

## Phylogeny of Salmonoid Fishes (Salmonoidei) Based on mtDNA *COI* Gene Sequences (Barcoding)

V. S. Artamonova<sup>a, b, \*</sup>, O. V. Kolmakova<sup>b</sup>, E. A. Kirillova<sup>a</sup>, and A. A. Makhrov<sup>a, b</sup>

<sup>a</sup>Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, 119071 Russia

<sup>b</sup>Institute of Biophysics, Krasnoyarsk Science Center, Siberian Branch, Russian Academy of Sciences, Krasnoyarsk, 660036 Russia

\*e-mail: valar99@mail.ru

Received November 22, 2017; in final form, December 22, 2017

**Abstract**—We have analyzed the partial sequences of the mitochondrial *COI* gene along with the amino acid sequences of cytochrome oxidase subunit I, encoded by this gene region, in representatives of 11 genera of salmonoid fish. For amino acid sequences, two alternative networks are constructed with outgroups represented by either Esocoidei or Osmeroidei as the supposed ancestral groups. This way, Osmeroidei appear to be closer to the salmonoid fish than Esocoidei, and their presence in the network as an outgroup explains the available data on the morphology and karyology of salmonoids much better. A number of the results of this study are fundamentally new. In particular, the slowing down of the molecular evolution of the grayling (*Thymallidae*) is shown. We conclude that the charr (*Salvelinus*) is one of the modern genera of salmonoids closest to their ancestor. The hypothesis of the phylogenetic proximity of the genera *Brachymystax*, *Hucho*, and *Salmo* has been confirmed. We also discuss the possibility that it is namely the changes in the amino acid sequence of cytochrome oxidase subunit I that lead to postzygotic reproductive isolation between taxa.

**Keywords:** evolution, network, molecular clock, amino acid sequence, reproductive isolation, immobilization, fishes

**DOI:** 10.1134/S1995425518030022

### INTRODUCTION

Researchers of various specialities have long been paying the closest attention to salmonoid fish. This is because many of these fish are key components of marine and/or freshwater ecosystems (Willson and Halupka, 1995) and have long been the main objects of commercial and amateur fishing and aquaculture (Kazakov, 1988; Karpevich et al., 1991; Chereshev et al., 2002; Montgomery, 2003; Shuntov and Temnykh, 2008; *Lyubitel'skoe rybolovstvo...*, 2010; Sidorov and Reshetnikov, 2014; Artamonova and Makhrov, 2015).

Due to its high ecological and morphological plasticity (Noakes et al., 1989; Pavlov and Savvaitova, 2008; Kuzishchin, 2010), salmonoid fish often serve as model objects for evolutionists (Viktorovskii, 1978; Mina, 1986; Altukhov et al., 1997; *Evolution Illuminated*, 2004). Thousands of publications are devoted to salmonoid fish, and in the present work it is impossible to list even review articles and monographs that generalize this data set.

Salmonoid fish are represented by a large number of genera and species, and researchers have very different views on the origin of large evolutionary lines: there are heated discussions about the systematic sta-

tus of many forms. In our study, we will use the classification according to which the suborder Salmonoidei includes families Salmonidae, Coregonidae, and Thymallidae (*Atlas presnovodnykh ryb...*, 2003), and we will try to shed light on the relationship between these families, as well as their constituting genera (Table 1).

The phylogeny and taxonomy of salmonoid fish were the topic of morphological studies (Regan, 1914; Chernavin, 1923; Norden, 1961; Vladukov, 1963; Rasadnikov, 1980; Alekseev, 1993), publications on the evolution of ontogenesis (Korovina, 1978; Soin, 1980; Kendall and Behnke, 1984; Pavlov and Osinov, 2004), generalizations of paleontological data (Sychevskaya, 1988; Wilson and Li, 1999), works presenting karyological data (Ojima et al., 1963; Rees, 1964; Chernenko, 1969; Vasil'ev, 1977; Allendorf and Thorgaard, 1984; Hartley, 1987; Frolov, 2000; Phillips and Rab, 2001; Zelinsky and Makhrov, 2002; Sutherland et al., 2016), allozymes (Osinov and Lebedev, 2000; Osinov and Lebedev, 2004), sequences of mitochondrial and nuclear DNA (Ramsden et al., 2003; Crespi and Fulton, 2004; Phillips et al., 2004; Matveev and Okada, 2009; Yasuike et al., 2010; Wang et al., 2011; Shedko et al., 2012; Macqueen and Johnston, 2014; Zhivotovskii, 2015; Lappin et al., 2016; Horreo, 2017), and papers summarizing and integrating data obtained

Table 1. Characteristics of the fish samples

Family	Genus	Species (subspecies)	Sample collection site	Collection date	Haplotype, number of its carriers, and number in GenBank	Variant of amino acid sequence		
Salmonids (Salmonidae)	Lenoks ( <i>Brachymystax</i> )	<i>Brachymystax lenok</i> (sharp-snouted form)	Ayakhta River (Yenisei basin)	June 14, 2016	Brlc (3) MG951554	BRLS		
			Komissarovka River (Amur basin)	May 24, 2017	Brlc (3) MG951555	BRLS		
		<i>Brachymystax lenok</i> (blunt-snouted form)	Komissarovka River (Amur basin)	May 24, 2017	Brsa (4) MG951556	BRLS		
			Muraveika River (Amur basin)	May 19, 2017	Brsa (1) MG951557	BRLS		
	Taimens ( <i>Hucho</i> )	<i>Taimen</i> ( <i>Hucho taimen</i> )	Ayakhta River (Yenisei basin)	June 14, 2016	Huch (2) MG951558	HUCH		
	Sakhalin taimens ( <i>Parahucho</i> )	Sakhalin taimen ( <i>Parahucho perryi</i> )	Langeri River (Sakhalin Island)	June–July 2015	PaHu1 (2) MG951559 PaHu2 (1) MG951560	PAHU		
			Severnol Lake (Novaya Zemlya)	July 2017	Salv1 (2) MG951561; Salv2 (1) MG951562	SALV		
	Arctic charrs ( <i>Salvelinus</i> )		Arctic charr ( <i>Salvelinus alpinus</i> )	Svyatloe Lake (Novaya Zemlya)	July–August 2015	Salv3 (1) MG951563	SALV	
				Gornoe Lake (Novaya Zemlya)	July–August 2010	Salv3 (2) MG951567	SALV	
				Yangoto Lake (Vaigach Island)	August 7–9, 2010	Salv1 (2) MG951564	SALV	
				Topozero Lake (White Sea basin)	2000	Salv1 (2) MG951565	SALV	
				Ladoga Lake	2000	Salv4 (4) MG951566	SALV	
	Brook trout ( <i>Salvelinus fontinalis</i> )			Yellowstone National Park	July 21, 2017	Safo (3) MG951571	SALV	
				Dolly Varden trout ( <i>Salvelinus malma</i> )	Ozernaya River (Kamchatka)	September 6, 2012	Sama (3) MG951572	SALV
	Bogomid charr ( <i>Salvelinus boganidae</i> )			Sobachye Lake (Taimyr)	September 2016	Salv1 (3) MG951568	SALV	
				Salvelinus sp. (goggle-eyed charr (puheglazka))	Sobachye Lake (Taimyr)	September 2016	Salv1 (2) MG951570; Salv5 (1) MG951569	SALV (2); SALL (1)
					Rofor breed	February 2009	Onmy1 (3) MG951595; Onmy2 (1) MG951598	ONMY
Pacific salmonids ( <i>Parasalmo</i> )		rainbow trout ( <i>Parasalmo mykiss</i> )	Rostal breed	February 2009	Onmy1 (2) MG951596	ONMY		
			Donaldson breed	February 2008	Onmy1 (2) MG951597; Onmy3 (1) MG951599	ONMY		

Table 1. (Contd.)

Family	Genus	Species (subspecies)	Sample collection site	Collection date	Haplotype, number of its carriers, and number in GenBank	Variant of amino acid sequence
Salmonids (Salmonidae)	Pacific trouts ( <i>Parasalmo</i> )	Rainbow trout ( <i>Parasalmo mykiss</i> )	Tao River (Yellow River Basin), fish that escaped hatchery	October 9–10, 2012	Onmy 1 (2) MG951594	ONMY
			Avgustin breed group	March 2008	Onmy4 (2) MG951601	ONMY
			Kamloops breed (Karachay-Cherkess Republic)	June 18, 2014	Onmy4 (3) MG951600	ONMY
	Noble Salmons ( <i>Salmo</i> )	Black Sea brown trout ( <i>Salmo trutta labrax</i> )	Dyurso River (Black Sea basin)	August–September 2015	SaTt11 (2) MG951578	SATR
			Mzymta River (Black Sea basin)	January 2008	SaTt11 (2) MG951579; SaTt12 (1) MG951580	SATR
			Azerbaijan, Chukhur-Kabalin-sky fish hatchery	October 1, 2012	SaTc (4) MG951581	SATR
			Varzina River (Kola Peninsula)	–	SaTt (2) MG951582	SATR
	Atlantic salmon ( <i>Salmo salar</i> )	Brown trout ( <i>Salmo trutta trutta</i> )	Drozdovka River (Kola Peninsula)	–	SaTt (1) MG951583	SATR
			Keret River (White Sea basin)	September 29, 2016	SaSr (2) MG951584	SASA
			Son River (White Sea basin)	July 6, 2011	SaSr(1) MG951585	SASA
	Pacific salmons ( <i>Oncorhynchus</i> )	Pink salmon ( <i>Oncorhynchus gorbusha</i> ); even line	Malaya Khuzy River (Sakhalin Island), downstream migrating juvenile fish	June 15, 2017	Ogev1 (1) MG951589; Ogev2 (1) MG951591	OGORI
			Langeri River (Sakhalin Island), downstream migrating juvenile fish	June 18, 2017	Ogev1 (1) MG951608; Ogev2 (1) MG951593	OGORI
			Keret River (White Sea basin)	June 25, 2016	Ogev1 (1) MG951590	OGORI

Table 1. (Contd.)

