Conference Paper

Morphological and Genetic Variability of the Mass Whitefish Forms in Lake Onega

Nikolay Ilmast[1], Dmitry Sendek[2], Elena Zuykova[3], Nikolay Milyanchuk[1], Denis Savosin[1], Aleksandra Borisovskaya[2], Maksim Alekseev[4], and Nikolay Bochkarev[3]

[1]Institute of Biology, Karelian Research Centre, Russian Academy of Sciences, Petrozavodsk, Russian Federation
[2]State Research Institute on Lake and River Fisheries, St. Petersburg, Russian Federation
[3]Institute of Systematic and Ecology of Animals, Siberian branch, Russian Academy of Sciences, Novosibirsk, Russian Federation
[4]Knipovich Polar Research Institute of Marine Fisheries and Oceanography, Murmansk, Russian Federation

Abstract

In Lake Onega, the whitefish *Coregonus lavaretus* has been shown to occur as a variety of forms. Medium- and sparsely-ranked whitefish are most abundant. Analysis of available data indicates that whitefish populations from Karelia's large lakes display the maximum values of various genetic variability indices. This fact seems to be due to the history of the colonization of the lake by the discrete evolutionary whitefish lineages from various Late Quaternary habitats followed by their hybridization. A great variety of Onega whitefish haplotypes is probably related to the genetic heterogeneity of the whitefish who until recently had occurred as five ecological forms ranking as subspecies. The median network obtained suggests that many of the populations studied have become less abundant. The well-defined ``star-like'' network structure is characteristic of populations that passed through a narrow ``bottleneck'' in the near past and then expanded rapidly, as indicated by the abundance of rare haplotype varieties. It seems that the retreat of the Scandinavian glacier was not a momentary event but took a long time during which the populations formed were subjected to demographic transformations.

Keywords: whitefish, Coregonus lavaretus complex, Lake Onega, morphological and genetic variability

OPEN ACCESS

1. Introduction

Fish of the *Coregonus* family, considered as valuable commercial species, make up a considerable portion of fish production in northern freshwater ecosystems. The whitefish *Coregonus lavaretus* L. is assumed to be represented in Fennoscandia's water bodies by many morphological and ecological forms that often occur sympatrically in large lakes. The intraspecific whitefish systematics for North-West Russia was generally

How to cite this article: Nikolay Ilmast, Dmitry Sendek, Elena Zuykova, Nikolay Milyanchuk, Denis Savosin, Aleksandra Borisovskaya, Maksim Alekseev, and Nikolay Bochkarev, (2020), ``Morphological and Genetic Variability of the Mass Whitefish Forms in Lake Onega'' in *International applied research conference «Biological Resources Development and Environmental Management»*, KnE Life Sciences, pages 141–151. DOI 10.18502/kls.v5i1.6037

Page 141

developed by L.S. Berg [1] and I.F. Pravdin [2] in the mid-20th century, but it was hard to use. A systematics, in which the structure of the complex species *Coregonus lavaretus*, described in a much simpler manner, was proposed later [3]. According to a new concept, the species consists of 6 subspecies. Most of the subspecies described earlier now rank as ecological forms. Yu.S. Reshetnikov [4] thinks that the diversity of whitefish forms observed has been produced by the adaptive radiation of one species to the heterogeneous conditions of the water bodies. However, there was another point of view, according to which a large proportion of morphs were produced by secondary intergradation [5]. The results of genetic studies, based on paleolimnological reconstructions, have shown that the origin of modern whitefish populations in North-West Russia is primarily due to the recolonization of water bodies by the phylogenetic lineages of species from several periglacial habitats [6].

Lake Onega is a large fishery water body which makes up about 40% of the total freshwater fish catch in the Republic of Karelia. The lake covers an area of 9890 km^2, of which 350 km^2 are occupied by islands. The lake has a maximum depth of 120 m and an average depth of 30 m. Its limnic heterogeneity is well-defined. The deepwater central zone, Great Onega Bay and Povenets Bay, retain an oligotrophic pattern. Large bays, such as Kondopozhskaya and Petrozavodskaya, have acquired a mesotrophic status. Some of the bays and zones are near-eutrophic. The lake zones are affected by human activities in various ways. Fifty-two rivers flow into Lake Onega and one river (Svir) flows out of the lake. The watershed basin area is 56340 km^2. The lake water is poorly mineralized. Total mineralization varies from 39 to 46 mg/l [7], [8].

