial maximum (26.5–19 kyr ago) within the Wurm glaciation. We can assume that the last glaciation to a lesser extent affected the demographics of chum salmon, which was more eurythermal and had a larger habitat. Sakhalin taimen has a limited range; obviously, its distribution is determined by a narrow range of temperature tolerance and greater association to the estuarine areas of the seas, which explains these differences [7, 21].

The typical marine deposits of the northeast coast of the Sakhalin Island indicate that the sea level during the Holocene came closer to the present level relatively early, approximately 11 kyr ago [4]. Due to the Holocene interglacial transgressions the sea water invaded much deepened Pleistocene riverine valleys of the Primorye coast and formed insulated bays with a striated shore line. These isolations also increased the accumulation of genetic variability.

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