Family	Genus	Species (subspecies)	Sample collection site	Collection date	Haplotype, number of its carriers, and number in GenBank	Variant of amino acid sequence
Salmonids (Salmonidae)	Pacific salmon ( <i>Oncorhynchus</i> )	Pink salmon ( <i>Oncorhynchus gorbuscha</i> ); odd line	Keret River (White Sea basin)	August 26, 2013	Ogod1 (7) MG951586; Ogod2 (1) MG951587; Ogod3 (4) MG951588	OGOR1 (11) OGOR2 (1)
		Coho salmon ( <i>Oncorhynchus kisutch</i> )	Langeri River (Sakhalin Island)	September 6–26, 2016	Onki1 (1) MG951602; Onki2 (2) MG951603; Onki3 (1) MG951604	ONKI
		Masu salmon ( <i>Oncorhynchus masou</i> )	Partizanskaya River (Primorsky krai)	May 28, 2017	Onma1 (2) MG951605; Onma2 (1) MG951606	ONMA
Whitefish (Coregonidae)	<i>Prosopium</i>	Round whitefish ( <i>Prosopium cylindraceum</i> )	Malaya Khuzi River (Sakhalin Island)	July 2, 2017	Onma3 (1) MG951607	ONMA
		Common whitefish ( <i>Coregonus lavaretus</i> )	Sobachye Lake (Taimyr)	2017	Pros1 (1) MG951550; Pros2 (1) MG951551	PROC
		Inconnu ( <i>Stenodus leucichthys</i> )	Ayakhta River (Yenisei basin)	June 14, 2016	Corg (2) MG951552	COST
Thymallidae	Graylings ( <i>Thymallus</i> )	Arctic grayling ( <i>Thymallus arcticus</i> )	Yenisei River	August 23, 2016	Sten (2) MG951553	COST
		Grayling ( <i>Thymallus thymallus</i> )	Ayakhta River (Yenisei basin)	June 14, 2016	ThAr1 (4) MG951573	THAT
		Amur grayling ( <i>Thymallus arcticus</i> grubei)	Onega Lake	August 2016	ThTh (3) MG951574	THAT
Pikes (Esocidae)	Pikes ( <i>Esox</i> )	Northern pike ( <i>Esox lucius</i> )	Muraveika River (Amur basin)	May 19, 2017	ThSp1 (2) MG951575; ThSp2 (1) MG951576	THSP
		Pond smelt ( <i>Hypomesus olidus</i> )	Poperechnaya River (Amur basin)	May 23, 2017	ThSp1 (2) MG951577	THSP
		European smelt ( <i>Osmerus eperlanus</i> )	Ayakhta River (Yenisei basin)	June 14, 2016	EsLu (1) MG951592	EXOS
Smelts (Osmeridae)	<i>Hypomesus</i>	Pond smelt ( <i>Hypomesus olidus</i> )	Varandey Bay (Barents Sea)	2016	Hyo1 (5) MG951609	HYOL
		European smelt ( <i>Osmerus eperlanus</i> )	Baltic Sea	2017	Osep1 (2) MG951610	OSEP

by different methods (Tchernavin, 1939; Berg, 1948; Behnke, 1970; Dorofeeva et al., 1980; Sanford, 1990; Makoedov, 1999; Alexandrou et al., 2013; Makhrov, 2017; Robertson et al., 2017). A huge number of publications are devoted to the evolution of individual groups of salmonoid fish.

There are works where solutions to taxonomy problems employ data on the sequence of the mitochondrial *COI* gene encoding the subunit I of the cytochrome oxidase complex (Complex IV), but these articles are devoted to solving an applied problem: identifying several practically important salmon species (Rasmussen et al., 2009; Cline, 2012; Muñoz-Colmenero et al., 2017, and references in that work). Meanwhile, Complex IV includes proteins encoded by both mitochondrial and nuclear genes, and these proteins must correspond to each other in their structure for the normal functioning of the system as a whole (Li et al., 2006). Moreover, *COI* is the only gene whose participation in speciation has been proved in a special study (Hebert et al., 2003).

All this makes the sequence of the mitochondrial *COI* gene an extremely valuable carrier of information in the reconstruction of events associated with the emergence of new taxa in evolution, especially since mitochondrial DNA does not normally recombine, and, therefore, stores information, including that about the sequence of events that led to emergence of a particular species.

We believe that combining the sequence of this gene with other sequences of mitochondrial DNA during analysis, as some researchers do (Crête-Lafrenière et al., 2012; Shedko et al., 2013), is unjustified, since the selection rate for different genes is different and the role of other mitochondrial genes in the speciation process is not shown.

The reconstruction of evolutionary events associated with changes in the sequence of mitochondrial DNA (substitutions, insertions, and deletions of nucleotides) is carried out by constructing a network of haplotypes that are variants of the nucleotide sequence of some region of mitochondrial DNA (Morrison, 2005). Originally, this method was used to study intraspecific diversity (Bandelt et al., 1999), but it is also successfully used to study the evolutionary relationships of large systematic groups (Huson and Bryant, 2006). However, to the best of our knowledge, this method has not been used to study the supraspecific evolution of salmonids.

In our work, we applied the haplotype network construction method suitable for studying evolutionary events at the level of the emergence of entire genera and families. In order to reconstruct evolutionary events of this scale, we took into account that only very rare substitutions in the sequence of the *COI* gene, whose product should work in conjunction with nuclear proteins, can lead to a change in the amino acid sequence of the protein and also not be lethal, and

constructed a network of haplotypes for the partial amino acid sequence of cytochrome oxidase I. This approach has long been known (Bandelt et al., 1999), but it is rarely used.

This paper discusses the features of taxonomy of salmonoid fish resulting from the presented reconstruction of evolutionary events, as well as the correspondence of the picture obtained to the existing concepts of the origin of the major evolutionary lines of this suborder.

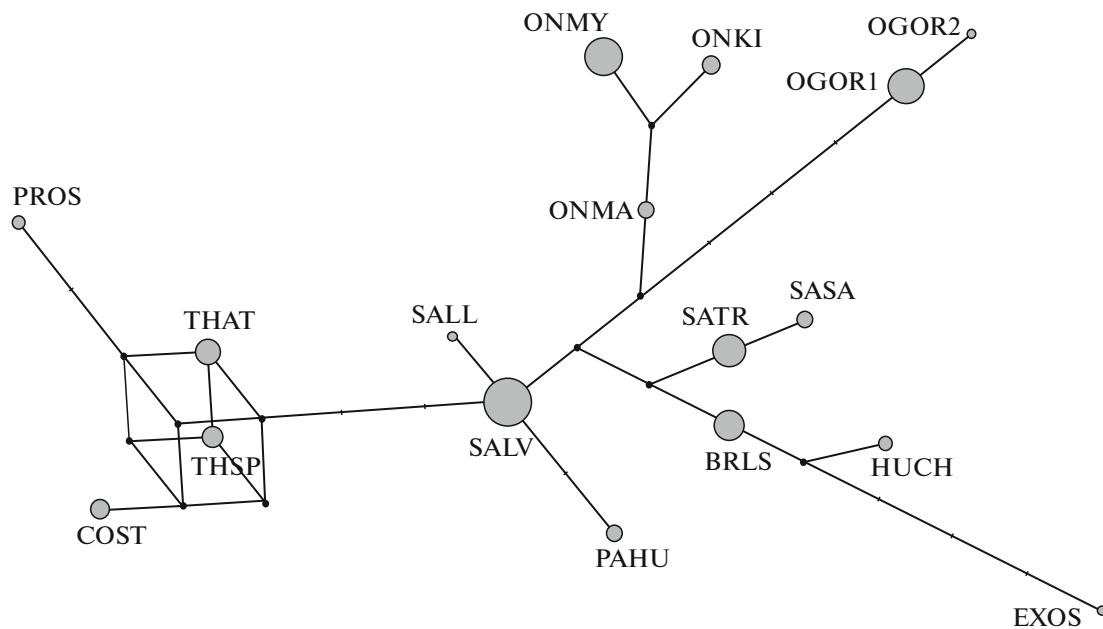
## MATERIALS AND METHODS

**Biological samples.** Biological material consisted of fragments of adipose fins or white fish muscles fixed with 96% ethanol. Information on the species affiliation of the fish studied, as well as the material collection sites, is provided in Table 1.

**Isolation of DNA and amplification of the partial sequence of the mitochondrial *COI* gene.** Total cellular DNA was isolated using the DNA-EXTRAN-2 reagent kit (produced by Synthol, Russia) according to the manufacturer's recommendations.