The fish fauna of Lake Onega consists of 36 species of 15 families [9], including some valuable species. The lake is inhabited by various forms of whitefish and European vendace. According to I.F. Pravdin [2], the whitefish *C. lavaretus* in the lake is representd by 5 subspecies: *C. lavaretus pallasi* -- densely-rakered whitefish (*Sp.br*=39-47), *C. lavaretus lavaretoides* -- medium-rakered whitefish (*Sp.br* =26-38), *C. lavaretus ludoga* -- Onega whitefish (*Sp.br*=23-33), *C. lavaretus widegreni* -- valaamka (*Sp.br*=23-32) and *C. lavaretus poljakowi* -- sparsely-rakered whitefish (*Sp.br*=21-31). Yu.S. Reshetnikov and A.A. Lukin [10] subdivide all the whitefish in the lake into three groups: sparsely-rakered whitefish (*Sp.br*=21-32), medium-rakered whitefish (*Sp.br*=26-38) and densely-rakered whitefish (*Sp.br*=40-55).

The goal of the present study is to assess the genetic diversity of the most common Onega pizhyan-like whitefish forms/species and to analyze the phylogenetic and phylogeographic relations of whitefish haplotypes from the water bodies of NW Europe.

2. Material and Methods

Onega whitefish samples were collected in March-November 2018 using fixed gill nets with a mesh size of 30 to 45 mm. Exposure time varied from 12 to 48 hrs, depending on weather. A total of 200 whitefish were caught. Twenty-four whitefish caught in Lake Onega were used for genetic analysis. The number of gill rakers and the number of punctured scales in the lateral lineage were calculated.

TABLE 1: Water bodies, sample size, coordinates of collecting the numbers of whitefish Coregonus lavaretus complex haplotypes in GenBank.

Water body	Form	*n*	Latitude	Longitude	Sequence numbers in GenBank
Lake Onega	1	31	N61°42'	N35°25'	MN227169-MN227179
Baltic Sea	2	37	N58°37'	N20°25'	KP123646 - KP123648* KP123652, KP123653* KP123658 - KP123666* DQ399863- DQ399868* JQ661382- JQ661385* JQ661387- JQ661389*
Lake Ladoga	1	4	N6109'	E31°20'	KP123667*, KP123668* KX010454*, KX010455*
Lake Kamennoe	2	16	N64°28'	E30°11'	KX010456 - KX010459*
Lake Hallstattsee	1	19	N47°26'	E13°39'	KP123674-KP123677* FM211036-FM211042*
Tuloma River	1	10	N68°53'	E33°00'	MN227179-MN227182
North Sea	2	92	N55°51'	E03°20'	JQ661390 -JQ661481* NC020765*

Note: Definition of whitefish by the external signs 1 -- lake whitefish, 2 -- lake--river whitefish. Asterisk indicated earlier used the haplotypes [14], [15].

Total genome DNA was extracted from the whitefish liver fixed by 96% ethanol using the phenol-chloroform method. The DNA extracted was stored at 4°C. We amplified the gene ND1 with mitochondrial DNA using our own developed external primers 5'GGCCTA AGCCCTTTTTCTC A 3' Forward and 5' GAGGGGACTTGAACCCCTAT 3' Reverse. A fragment was amplified in reaction mixture, 30 µl in volume. As a result of the amplification, we obtained sequences 1091 nucleotide bases in length. PCR products (Polymerase chain reaction) were purified with a set of BIOSILICA reagents (Novosi-birsk, Russia) and sequenced in both directions on an automatic ABI 3130xl Genetic Analyser (Applied Biosystems, Inc., USA) at Genomica CKP, RAS, Siberian Branch (Novosibirsk, Russia, http://sequest.niboch.nsc.ru) (Novosibirsk, Russia, http://sequest.niboch.nsc.ru) using a BigDye terminator (Applied Biosystems). Nucleotide sequences were straightened with the algorithm ClustalW and edited manually. To analyze the poly-morphism of the nucleotide sequences obtained, we calculated the following standard