A PCR product containing a partial sequence of the mitochondrial *COI* gene was obtained using the Tertsik thermal cycler (DNA Technology, Russia) using universal primers designed specifically for fish (Ward et al., 2005). For representatives of the genera *Salvelinus*, *Salmo*, *Parasalmo*, and *Oncorhynchus*, we used primers FishF1 and FishR1; for all others we used FishF2 and FishR2. Amplification was carried out in 25  $\mu$ L of buffer manufactured by Fermentas (Lithuania, Vilnius) containing 75 mM Tris-HCl (pH 8.8), 20 mM  $(\text{NH}_4)_2\text{SO}_4$ , 0.1% Tween 20, and 2 mM  $\text{MgCl}_2$ . The amplification mixture contained about 300 ng of total cell DNA, 200 nmol each of four deoxyribonucleotides, 10 pmol of forward and reverse primers, and 0.5–0.7 polymerase units (produced by Bionem, Russia). To prevent evaporation during the reaction, about 12  $\mu$ L of mineral oil was layered on top of the mixture. The amplification program included the stage of initial denaturation of DNA at +95°C for 4 min, 31 cycles of DNA fragment amplification at +95°C for 30 s, +50°C for 30 s, and +72°C for 50 s, as well as the final chain elongation stage at +72°C for 5 min.

**PCR product sequencing.** The resulting PCR product was reprecipitated under mild conditions, adding ammonium acetate to the sample extracted from the mineral oil to a final concentration of 0.125 M and ethanol to 70%; the mixture was kept for 20 min at room temperature and then centrifuged for 20 min at 13000 rpm (centrifuge Eppendorf 5415R). The precipitate was washed with 70% cooled ethanol, dried, and redissolved in bidistilled water. The sequencing reaction was carried out taking 20 ng of the PCR product and 3.2 pmol of the corresponding primer. Each sample was sequenced twice, from the forward and reverse



**Fig. 1.** Network for the partial amino acid sequence of cytochrome oxidase subunit I, where the pike, a representative of the suborder Esocoidei, is selected as an outgroup with respect to the subfamily Salmonoidei. The lengths of the branches are proportional to the number of amino acid changes in the protein sequence. For the notation of the sequence variants, see Table 1.

primer. The sequencing reaction was carried out using the ABI PRISM® BigDye™ Terminator v. 3.1 reagents with the subsequent analysis of the reaction products on the ABI PRISM 3730 Applied Biosystems sequencer of the Institutional Collective Use Center “Genome” of the Institute of Molecular Biology of the Russian Academy of Sciences.

**Analysis of sequencing results and construction of haplotype networks for mtDNA COI sequences and their amino acid sequences.** The sequencing results were analyzed using a specialized BioEdit v. 7.0.5. editor (Hall, 1999). To translate nucleotide sequences into amino acids, the option *Translate in Selected Frame (Permanent)* was used in employing the genetic code of mitochondria.

Network 5.0.0.1 software was used (<http://www.fluxus-engineering.com>) for the construction of haplotype networks. For partial mtDNA COI sequences, the option *Median Joining* was used and, for the amino acid sequences, the option *Reduced Median* was used.

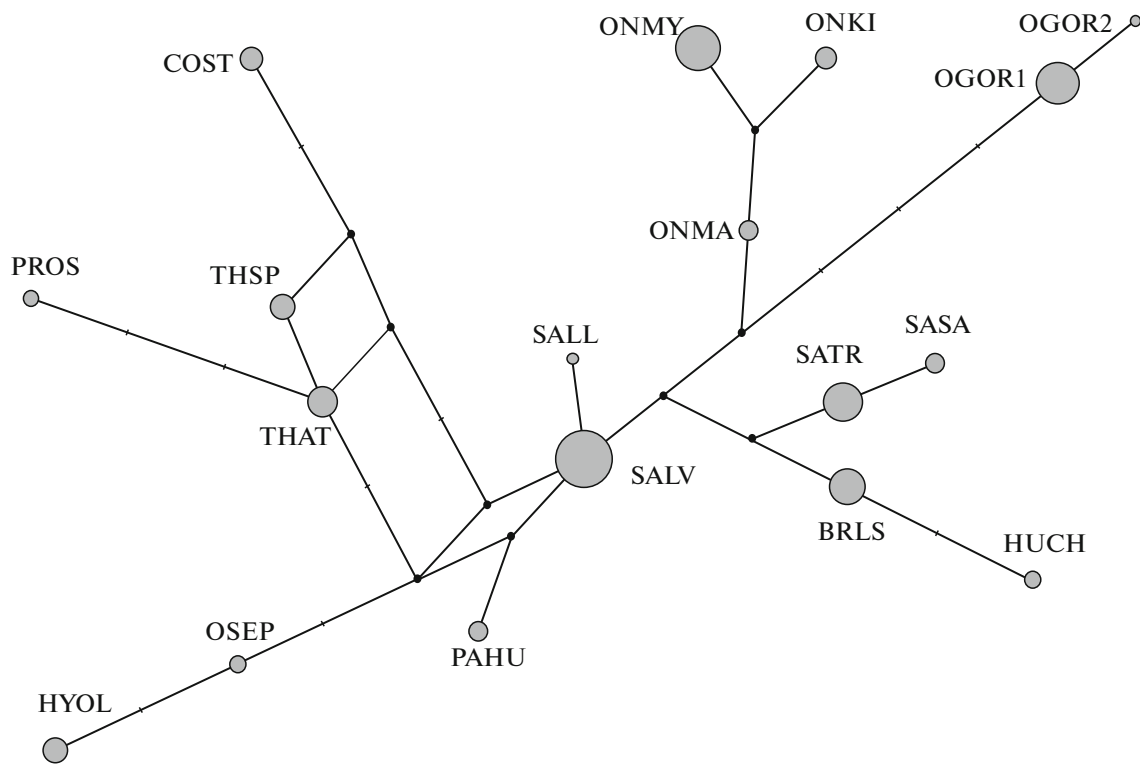
## RESULTS

Nucleotide and amino acid sequences of 124 representatives of different species of fish belonging to 14 genera were studied. For the construction of networks, partial sequences of the mitochondrial COI gene of 651 bp encoding the amino acid sequences of 217 amino acid residues were used, which belonged to 45 different haplotypes and 19 variants of the amino acid sequence of cytochrome oxidase subunit I. The

sequences representing these haplotypes are placed in the international GenBank database; the sequence numbers are given in Table 1.

Networks for variants of the partial amino acid sequence of the mitochondrial COI protein are shown in Figs. 1 and 2. They take into account the sequences of representatives of all salmonoid genera except for *Salvethymus*, the genus whose only representative is longfin Svetovidov's charr (*Salvethymus svetovidovi*). However, the nucleotide sequence of the mitochondrial COI gene presented in the international Genbank database (JX960966.1) suggests that the partial amino acid sequence of cytochrome oxidase subunit I in this single representative of the genus *Salvethymus* does not differ from the corresponding amino acid sequence fragment characteristic of the vast majority of representatives of the genus *Salvelinus*. Thus, adding this species into analysis does not change the topology of the branches of the network.

The networks shown in Figs. 1 and 2 were constructed using the sequences for the same representatives of salmonoid fish. Data groups differed only in that, in the first case, pike belonging to the suborder Esocoidei was selected as an outgroup with respect to the suborder Esocoidei and, in the second case, representatives of two genera of the suborder of Osmeroidei, *Osmerus* and *Hypomesus*, were selected. The choice of the representatives of these suborders was due to their proximity to salmonoid fish in terms of morphological features. Osmeroidei are considered immediate ancestors of salmonoid fish: it is assumed that tetraploidization of the genome occurred in one of the smeltlike



**Fig. 2.** Network for the partial amino acid sequence of cytochrome oxidase subunit I, where representatives of two genera of the suborder Osmeroidei are selected as an outgroup with respect to the subfamily Salmonoidei. The lengths of the branches are proportional to the number of amino acid changes in the protein sequence. For the notation of the sequence variants, see Table 1.

ancestors, and this ancestor gave rise to a new fish suborder, Salmonidae. This assumption is favored by karyological data (Frolov, 2000).

As for Esocoidei, according to the results of an analysis of a number of mitochondrial and some nuclear genes, they turned out to be more similar to salmonoid fish than Osmeroidei are (Ramsden et al., 2003; Asinov and Lebedev, 2004, Li et al., 2010; Campbell et al., 2013; and references in these works). Therefore, we found it necessary to test, among other things, the hypothesis of the origin of salmonoid fish from the ancient representatives of the suborder Esocoidei.

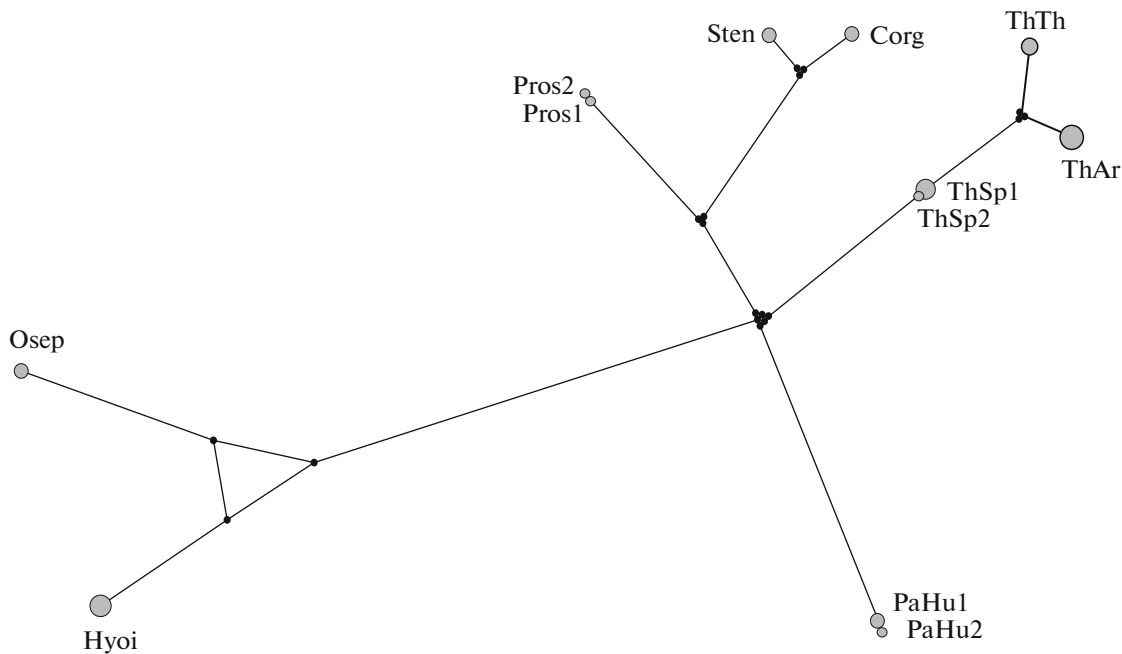
A comparison of the two variants of the network shows that its overall topology, in general, remains independent of the choice of the external group. Moreover, in both cases, there is incomplete unambiguity in the arrangement of branches for a group of fish that includes *Thymallus* and *Coregonus*, although possible variants of the origin of phylogenetic lines look somewhat different in the case of two different outgroups.

In order to clarify this issue, Fig. 3 presents a network of haplotypes for a partial nucleotide sequence of *COI*, which includes data for *Coregonus* and *Thymallus*, as well as for their nearest neighbors, smelts and Sakhalin taimen. Figure 3 clearly shows that the arrangement of branches for *Coregonus* and *Thymallus* makes it possible to conclude that these two families

independently originate from a common smeltlike ancestor, which also independently gave rise to the Sakhalin taimen, a representative of the family Salmonidae.

We assume that expanding the network with the inclusion of a larger number of *Coregonus* species would make it possible to clarify this issue at the level of the amino acid sequence of the *COI* protein, which generally speaking would be more preferable. This is due to the fact that a large number of nucleotide variations in similar sequences of phylogenetically distant species usually does not allow for the reconstruction of evolutionary events at the level of the nucleotide sequence. Our attempt to do this was successful most likely due to the correct choice of the outgroup, and also the fact that the rate of the mutation process in the coding sequence of the *COI* gene is relatively low; because the need for the coordinated work of cytochrome oxidase subunit I with the proteins encoded by nuclear genome sequences, this gene is under severe pressure of selection.

In order to assess how the rate of nucleotide substitutions correlates with the rate of amino acid substitutions, we constructed a network of haplotypes of the partial sequence of *COI* for the genus *Salvelinus*, including as an outgroup a similar sequence for the sole representative of the genus *Parahucho*, the Sakhalin taimen. These data are presented in Fig. 4.



**Fig. 3.** Median network of haplotypes of the partial sequence of mitochondrial *COI* gene for representatives of families Coregonidae and Thymallidae. Sequences of two genera (*Hypomesus* and *Osmerus*) of Osmeroidei, the supposed diploid ancestors of these families, are used as outgroups. Additionally, the network of haplotypes includes sequences of this gene of the Sakhalin taimen, the only representative of the genus *Parahucho*, belonging to the family Salmonidae, which is related to genera *Coregonus* and *Thymallus*. The lengths of branches between the points separating the representatives of different genera, as well as between the branching points of the network (median vectors) and the closest haplotypes of representatives of the genera, are proportional to the number of nucleotide substitutions in the gene sequence. For the notation of haplotypes, see Table 1.

## DISCUSSION

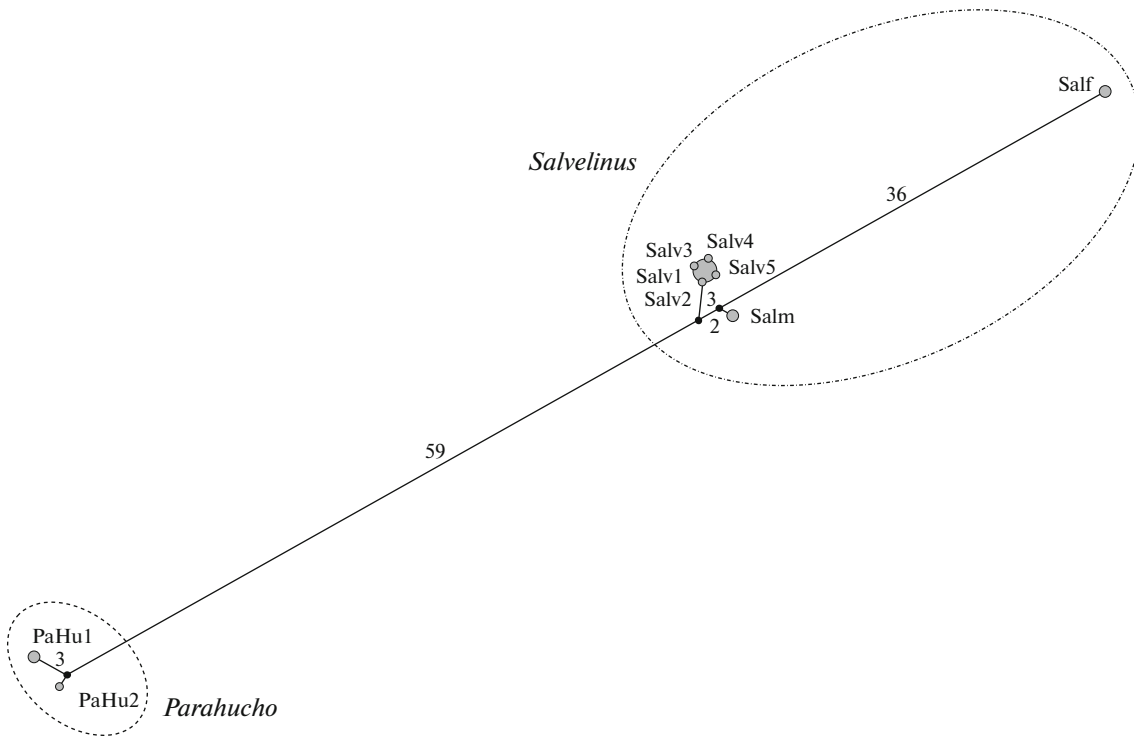
**Amino acid substitutions, taxonomy, and reproductive isolation.** First, it should be noted that, regardless of which particular variant of the haplotype network for the amino acid sequence of cytochrome oxidase subunit I is considered (Fig. 1 or Fig. 2), most genera of the suborder of salmonoid fish are separated from each other by at least two substitutions in the considered partial amino acid sequence. There is no univocal correspondence between the number of nucleotide and amino acid substitutions.

Thus, Fig. 4 shows the network of haplotypes of the partial sequence of the *COI* gene for the genus *Salvelinus*, where the outgroup includes a sequence for the Sakhalin taimen, the only representative of the genus *Parahucho*. The nearest haplotypes for the genera *Salvelinus* and *Parahucho* are separated by 63 nucleotide and two amino acid substitutions, and the most distant from each other haplotypes of representatives of the genus *Salvelinus*, which have absolutely identical amino acid sequences of cytochrome oxidase subunit I, are separated by 43 nucleotide substitutions (see Fig. 4). Moreover, only 38 nucleotide substitutions separate haplotypes of representatives of the genera *Brachymystax* and *Hucho*, which are separated by two amino acid substitutions.

These data make it possible to suggest that cytochrome oxidase is one of the most important proteins that provides postzygotic reproductive isolation: the disparity between proteins of Complex IV, which are encoded by the nuclear and mitochondrial genomes, leads to the death of intergeneric hybrids. At the same time, many hybrids between species that are part of the same genus are viable, and often fertile. This is particularly true in cases where amino acid sequences of cytochrome oxidase subunit I coincide in the two species: thus, hybrids between the Arctic charr and the brook trout are viable and fertile (Gross et al., 2004, and references in that work). Moreover, whitefish and inconnu, which formally belong to different genera (*Coregonus* and *Stenodus*, respectively) but have identical amino acid sequences, also hybridize, even under natural conditions (Reist et al., 1992).

**The problem of the diploid ancestor of salmonoid fish.** There is no doubt that the entire group of salmonoid fish has a tetraploid origin (reviews: Allendorf and Thorgaard, 1984; Hartley, 1987; Frolov, 2000). Extensive data on comparative morphology (Norden, 1961; Dorofeeva et al., 1980) led ichthyologists to the conclusion that the nearest diploid ancestors of salmonoids were fish similar to modern Osmeridae. However, molecular genetic data based on a joint analysis of the sequences of mitochondrial and nuclear genes, which resulted in the construction of various dendrograms,





**Fig. 4.** Median network of haplotypes of the partial sequence of the mitochondrial *COI* gene for the studied representatives of the phylogenetically related genera *Parahucho* and *Salvelinus*. Numbers indicate the number of nucleotide substitutions in the gene sequence between the closest representatives of genera or between haplotypes and branching points of the network (median vectors). For the notation of haplotypes, see Table 1.

led some researchers to the conclusion that the sister group of Salmoniformes (including salmonids, whitefishes, and graylings) is Esociformes (Ramsden et al., 2003; Osinov and Lebedev, 2004, Li et al., 2010; and references in those works).