genetic parameters: the number of polymorphic (segregating) sites (S), the number of haplotypes (h), haplotypic diversity (H_d), nucleotide diversity (π) and the average number of nucleotide differences (k). All calculations were made using the software DnaSP v. 5.10. To estimate genetic distances between the populations, paired F_{ST} values were calculated. Corresponding probability values were calculated using 10000 rearrangements in the software. The statistical significance of the results was calculated with 1000 replications. To assess evolution neutrality of evolution, Tajima's (Tajima's D) and Fu (Fu's Fs) tests were used [11], [12]. To support one or another hypothesis, the sum of squares (SSD) and Harpending's index of roughness (r), calculated using the software ARLEQUIN v.3.5, was assessed. To assess genealogical relations between the haplotypes of assumed whitefish forms/species, a median network was constructed using the software Network v.4.5 and the Median joining algorithm (Median--Joining) [13]. In addition to Onega whitefish, we used whitefish samples from adjacent water bodies. Some of them were analyzed for the first time, while others were used in earlier studies or were taken from GenBank (Table 1).

3. Results

Onega whitefish from our catches was represented by two forms: sparsely-rakered and medium-rakered. A sparsely-rakered form is represented by pizhyan-like whitefish such as Onega whitefish and Upper Svir whitefish, small dominantly benthos-eating whitefish. These forms often occur in many water bodies of Karelia, the Murmansk Region and Norway. In Lake Onega, this group comprises valaamka and Onega whitefish (ludoga) which occur at great depths and mainly eat crustaceans. There are four forms of medium-rakered whitefish: 1) medium-rakered lake whitefish, 2) Shala whitefish, 3) Suna whitefish and 4) Shuya whitefish. The mean linear-weight indices of the whitefish caught in 2018 are shown in Table 2.

TABLE 2: Linear-weight parameters of Lake Onega whitefish (March-November, 2018).

Indicator	Fish age, years										
	2	3	4	5	6	7	8	9	10	11	12
Sparsely-rakered whitefish -- 23 gill rakers (range 22-24), n=32											
Smith length, cm	--	27,3	29,8	33,1	35,0	37,4	39,3	--	--	--	--
Mass, g	--	190	270	360	458	580	730	--	--	--	--
Medium-rakered whitefish -- 29 gill rakers (range 26--36), n=168											
Smith length, cm	24,3	27,0	29,7	33,0	35,0	37,7	39,6	42,8	44,0	47,8	51,5
Mass, g	142	190	264	351	450	580	750	870	1070	1260	1773

Sixteen whitefish samples (combined morphological and genetic analyses were conducted) with 22 to 31 gill rakers on a gill arch and 87 to 102 punctured scales in the lateral lineage were used in this study (Figure 1).