A comparison of the haplotype networks for the partial amino acid sequence of cytochrome oxidase subunit I, shown in Figs. 1 and 2, shows that this assumption is false. Since mitochondrial DNA, unlike nuclear DNA, does not recombine, its genes are not able to duplicate and change their function: it keeps complete information about the sequence of evolutionary events. If we assume that the diploid ancestor of salmonoid fish was pikelike, then the families of whitefishes and graylings are much younger than the family of salmonids, from which in this case whitefishes and graylings must trace their origin. This contradicts not only the morphological (Norden, 1961; Dorofeeva et al., 1980) but also karyological (Frolov, 2000; Phillips and Rab, 2001) and paleontological (Wilson and Li, 1999) data. Moreover, even if one proceeds from formal estimates and considers that the ancestor genus is the one that is separated from salmonoid fish by a smaller number of changes in the amino acid sequence, it turns out that the pike is separated from salmonoid fish by five amino acid substitutions (Fig. 1) and the smelt is separated only by four (Fig. 2).

In addition, the situation presented in Figs. 2 and 3 is in good agreement with modern taxonomy: it demonstrates the independent origin of graylings, whitefishes, and salmonids from their common smelt-like ancestor. At the level of individual lines representing the evolution of families, there is a tendency to a decrease in the number of chromosomes towards the periphery (examples are Atlantic salmon and pink salmon).

Proceeding from the foregoing, in further analysis of the results, we proceeded from the assumption that the ancestor of salmonoid fish was smeltlike and considered only a network of haplotypes that included Osmeridae as an outgroup (Fig. 2).

**Slowing down of the molecular evolution of graylings.** The key advantage of a haplotype network (for both nucleotide and amino acid sequences) against a dendrogram is the ability to distinguish more ancient haplotypes from younger ones. An examination of the network that we obtained (Fig. 2) shows that amino acid sequences of modern graylings are much more similar to sequences of smelts, ancestors of salmonoid fish, than sequences of representatives of two other families. This means, in turn, that the molecular evolution of graylings at the level of the amino acid sequence was much slower than the molecular evolution of whitefishes and salmonids (although Arctic charrs, representatives of salmonids, also have haplotypes similar

to haplotypes of Osmeridae). The molecular evolution of pink salmon was particularly rapid.

It should be noted that, in terms of the characteristics of the karyotype, the graylings are also closer to their common ancestor than other salmonoids (Frolov, 2000); therefore, it can be assumed that these fish are also ecologically and morphologically closer to their common ancestor than salmonoids and whitefishes.

The fact of differences in the rates of evolution of different groups of salmonoids, as well as many other facts (Artamonova and Makhrov, 2006; Rol'skii et al., 2017; Bolotov et al., 2017; Makhrov and Laius, 2018), refute the molecular clock hypothesis, which has become widespread in recent decades. Meanwhile, with the accumulation of facts that contradict it, supporters of this hypothesis only complicate their models more and more (like supporters of the geocentric model of the world once did) instead of trying to confirm it experimentally.

**Arctic charrs are the closest modern group to the ancestor of salmonids.** The principal difference in the phylogeny of salmonoids, resulting from the haplotype network that we represented for the partial amino acid sequence of cytochrome oxidase subunit I, from the ideas typical of most evolutionary geneticists (review: Zhivotovskii, 2015) is the proximity of the ancestors of this family to modern Arctic charrs. However, the network of haplotypes undoubtedly more adequately describes the evolutionary process when compared to dendrograms, on which genetics at the present rely heavily.

The origin of other salmonid species from fish similar to Arctic charrs contradicts the most widespread concepts of salmonid evolution that morphologists have (Norden, 1961; Glubokovskii, 1995). However, evolution schemes of this group, which have been proposed on the basis of morphological data, include some that are in good agreement with our data. Thus, Arctic charrs are close to the ancestor of salmonoids in Fig. 2.14 (b) in the monograph of Glubokovskii (1995). This figure is a treelike graph constructed by the Lee method using data on 45 morphological traits. In addition, representatives of the genus *Salvelinus*, unlike other salmonoids, are similar to representatives of the genus *Thymallus* in the structure of the lateral line system (Rassadnikov, 1980).

Sanford (1990) attempted to construct a phylogeny of salmonoids in terms of different traits on the basis of a cladistic approach, noted the unresolved trichotomy of the branch leading to *Salvelinus* and the branches leading to *Brachymystax* + *Hucho* and *Salmo* + *Oncorhynchus*. The reason for this is both the osteological trait common for *Salvelinus*, *Brachymystax*, and *Hucho* and the osteological trait common for *Salvelinus*, *Salmo*, and *Oncorhynchus* (Sanford, 1990). Now the reason for this trichotomy is clear: Arctic charrs are an ancestor of the other four genera.

The assumption that representatives of the genus *Salvelinus* are closest to the ancestor of salmonoids is favored by the fact that Arctic charrs are distinguished by a very high ecological and morphological diversity (reviews: Savvaitova, 1989; Adams and Maitland, 2007; Knudsen et al., 2016), which is characteristic of the ancient groups (Mamkaev, 1968; Rozhnov, 2006).

At the same time, it should be noted that, from the point of view of the well-established pattern concerning the decrease in the number of chromosomes during the evolution of salmonoids (Viktorovskii, 1978), the origin of the karyotype of lenoks, *Brachymystax*, with the number of chromosomes  $2n = 90-92$ , from the modern karyotype of Arctic charrs, *Salvelinus* ( $2n = 76-84$ ), is extremely unlikely. Apparently, the first representatives of salmonoids had a mitochondrial haplotype close to the most common haplotype of modern *Salvelinus*, but at the same time their karyotype was significantly different from the modern one and was close to the karyotype of modern *Brachymystax*.

**A decrease in the number of chromosomes is accompanied by a decrease in the evolutionary plasticity of salmonoids.** The network that we constructed for the partial amino acid sequence of cytochrome oxidase subunit I shows that salmonoid species characterized by a low number of chromosomes (Sakhalin taimen, Atlantic salmon, and pink salmon) are peculiar evolutionary dead ends: they have not given rise to new taxa.

Earlier we have shown (Makhrov, 2017) that these groups have, as a rule, a narrower spectrum of morphological and ecological diversity in comparison with the closest ancestors. The cited work also suggested that the genome rearrangements accompanied by the fusion of several chromosomes at once led, inter alia, to the disruption of the functioning of genetic systems responsible for the formation of certain ecological groups and morphotypes. As a result, taxa that underwent such genome rearrangements have fewer ecological groups and morphotypes than their immediate ancestors.

Thus, groups of salmonoids with a low number of chromosomes can serve as a good example of a decline in evolutionary plasticity. This phenomenon, rather than its natural occurrence in the course of evolution within a single family, has been known for a long time, and different authors have called it different things: immobilization (Shmal'gauzen, 1945), routinization (Lyubishchev, 1982), and frozen evolution (Fleg, 2008).

**Phylogenetic proximity of genera *Brachymystax*, *Hucho*, and *Salmo*.** Different geneticists treat the evolutionary relationships of these three genera differently (Phillips and Oakley, 1997; Oakley and Phillips, 1999; Osinov and Lebedev, 2000; Crespi and Fulton, 2004; Phillips et al., 2004; Matveev et al., 2007; Crête-Lafrenière et al., 2012; Shedko et al., 2012, 2013; Pankova and Brykov, 2013). According to our data, *Bra-*

*chymystax*, *Hucho*, and *Salmo* belong to a single phylogenetic clade, as was previously revealed in a phylogenetic study that employed a large set of genes encoding proteins (Osinov and Lebedev, 2000).

Our research is another piece of evidence indicating that these ideas are true, and more and more such evidence has appeared in recent years. Thus, for example, according to molecular genetic data, *Margaritifera dahurica*, whose larvae are parasites of lenoks, *Brachymystax* (I.V. Vikhrev, personal communication), is a sister species for the freshwater pearl mussel (*Margaritifera margaritifera*), whose larvae are parasites mainly in salmon of the genus *Salmo* (Bolotov et al., 2016). This fact to some extent confirms the evolutionary proximity of the genera *Salmo* and *Brachymystax*.

**The origin of Atlantic salmon.** The hypothesis about the origin of the Atlantic salmon (*Salmo salar*) from an ancestor similar to the modern brown trout (*Salmo trutta*) was previously expressed on the basis of morphological and karyological data (Dorofeeva, 1998). Our network for the partial amino acid sequence of cytochrome oxidase subunit I fully supports this assumption.

Understanding the evolutionary history of Atlantic salmon makes it possible to explain several important issues of biology of this species. In particular, since the formation of landlocked forms is very characteristic of the brown trout and the landlocked forms of the Atlantic salmon are rare, the hypothesis of the relic nature of the landlocked forms of this species seems quite reasonable (Kuderskii, 1977; Lysenko, 1994; Makhrov et al., 2013).

In addition, since the brown trout can repeatedly become infected with larvae of the freshwater pearl mussel, while the Atlantic salmon develops immunity after a single infection, the hypothesis about the symbiosis of the Atlantic salmon and the freshwater pearl mussel is questionable (Makhrov and Bolotov, 2010). On the contrary, it is rather possible to talk about the intensification of the immune response to infection by larvae of the freshwater pearl mussel during the evolution of the Atlantic salmon.

**Evolutionary relationships between genera *Oncorhynchus* and *Parasalmo*.** The systematic status of *Parasalmo* has been discussed for many years. Most researchers do not distinguish the genus *Parasalmo*, attributing the species of this group to the genus *Oncorhynchus*. However, other specialists (including some of the authors of this paper) defended the notion that *Parasalmo* is a separate genus (Zelinsky and Makhrov, 2001; Pavlov et al., 2001; *Atlas presnovodnykh ryb...*, 2003).