Five whitefish samples were sparsely-rakered and 11 samples were medium-rakered. Most of the samples are medium-rakered, except for 4 samples with 87 to 90 punctured scales in the lateral lineage. According to I.F. Pravdin [2], the whitefish fall into the following forms, based on its appearance: sparsely-rakered -- *C. lavaretus poljakovi* (*Sp.br* 22-24); and medium-rakered -- *C. lavaretus lavaretoides* (*Sp.br* 27-31). The average length (ac) of the sparsely-rakered whitefish was 33.9 cm (varying from 31.3 to 37.4) and the average body mass was 407.4 g (variation: 235 to 593); the corresponding values for the medium-rakered whitefish were 29.8 cm (variation: 25.8 to 33.3) and 285.5 g (variation: 155 to 406), respectively.
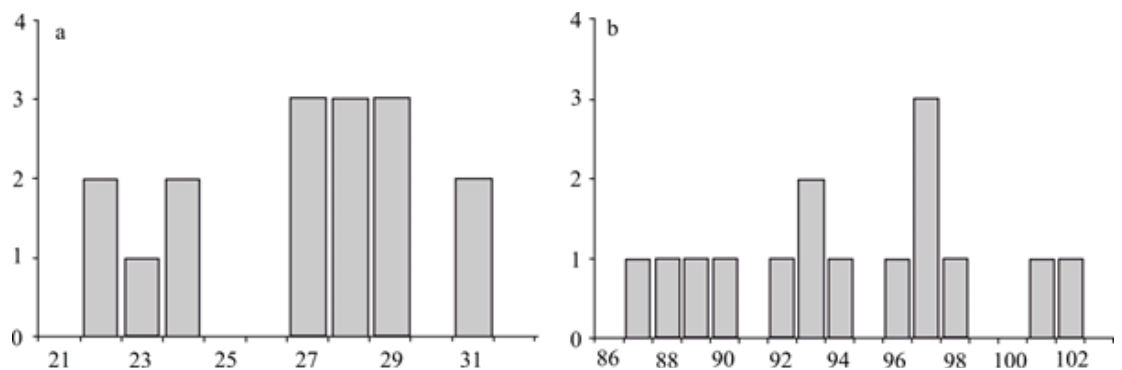

Figure 1: Meristic signs of whitefish (a - the number of gill rakers, b - the number of perforated scales in the lateral line).

Genetic variability. Analysis of the polymorphism of the nucleotide sequences has shown that the whitefish populations/forms analyzed display great haplotypic diversity (Table 3). The greatest haplotypic diversity is shown by whitefish populations/forms from large water bodies, the Gulf of Finland, Lake Ladoga, the North Sea basin and the River Tuloma. Whitefish populations from Lake Onega, an Alpine lake and Lake Kamennoye are slightly less diverse.

The highest nucleotide diversity, roughly equal to that of all the populations studied, is displayed by whitefish from the Gulf of Finland, the Baltic Sea. Much lower nucleotide diversity is exhibited by whitefish from the North Sea basin and the western Baltic Sea. The smallest number of nucleotide differences was displayed by whitefish from the River Tuloma, the Barents Sea basin. The greatest average number of nucleotide differences (k) was shown by whitefish from the Gulf of Finland, the Baltic Sea (5,066). Whitefish from the Austrian mountain lakes of the Danube River basin system shows average

TABLE 3: Polymorphism of sequences of ND1 mtDNA gene fragment in whitefish populations from some water bodies of the North-West Eurasia Peninsula.

Species/Form	n	S	h	H_d	π	k
Lake Onega	31	14	10	0.684	0.00215	2.095
Gulf of Finland	37	23	15	0.839	0.00520	5.066
Lake Ladoga	4	3	4	1.000	0.00171	1.667
Lake Kamennoe	16	4	4	0.642	0.00172	1.675
Lake Hallstattsee	19	16	8	0.673	0.00299	2.912
Tuloma River	10	3	4	0.733	0.00093	0,911
North Sea	92	17	9	0.779	0.00340	3.301
All together	209	45	39	0.902	0.00580	5.652

Note: n -- number of specimens, S -- number of polymorphic (egregiously sites), h - number of haplotypes, Hd -- gipotermicescoe diversity, π -- nucleotide diversity, k - average number of nucleotide differences (per site).

values for its parameter (2.912). The smallest average number of nucleotide differences was exhibited by Tuloma River whitefish.

Evolution neutrality and demography tests. The negative values of Tajima and Fu tests, obtained for most samples, are common for populations with relatively recent demographic expansion (Table 4).

TABLE 4: Test of neutrality for whitefish populations.