It should be recognized that the data we have obtained is a strong indication in favor of the fact that *Parasalmo* is not a separate genus but part of the genus *Oncorhynchus*. According to the data, a representative of this genus, the rainbow trout, originates from an ancestor similar to the masu salmon (*Oncorhynchus*

*masou*). At the same time, *Parasalmo* and *Oncorhynchus* have significant differences in morphological traits and in ecology (in particular, *Oncorhynchus*, with rare exceptions, is characterized by monocyclic-ity); this must be taken into account when deciding on the systematic position of *Parasalmo*.

## CONCLUSIONS

The network obtained for the partial amino acid sequence of cytochrome oxidase subunit I makes it possible to draw several conclusions about the phylogeny of salmonoid fish. There is a fundamentally new idea about the origin of all salmonids from an ancestor close to the modern Arctic charrs (*Salvelinus*). We confirm the ideas expressed earlier in the literature but still discussed the origin of salmonoid fish from Osmeroidei, the phylogenetic proximity of the genera *Brachymystax*, *Hucho*, and *Salmo*; the origin of the Atlantic salmon from a brown trout-like ancestor; and the belonging of the *Parasalmo* group to the genus *Oncorhynchus*.

Some of the results obtained in this work have a general biological significance. In particular, the data indicate that the evolution of salmonids, starting with highly polymorphic Arctic charrs, ultimately leads to taxa with a small ecological and morphological diversity and with a low evolutionary potential (which experienced immobilization). This process is accompanied by the fusion of entire chromosome groups.

Another interesting phenomenon noted in this work is the preservation in an almost unchanged form of several ancient haplotypes and the slowing down of the molecular evolution of some groups of salmonoid fish. In particular, it has been shown that haplotypes of grayling fish are very close to haplotypes of the ancestor of all salmonoid fish. Contrary to the molecular-clock hypothesis, the evolution of graylings was much slower than the evolution of whitefish and salmonids.

It is also suggested that the main mechanism of postzygotic reproductive isolation is the discrepancy between the amino acid sequence of cytochrome oxidase subunit I encoded by the mitochondrial genome and the amino acid sequences of nuclear proteins, which together with this protein constitute so-called Complex IV.

## ACKNOWLEDGMENTS

We are very grateful to colleagues who helped collect samples: E.G. Berestovskii, I.N. Bolotov, E.A. Borovikova, I.V. Vikhrev, L.A. Glushchenko, V.V. Ignatenko, D.P. Karabanov, A.P. Novoselov, V.M. Spitsyn, V.A. Shirokov, and I.L. Shchurov; employees of Trout Hatchery "Adler", the Federal Breeding and Genetic Center for Fish Culture, and Vygsky and Kemsy fish hatcheries; and residents of Barabash-Levada, Len-lu, and Chupa settlements. We also thank S.S. Alekseev for identifying sharp-

snouted and blunt-snouted lenoks. This work was supported by the Russian Science Foundation, project no. 16-14-10001.

## REFERENCES

- Adams, C.E. and Maitland, P.S., Arctic charr in Britain and Ireland—15 species or one? *Ecol. Freshwater Fish*, 2007, vol. 16, pp. 20–28.
- Alekseev, S.S., The data on vomer initiation in some salmon fishes (Salmonoidei) related to their phylogeny, *Zool. Zh.*, 1993, vol. 72, no. 4, pp. 97–105.
- Altukhov, Yu.P., Salmenkova, E.A., and Omel'chenko, V.T., *Populyatsionnaya genetika lososevykh ryb* (Population Genetics of Salmon Fishes), Moscow: Nauka, 1997.
- Alexandrou, M.A., Swartz, B.A., Matzke, N.J., and Oakley, T.H., Genome duplication and multiple evolutionary origins of complex migratory behavior in Salmonidae, *Mol. Phylogenet. Evol.*, 2013, vol. 69, pp. 514–523.
- Allendorf, F.W. and Thorgaard, G.H., Tetraploidy and the evolution of Salmonid fishes, in *Evolutionary Genetics of Fishes*, New York: Plenum, 1984, pp. 1–53.
- Artamonova, V.S. and Makhrov, A.A., Unintentional genetic processes in artificially maintained populations: proving the leading role of selection in evolution, *Russ. J. Genet.*, 2006, vol. 42, no. 3, pp. 234–246.
- Artamonova, V.S. and Makhrov, A.A., *Geneticheskie metody v lososevodstve i forelevodstve: ot traditsionnoi selektsii do nanobiotekhnologii* (Genetic Analysis in Salmon and Trout Farming: From Classical Breeding to Nanotechnologies), Moscow: KMK, 2015.
- Atlas presnovodnykh ryb Rossii* (Atlas of Freshwater Fishes of Russia), Reshetnikov, Yu.S., Ed., Moscow: Nauka, 2003, vol. 1.
- Bandelt, H.-J., Forster, P., and Röhl, A., Median-joining networks for inferring intraspecific phylogenies, *Mol. Biol. Evol.*, 1999, vol. 16, no. 1, pp. 37–48.
- Behnke, R.J., The application of cytogenetic and biochemical systematics to phylogenetic problems in the family Salmonidae, *Trans. Am. Fish. Soc.*, 1970, vol. 99, no. 1, pp. 237–248.
- Berg, L.S., Origin of trouts and other freshwater salmon fishes, in *Pamyati akademika Segeya Alekseevicha Zernova* (In Memoriam of Academician Sergei Alekseevich Zernov), Moscow: Akad. Nauk SSSR, 1948, pp. 159–172.
- Bolotov, I.N., Aksenova, O.V., Bepalaya, Y.V., Gofarov, M.Y., Kondakov, A.V., Paltser, I.S., Stefansson, A., Travina, O.V., and Vinarski, M.V., Origin of a divergent mtDNA lineage of a freshwater snail species, *Radix balthica*, in Iceland: cryptic glacial refugia or a postglacial founder event? *Hydrobiologia*, 2017, vol. 787, pp. 73–98.
- Bolotov, I.N., Vikhrev, I.V., Bepalaya, Yu.V., Gofarov, M.Y., Kondakov, A.V., Konopleva, E.S., Bolotov, N.N., and Lyubas, A.A., Multi-locus fossil-calibrated phylogeny, biogeography and a subgeneric revision of the Margaritiferidae (Mollusca: Bivalvia: Unionoida), *Mol. Phylogenet. Evol.*, 2016, vol. 103, pp. 104–121.
- Campbell, M.A., López, J.A., Sado, T., and Miya, M., Pike and salmon as sister taxa: detailed intraclade resolution and divergence time estimation of Esociformes + Salmoniformes based on whole mitochondrial genome sequences, *Gene*, 2013, vol. 530, pp. 57–65.
- Chereshnev, I.A., Volobuev, V.V., Shestakov, A.V., and Frolov, S.V., *Lososevidnye ryby severo-vostoka Rossii* (Salmonidae Fishes of the Northeast of Russia), Vladivostok: Dal'nauka, 2002.
- Chernavin, V.V., Systematic grouping of some Salmonoidei fishes based on their osteological features, *Izv. Gos. Inst. Opyt. Agron.*, 1923, vol. 1, no. 3, pp. 103–106.
- Chernenko, E.V., Evolution and cytotaxonomy of salmon fishes of family Salmonidae, *Vopr. Ikhtiol.*, 1969, vol. 9, no. 6, pp. 971–980.
- Cline, E., Marketplace substitution of Atlantic salmon for Pacific salmon in Washington State detected by DNA barcoding, *Food Res. Int.*, 2012, vol. 45, pp. 388–393.
- Crespi, B.J. and Fulton, M.J., Molecular systematics of Salmonidae: combined nuclear data yields a robust phylogeny, *Mol. Phylogenet. Evol.*, 2004, vol. 31, pp. 658–679.
- Crête-Lafrenière, A., Weir, L.K., and Bernatchez, L., Framing the Salmonidae family phylogenetic portrait: A more complete picture from increased taxon sampling, *PLoS One*, 2012, vol. 7, no. 10, p. e46662.
- Dorofeeva, E.A., Systematics and the history of distribution of European salmon of genus *Salmo*, *Vopr. Ikhtiol.*, 1998, vol. 38, no. 4, pp. 437–447.
- Dorofeeva, E.A., Zinov'ev, E.A., Klyukanov, V.A., Reshetnikov, Yu.S., Savvaitova, K.A., and Shaposhnikova, G.Kh., Modern studies in phylogeny and classification of salmon fishes, *Vopr. Ikhtiol.*, 1980, vol. 20, no. 5, pp. 771–791.
- Evolution Illuminated. Salmon and Their Relatives*, Hendry A.P. and Stearns S.C., Eds., Oxford: Oxford Univ. Press, 2004.
- Flegr, J., *Frozen Evolution*, Prague: Charles Univ., 2008.
- Frolov, S.V., *Izmenchivost' i evolyutsiya kariotipov lososevykh ryb* (Variability and Evolution of Karyotypes of Salmon Fishes), Vladivostok: Dal'nauka, 2000.
- Glubokovskii, M.K., *Evolutsionnaya biologiya lososevykh ryb* (Evolutionary Biology of Salmon Fishes), Moscow: Nauka, 1995.
- Gross, R., Gum, B., Reiter, R., and Kühn, R., Genetic introgression between Arctic charr (*Salvelinus alpinus*) and brook trout (*Salvelinus fontinalis*) in Bavarian hatchery stocks inferred from nuclear and mitochondrial DNA markers, *Aquacult. Int.*, 2004, vol. 12, pp. 19–32.
- Hall, T.A., BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT, *Nucleic Acids Symp. Ser.*, 1999, vol. 41, pp. 95–98.
- Hartley, S.E., The chromosomes of salmonid fishes, *Biol. Rev.*, 1987, vol. 62, no. 3, pp. 197–214.
- Hebert, P.D.H., Cywinska, A., Ball, S.L., and De Waard, J.R., Biological identification through DNA barcodes, *Proc. R. Soc. London, Ser. B*, 2003, vol. 270, pp. 313–321.
- Horreo, J.L., Revisiting the mitogenomic phylogeny of Salmoninae: new insights thanks to recent sequencing advances, *PeerJ.*, 2017, vol. 5, art. ID e3828.
- Huson, D.H. and Bryant, D., Application of phylogenetic networks in evolutionary studies, *Mol. Biol. Evol.*, 2006, vol. 23, pp. 254–267.

- Karpevich, A.F., Agapov, V.S., and Magomedov, G.M., *Akklimatizatsiya i kul'tivirovanie lososevykh ryb-introduitsintov* (Naturalization and Farming of Introduced Salmon Fishes), Moscow: VNIRO, 1991.
- Kazakov, R.V., The history of exploration and economic value of Atlantic salmon and brown trout, *Sb. Nauchn. Tr.-Gos. Nauchno-Issled. Inst. Ozern. Rechn. Rybn. Khoz.*, 1988, no. 286, pp. 111–129.
- Kendall, A.W., Jr. and Behnke, R.J., Salmonidae: development and relationships, in *Ontogeny and Systematics of Fishes*, Moser, H.G., , Eds., Lawrence: Allen Press, 1984, pp. 142–149.
- Knudsen, R., Klemetsen, A., Alekseyev, S., Adams, C.E., and Power, M., The role of *Salvelinus* in contemporary studies of evolution, trophic ecology and anthropogenic change, *Hydrobiologia*, 2016, vol. 783, pp. 1–9.
- Korovina, V.M., The structure of fishes of family Salmonidae: egg structure and specific morphogenesis, in *Morfologiya i sistematika ryb* (Morphology and Systematics of Fishes), Leningrad: Zool. Inst., Akad. Nauk SSSR, 1978, pp. 40–52.
- Kuderskii, L.A., Origin of the lake salmons, in *Rybnokhozyaistvennoe izuchenie vnutrennikh vodoemov* (Fishery Study of Inland Reservoirs), Leningrad: Gos. Nauchno-Issled. Inst. Ozern. Rechn. Rybn. Khoz., 1977, no. 19, pp. 34–39.
- Kuzishchin, K.V., Development and adaptive role of intraspecific ecological diversity of salmon fishes (family Salmonidae), *Doctoral (Biol.) Dissertation*, Moscow: Moscow State Univ., 2010.
- Lappin, F.M., Shaw, R.L., and Macqueen, D.J., Targeted sequencing for high-resolution evolutionary analyses following genome duplication in salmonid fish: Proof of concept for key components of the insulin-like growth factor axis, *Mar. Genomics*, 2016, vol. 30, pp. 15–26.
- Li, J., Xia, R., McDowall, R.M., Lypez, J.A., Lei, G., and Fu, C., Phylogenetic position of the enigmatic *Lepidogalaxias salamandroides* with comment on the orders of lower euteleostean fishes, *Mol. Phylogenet. Evol.*, 2010, vol. 57, pp. 932–936.
- Li, Y., Park, J.-S., Deng, J.H., and Bai, Y., Cytochrome c oxidase subunit IV is essential for assembly and respiratory function of the enzyme complex, *J. Bioenerg. Biomembr.*, 2006, vol. 38, pp. 283–291.
- Lysenko, L.F., *Atlanticheskii losos': biologiya i proiskhozhdenie* (Atlantic Salmon: Biology and Origin), Murmansk: Polar. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr., 1994.
- Lyubitel'skoe rybolovstvo i sokhranenie lososevykh ryb v Rossii (Amateur Fishing and Conservation of Salmon Fishes in Russia), Moscow: Fond Russkii Losos', 2010.
- Lyubishchev, A.A., *Problemy flory, sistematiki i evolyutsii organizmov* (Forms, Systematics, and Evolution of Organisms), Moscow: Nauka, 1982.
- Macqueen, D.J. and Johnston, I.A., A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification, *Proc. R. Soc. London, Ser. B*, 2014, vol. 281, art. ID 20132881.
- Makhrov, A.A., A narrowing of the phenotypic diversity range after large rearrangements of the karyotype in Salmonidae: The relationship between saltational genome rearrangements and gradual adaptive evolution, *Genes*, 2017, vol. 8, p. 297.
- Makhrov, A.A. and Bolotov, I.N., Does freshwater pearl mussel (*Margaritifera margaritifera*) change the lifecycle of Atlantic salmon (*Salmo salar*)? *Adv. Gerontol.*, 2011, vol. 1, no. 2, pp. 186–194.
- Makhrov, A.A. and Lajus, D.L., Post-glacial colonization of the North European seas by Pacific fishes and lamprey, *Contemp. Probl. Ecol.*, 2018, vol. 11, no. 3, pp. 247–258.
- Makhrov, A.A., Ponomareva, M.V., Khaimina, O.V., Gilepp, V.E., Efimova, O.V., Nechaeva, T.A., and Vasilenkova, T.I., Abnormal development of gonads of dwarf females and low survival of their offspring as the cause of rarity of resident populations of Atlantic salmon (*Salmo salar* L.), *Russ. J. Dev. Biol.*, 2013, vol. 44, no. 6, pp. 326–335.
- Makoedov, A.N., *Kariologiya, biokhimicheskaya genetika i populyatsionnaya fenetika lososevidnykh ryb Sibiri i Dal'nego Vostoka: sravnitel'nyi aspekt* (Comparison of Caryology, Biochemical Genetics, and Population Phenetics of Salmon Fishes of Siberia and Far East), Moscow: Psikhologiya, 1999.
- Mamkaev, Yu.V., Comparative morphology of higher and lower groups of the same phylogenetic branch, *Zh. Obshch. Biol.*, 1968, vol. 29, no. 1, pp. 48–56.
- Matveev, V. and Okada, N., Retroposons of salmonoid fishes (Actinopterygii: Salmonoidei) and their evolution, *Gene*, 2009, vol. 434, pp. 16–28.
- Matveev, V., Nishihara, H., and Okada, N., Novel SINE families from salmons validate *Parahucho* (Salmonidae) as a distinct genus and give evidence that SINEs can incorporate LINE-related 3'-tails of other SINEs, *Mol. Biol. Evol.*, 2007, vol. 24, no. 8, pp. 1656–1666.
- Mina, M.V., *Mikroevolyutsiya ryb. Evolyutsionnye aspekty feneticheskogo raznoobraziya* (Microevolution of Fishes: Evolutionary Aspects of Phenetic Diversity), Moscow: Nauka, 1986.
- Montgomery, D.R., *King of Fish. The Thousand-Year Run of Salmon*, New York: Westview, 2003.
- Morrison, D.A., Networks in phylogenetic analysis: new tools for population biology, *Int. J. Parasitol.*, 2005, vol. 35, pp. 567–582.
- Muñoz-Colmenero, M., Juanes, F., Dopico, E., Martinez, J.L., and Garcia-Vazquez, E., Economy matters: A study of mislabeling in salmon products from two regions, Alaska and Canada (Northwest of America) and Asturias (Northwest of Spain), *Fish. Res.*, 2017, vol. 195, pp. 180–185.
- Noakes, D.L.G., Skulason, S., and Snorrason, S.S., Alternative life-history styles in salmonine fishes with emphasis on arctic charr, *Salvelinus alpinus*, in *Alternative Life-History Styles of Animals*, Dordrecht: Kluwer, 1989, pp. 329–346.
- Norden, C.R., Comparative osteology of representative salmonid fishes, with particular reference to the grayling (*Thymallus arcticus*) and its phylogeny, *J. Fish. Res. Board Can.*, 1961, vol. 18, no. 5, pp. 679–791.
- Oakley, T.H. and Phillips, R.B., Phylogeny of salmonine fishes based on growth hormone introns: Atlantic (*Salmo*) and Pacific (*Oncorhynchus*) salmons are not