Species	n	Tajima's D	Fu's Fs
Lake Onega	31	-1.333 (0,071)	-2.856 (0,067)
Gulf of Finland	37	-0.274 (0,454)	-2.275 (0,200)
Lake Ladoga	4	0.168 (0,725)	**-2.181** (0,0207)
Lake Kamennoe	16	1.218 (0,880)	0.952 (0,733)
Lake Hallstattsee	19	-1.370 (0,086)	-1.080 (0,299)
Tuloma River	10	-0.507 (0,313)	-1.071 (1,134)
North Sea	92	-0.031 (0,548)	1.892 (0,771)
All together	209	-0.850 (0,226)	**-10.812** (0,017)

Note: Reliable values are shown in bold.

Negative test values commonly suggest directed selection. However, low and unreliable test values may indicate test neutrality. We assume that low and invalid test values obtained for the populations analyzed corroborate the completeness of hybridization processes in whitefish populations from most of the water bodies studied, while high negative test values for Ladoga whitefish populations (a very small group of samples) suggest ongoing demographic expansion (Table 5).

A low Fst value between neighbouring populations supports the existence of gene flow between the populations. The gene flow between Baltic Sea, Lake Onega, Lake Ladoga and Lake Kamennoye populations and whitefish populations from Alpine lakes

TABLE 5: Pairwise comparison of Fst between studied populations.

Population	1	2	3	4	5	6
Lake Onega	0.000					
Gulf of Finland	0.039	0.000				
Lake Ladoga	0.026	0.063	0.000			
Lake Kamennoe	0.102	0.085	0.078	0.000		
Lake Hallstattsee	0.187	0.097	0.112	0.192	0.000	
Tuloma River	0.624	0.478	0.632	0.671	0.528	0.000
North Sea	0.638	0.476	0.684	0.660	0.545	0.768

is unlimited, but it is limited between North Sea whitefish populations and all other populations (Fst 0.476-0.768), and those from the River Tuloma (0. 478-0.671).

To assess genealogical relations between the haplotypes, median networks were constructed (Fig. 2). This analysis has shown that all the samples fall into two haplogroups. One haplogroup consists of two dominant star-like structures of whitefish haplotypes from the northwestern Baltic Sea basin and the North Sea with a small number of closely related minor haplotypes. They are connected with each other through 1-2 mutation steps. The dominant group of haplotypes, consisting of whitefish haplotypes from the North Sea basin, comprises whitefish haplotypes from Lake Onega and the Gulf of Finland, the Baltic Sea.

A small number of minor haplotypes comprise whitefish haplotypes from the Gulf of Finland (H_15) and whitefish haplotypes from Alpine lakes (H_22, 24). The other haplogroup is connected with the first one via 5 mutation steps and the central haplotype H_11. The haplogroup consists of first- (H_1, 2,11) and second (H_28)-order star-like structures that comprise many minor haplotypes located at a distance of 1-3 mutation steps from the central haplotype. The haplogroup displays a complex haplotypic network structure, several central haplotypes and many other connections of varying length. Onega whitefish haplotypes occur in two star-like structures located at a distance of 2-3 mutation steps from star-like structures (H_1,2). Both structures are surrounded by numerous minor haplotypes located at a distance of 1-2 mutation steps from the central structure.

4. Discussion

Morphological analysis has shown that the medium-rakered whitefish *C. lavaretus mediospinatus* is most abundant of all the fish studied in Lake Onega. The sparsely-rakered whitefish *C. lavaretus pidschian* is less common. No densely-rakered whitefish
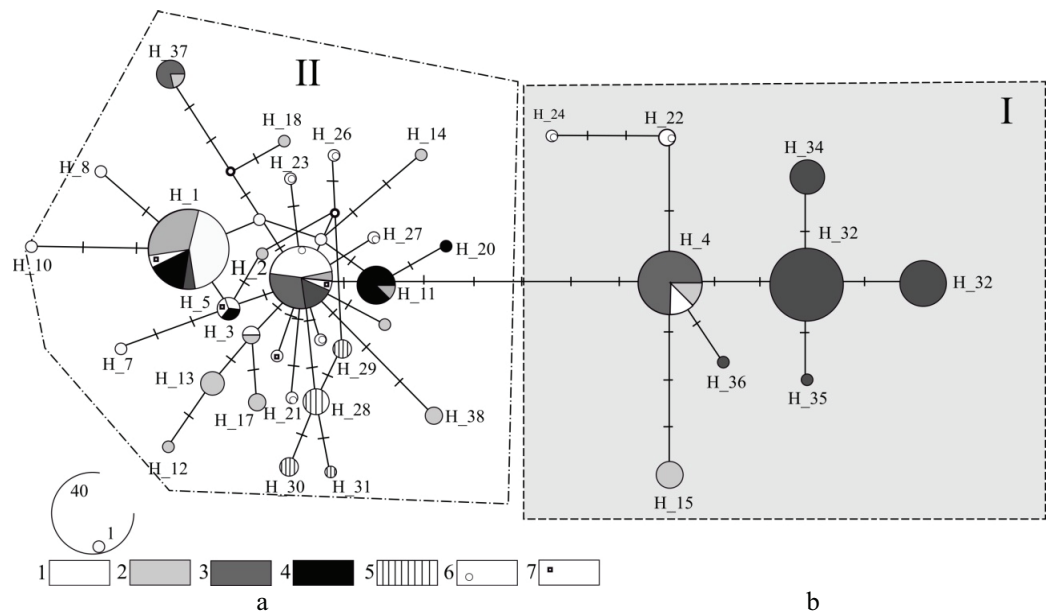
Figure 2: The median network of whitefish haplotypes of North-Western Europe. 1 -- whitefish haplotypes of Lake Onega, 2 -- whitefish haplotypes of Gulf of Finland, 3 -- whitefish haplotypes of Western Baltic and North Sea, 4- whitefish haplotypes of Lake Kamennoe, 5 - whitefish haplotypes of Tuloma River, 6 - whitefish haplotypes of Alpine lakes, 7 -- whitefish haplotypes of Lake Ladoga.

C. lavaretus pallasi has been found in the lake. Analysis of the growth rate of the two whitefish forms caught has shown that they both display similar linear-weight indices and that the age sequence of medium-rakered whitefish catches is much wider. The age structure of medium-rakered whitefish catches was represented by 11 age groups and that of sparsely-rakered whitefish by 6 groups.

Whitefish populations from large lakes commonly display the maximum values of various genetic variability indices. This seems to be due to the history of colonization of this water body by discrete evolutionary whitefish lineages from various Late Quaternary habitats followed by their hybridization. The great diversity of Onega whitefish haplotypes seems to be due to the genetic heterogeneity of the whitefish which had occurred in the lake until recently as five subspecies-ranking ecological forms [2]. Furthermore, the migration pathways of many European whitefish forms/species could have been intersected as the colonization continued. Fairly high indices for some genetic characteristics were also displayed by whitefish from Alpine lakes, as has been noted earlier [15]. However, a considerable portion of this variability was acquired by the introduction of Baltic whitefish into Alpine lakes [16].

The development of whitefish forms with the varying number of gill rakers in large freshwater bodies is due to one of two major ways of form evolution. According to one hypothesis, the occurrence of forms with varying number of gill rakers in Lake Onega is due to the differentiation of an ancestral form by selection in a heterogeneous aquatic

environment (sympatry). In accordance with another scenario, two or more genetically discrete whitefish forms (allopatry) colonized the ice-free lake basin and hybridized, giving rise to several forms. The allopatry-type scenario of whitefish form/species evolution has been described by P.Ya Pervozvansky [17]. This scenario is quite possible, as indicated by the genetic data we have obtained.

The median network we have constructed shows that many of the populations we studied have been reduced, which is now obvious. The well-defined star-like network structure is characteristic of populations that passed through a narrow bottleneck recently and rapidly expanded, as indicated by the occurrence of rare haplotype varieties. In all probability, the retreat of the Scandinavian glacier was not a momentary event but took a long time when demographic transformations in the populations were taking place. The haplogroups studied display many star-like structures and other links of varying length produced by inverse or parallel mutations. Such structures commonly indicate the long evolution of the whitefish populations that combined under variable conditions, suffered frequent variations in abundance, existed simultaneously if there were many adjacent refugia and were then subjected to intergradation [15].