- sister taxa, *Mol. Phyl. Evol.*, 1999, vol. 11, no. 3, pp. 381–393.
- Ojima, Y., Maeki, K., Takayama, S., and Nogusa, S., A cytotoxic study on the Salmonidae, *Nucleus*, 1963, vol. 6, no. 2, pp. 91–98.
- Osinov, A.G. and Lebedev, V.S., Genetic divergence and phylogeny of the Salmoninae based on allozyme data, *J. Fish Biol.*, 2000, vol. 57, no. 2, pp. 354–381.
- Osinov, A.G. and Lebedev, V.S., Salmonid fishes (Salmonidae, Salmoniformes): the systematic position in the superorder Protacanthopterygii, the main stages of evolution, and molecular dating, *J. Ichthyol.*, 2004, vol. 44, no. 9, pp. 690–715.
- Pankova, M.V. and Brykov, V.I.A., Divergence of introns in the paralogous growth hormone genes of salmonid fish indicates the effect of selection, *Dokl. Biol. Sci.*, 2013, vol. 451, no. 1, pp. 231–234.
- Pavlov, D.A. and Osinov, A.G., Main features of early ontogeny in salmonids (Salmoniformes) and other representatives of the Protacanthopterygii in relation to the phylogeny, *J. Ichthyol.*, 2004, vol. 44, no. 4, pp. 267–283.
- Pavlov, D.S. and Savvaitova, K.A., On the problem of ratio of anadromy and residence in salmonids (Salmonidae), *J. Ichthyol.*, 2008, vol. 48, no. 9, pp. 778–791.
- Pavlov, D.S., Savvaitova, K.A., Kuzishchin, K.V., Gruzdeva, M.A., Pavlov, S.D., Mednikov, B.M., and Maksimov, S.V., *Tikhookeanskije blagorodnye lososi i foreli Azii* (Pacific Noble Salmon and Trouts in Asia), Moscow: Nauchnyi Mir, 2001.
- Phillips, R.B. and Oakley, T.H., Phylogenetic relationships among the Salmoninae based on nuclear and mitochondrial DNA sequences, in *Molecular Systematics of Fishes*, Kocher, T.D. and Stepien, C.A., Eds., London: Academic, 1997, pp. 145–162.
- Phillips, R. and Rab, P., Chromosome evolution in Salmonidae (Pisces): an update, *Biol. Rev.*, 2001, vol. 76, pp. 1–25.
- Phillips, R.B., Matsuoka, M.P., Konkol, N.R., and McKay, S., Molecular systematics and evolution of the growth hormone introns in the Salmoninae, *Environ. Biol. Fish.*, 2004, vol. 69, pp. 433–440.
- Ramsden, S.D., Brinkmann, H., Hawryshyn, C.W., and Taylor, J.S., Mitogenomics and the sister of Salmonidae, *Trends Ecol. Evol.*, 2003, vol. 18, pp. 607–610.
- Rasmussen, R.S. Morrissey, M.T., and Hebert, P.D.N., DNA barcoding of commercially important salmon and trout species (*Oncorhynchus* and *Salmo*) from North America, *J. Agric. Food Chem.*, 2009, vol. 57, pp. 8379–8385.
- Rassadnikov, O.A., Morphology of seismosensory system of some salmon fishes, in *Populyatsionnaya biologiya i sistematika lososevykh* (Population Biology and Systematics of Salmon Fishes), Vladivostok: Dal'nevost. Nauch. Tsentr, Akad. Nauk SSSR, 1980, pp. 104–112.
- Rees, H., The question of polyploidy in the Salmonidae, *Chromosoma*, 1964, vol. 15, no. 3, pp. 275–279.
- Regan, C.T., The systematic arrangement of the fishes of the family Salmonidae, *Ann. Mag. Nat. Hist.*, 1914, vol. 13, pp. 405–408.
- Reist, J.D., Vuorinen, J., and Bodaly, R.A., Genetic and morphological identification of coregonid hybrid fishes from arctic Canada, *Pol. Arch. Hydrobiol.*, 1992, vol. 39, pp. 551–561.
- Robertson, F.M., Gundappa, M.K., Grammes, F., Hvidsten, T.R., Redmond, A.K., Lien, S., Martin, S.A.M., Holland, P.W.H., Sandve, S.R., and Macqueen, D.J., Lineage-specific rediploidization is a mechanism to explain time-lags between genome duplication and evolutionary diversification, *Genome Biol.*, 2017, vol. 18, p. 111.
- Rol'skii, A.Yu., Makhrov, A.A., and Artamonova, V.S., Speciation of marine rockfishes of genus *Sebastes* from Atlantic and Arctic oceans, *Materialy III mezh-dunarodnoi konferentsii posvyashchennoi 130-letiyu sa dnya rozhdeniya N.I. Vavilova i 110-letiyu so dnya osnovaniya Gosudarstvennogo Darvinovskogo muzeya "Sovremennye problemy biologicheskoi evolyutsii," g. Moskva, 16–20 oktyabrya 2017 g.* (Proc. III Int. Conf. Dedicated to the 130th Anniversary of N.I. Vavilov and 110th Anniversary of Establishment of the State Darwin Museum "Modern Biological Evolution," Moscow, October 16–20, 2017), Moscow, 2017, pp. 101–104.
- Rozhnov, S.V., Vavilov's law of homologous series and archaic diversity according to paleontology, in *Evolutsiya biosfery i bioraznoobrazie* (Evolution of Biosphere and Biological Diversity), Moscow: KMK, 2006, pp. 134–146.
- Sanford, C.P.J., The phylogenetic relationships of salmonoid fishes, *Bull. Br. Mus. Nat. Hist. Zool.*, 1990, vol. 56, no. 2, pp. 145–153.
- Savvaitova, K.A., *Arkticheskie gol'tsy* (Arctic Chars), Moscow: Agropromizdat, 1989.
- Shedko, S.V., Miroshnichenko, I.L., and Nemkova, G.A., Phylogeny of salmonids (Salmoniformes: Salmonidae) and its molecular dating: analysis of nuclear *RAG1* gene, *Russ. J. Genet.*, 2012, vol. 48, no. 5, pp. 575–579.
- Shedko, S.V., Miroshnichenko, I.L., and Nemkova, G.A., Phylogeny of salmonids (Salmoniformes: Salmonidae) and its molecular dating: analysis of mtDNA data, *Russ. J. Genet.*, 2013, vol. 49, pp. 623–637.
- Shmal'gauzen, I.I., Resistance of organic forms (ontogenesis) during evolution, *Zh. Obshch. Biol.*, 1945, vol. 6, no. 1, pp. 3–25.
- Shuntov, V.P. and Temnykh, O.S., *Tikhookeanskije lososi v morskikh i okeanicheskikh ekosistemakh* (Pacific Salmon in Marine and Ocean Ecosystems), Vladivostok: TINRO-Tsentr, 2008, vol. 1.
- Sidorov, G.P. and Reshetnikov, Yu.S., *Lososeobraznye ryby vodoemov evropeeskogo Severo-Vostoka* (Salmoniformes Fishes from Reservoirs of European Northeast), Moscow: KMK, 2014.
- Soin, S.G., Types of development of salmon fishes and their taxonomic role, *Vopr. Ikhtiol.*, 1980, vol. 20, no. 1, pp. 65–72.
- Sutherland, B.J.G., Gosselin, T., Normandeau, E., Lamothé, M., Isabel, N., Audet, C., and Bernatchez, L., Salmonid chromosome evolution as revealed by a novel method for comparing RADseq linkage maps, *Genome Biol. Evol.*, 2016, vol. 8, pp. 3600–3617.
- Sychevskaya, E.K., Origin of *Coregonus* fishes in terms of historical development of salmonids (Salmonoidea), in

- Biologiya sigovykh ryb* (Biology of Coregonus Fishes), Moscow: Nauka, 1988, pp. 17–28.
- Tchernavin, V., The origin of salmon. Is its ancestry marine or freshwater? *Salmon Trout Mag.*, 1939, vol. 95, pp. 120–140.
- Vasil'ev, V.P., Polyploidy of fishes and evolution of karyotypes of salmon fishes (Salmonidae), *Zh. Obshch. Biol.*, 1977, vol. 38, no. 3, pp. 380–392.
- Viktorovskii, R.M., *Mekhanizmy vidoobrazovaniya u gol'tsov Krobotskogo ozera* (Speciation Mechanisms of Chars from the Kronotskoe Lake), Moscow: Nauka, 1978.
- Vladukov, V.D., *A Review of Salmonid Genera and Their Broad Geographical Distribution*, Transactions of the Royal Society of Canada, Series 4, Section 3, Ottawa: *R. Soc. Can.*, 1963, vol. 1, pp. 459–504.
- Wang, Y., Guo, R., Li, H., Zhang, X., Du, J., and Song, Z., The complete mitochondrial genome of the Sichuan taimen (*Hucho bleekeri*): repetitive sequences in the control region and phylogenetic implications for Salmonidae, *Mar. Genomics*, 2011, vol. 4, pp. 221–228.
- Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., and Hebert, P.D.N., DNA barcoding Australia's fish species, *Philos. Trans. R. Soc., B*, 2005, vol. 360, pp. 1847–1857.
- Willson, M.F. and Halupka, K.C., Anadromous fish as keystone species in vertebrate communities, *Conserv. Biol.*, 1995, vol. 9, no. 3, pp. 489–497.
- Wilson, M.V.H. and Li, G.-Q., Osteology and systematic position of the Eocene salmonid †*Eosalmo driftwoodensis* Wilson from western North America, *Zool. J. Linn. Soc.*, 1999, vol. 125, no. 3, pp. 279–311.
- Yasuike, M., Jantzen, S., Cooper, G.A., Leder, E., Davidson, W.S., and Koop, B.F., Grayling (Thymallinae) phylogeny within salmonids: complete mitochondrial DNA sequences of *Thymallus arcticus* and *Thymallus thymallus*, *J. Fish Biol.*, 2010, vol. 76, pp. 395–400.
- Zelinsky, Yu.P. and Makhrov, A.A., Chromosomal variability, genome reorganization in phylogeny, and the systematics of *Salmo* and *Parasalmo* species (Salmonidae), *J. Ichthyol.*, 2001, vol. 41, no. 3, pp. 209–216.
- Zelinsky, Yu.P. and Makhrov, A.A., Homological series by chromosome number and the genome rearrangements in the phylogeny of Salmonoidei, *Russ. J. Genet.*, 2002, vol. 38, no. 10, pp. 1115–1120.
- Zhivotovskii, L.A., Evolutionary history of Pacific salmon and trouts, *Tr. VNIRO*, 2015, vol. 157, pp. 4–23.

Translated by K. Lazarev