Funding

Acknowledgement

Conflict of Interest

The authors have no conflict of interest to declare.

References

[1] Berg, L.S. (1948). *Freshwater fish of the USSR and neighboring countries.* Moscow-Leningrad: Publishing House of the Academy of Sciences of the USSR.

[2] Pravdin, I. F. (1954). *Whitefish of water bodies of the Karelian-Finnish SSR*. Moscow-Leningrad: Academy of Sciences of the USSR.

[3] Reshetnikov, Yu. S., Slugin, I. V., Mamontova, T. G. (1979). About sympatric populations of whitefish of Anadyr River. *Variability of fish in freshwater ecosystems*, Moscow: Nauka. pp. 113--136.

[4] Reshetnikov, Yu. S. (1980). *Ecology and taxonomy of whitefish*. Moscow: Nauka.

[5] Bochkarev, N. A., Zuykova, E. I., Solovyev, M. M. (2018). Secondary intergradation of various forms of pidschian-like whitefishes (Coregonus lavaretus sensu lato, Coregonidae) in the water bodies of the Altai-Sayan mountains. *Russian Journal of Genetics: Applied Research*, vol. 8, No. 2, pp. 178--189.

[6] Sendek, D.S. (2004). The origin of sympatric forms of -- European whitefish (Coregonus lavaretus (L.) in Lake Ladoga based on comparative genetic analysis of populations in North-West Russia. *Ann. Zool. Fennici*., No 41, pp. 25--39.

[7] Xu, R. (2002). *Particle Characterization: Light Scattering Methods*. New York, NY: Kluwer Academic Publishers.

[8] (1999). *Onega Lake. Ecological problems* Petrozavodsk: Karelian Research Centre, Russian academy of Sciences.

[9] *Indexers*. Port Aransas, TX: American Society of Indexers.

[10] (2013). *Lakes of Karelia. Reference Book.* Petrozavodsk: Karelian Research Centre, Russian academy of Sciences.

[11] Reshetnikov, Yu. S., Lukin, A. A. (2006). Modern state of the diversity of Coregonids from Onega Lake and the problems of their species identification. Journal of Ichthyology*, vol. 46, iss. 9, pp. 694--708.

[12] Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics,* vol. 123(3), pp. 585--595.

[13] Fu, Y. X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics,* vol. 147(2), pp. 915--925.

[14] Bandelt, H. J., Forster, P., Röhl, A. (1999). Median--joining networks for inferring intraspecific phylogenies. *Mol. boil. and Evol.*, 1999. vol. 16, pp. 37--48.

[15] Jacobsen, M. W., Hansen, M. M., Orlando, L., Bekkevold, D., Bernatchez, L., Willerslev, E. and Gilbert, M.T. (2012). Mitogenome sequencing reveals shallow evolutionary

histories and recent divergence time between morphologically and ecologically distinct European whitefish (Coregonus spp.). *Mol. Ecol.,* vol. 21(11), pp. 2727--2742.

[16] Ilmast, N. V., Sendek, D. S., Titov, S. F., Abramov, S. A., Zuykova, E. I., Bochkarev, N. A. (2016) On the differentiation of the ecological forms/subspecies of the whitefish Coregonus lavaretus from Lake Kamennoye. *Proceedings of Petrozavodsk State University,* No 4 (157), pp. 42--53.

[17] Winkler, K., Pamminger-Lahnsteiner, B., Wanzenböck, J. and Weiss, S. (2011). Hybridization and restricted gene flow between native and introduced stocks of Alpine whitefish (Coregonus sp.) across multiple environments. *Mol. Ecol.,* vol. 20(3), pp. 456 -- 472.

[18] Pervozvansky, V. Y. (1983). Whitefish Coregonus lavaretus (L.) in water bodies of the Kamennaya river system. *Salmonids (Salmonidae) of Karelia*. Petrozavodsk: Karelian branch of the USSR Academy of Sciences, pp. 42--74.