Evidence of dispersal between the Yenisei and Lena river basins during the late Pleistocene within the whitefish complex (*Coregonus lavaretus pidschian*)

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Abstract

The *Coregonus lavaretus* complex is a morphologically and genetically diverse group of white-fish. Its taxonomic structure causes controversy for almost a century. At least 25 forms of species *C. lavaretus* were described in Siberia, but there is still no consensus on their intraspecific structure and taxonomy. *C.l. pidschian* was described as one of the subspecies of *C. lavaretus*. Lately it was assumed that this subspecies is also a complex. The purpose of this study was to compare the distributions of *pidschian*-like whitefish haplotypes in two basins of large Siberian rivers: Yenisei and Lena, and to assess the gene flow between basins of these rivers, which were connected after the last glaciation. The sequence of the following mitochondrial DNA genes: 16S rRNA (partial), tRNA-Leu (full), NADH dehydrogenese subunit 1 (full), tRNA-Ile (full) and tRNA-Gln (partial) were used for the inference of intraspecific genetic structure of *C. lavaretus pidschian*. Whitefish haplotypes were clustered into two groups according to their distribution between two large Siberian river basins; but nevertheless there were shared haplotypes indicating events of migration and hybridization, which could happen when Yenisei and Lena river systems were connected after the last glaciation in the Late Pleistocene.

Key words: *Coregonus lavaretus pidschian*, mtDNA, gene flow, Siberia, Late Pleistocene

Introduction

The intraspecific taxonomy of the *C. lavaretus* complex was traditionally based on morphological and meristic traits leading to the assignment of forms/species rank to more than 100 whitefishes, but later many of them were rejected (Svetovidov 1934; Kirillov 1955; 1962; 1964; 1972; Mikhin 1959; Vershinin 1961; Novikov 1966; Kalashnikov 1968; Skryabin 1977; Karasev 1987). It is known that one of subspecies, *C. lavaretus pidschian* (Gmelin, 1788), inhabits lakes and rivers of Eurasia, from the North Sea basin in the west to the basin of the Bering Sea in the northeast (Pravdin 1954; Bochkarev et al., 2017). In this work we rely on the taxon of *C. l. pidschian*, sometimes called «siberian whitefish», which still has its status of a subspecies and, moreover, also consists of a number of morphologically and ecologically distinguishable forms. Later, it was shown that several of these forms were genetically distinct from each other according to sequence of mtDNA (Bochkarev et al. 2017). Such forms as *C.l.* n. *fluviatilis* and *C. l. pidschian* n. *anaulorum* were genetically identified as potential species and deposited in NCBI as *C. fluviatilis* and *C. anaulorum* respectively (Bochkarev et al. 2017) as they were originally described (Isachenko1925; Kaganovskij 1933). But, by the present time, the scientific redescription of these forms/species as a species is absent. Thus, due to very putative taxonomy of different whitefishes belonging to complex *C. l. pidschian*, we will name them further as a "forms/species".

Most of the known forms of *C. l. pidschian* were described in the mountain lakes located in upstream reaches of the large Siberian rivers: Ob, Yenisei, and Lena (Issatchenko 1925; Ioganzen and Moiseev 1955; Mikhin 1959; Lobovikova 1959; Kirillov 1972; Gundrizer 1978; Bochkarev et al. 2011). These three large rivers basins are main migration routes for whitefish on the east part of the Eurasia. These rivers, and other river basins of the arctic migration route, significantly effect the level of salinity of arctic seas. The low level of salinity around the mouth part of rivers permits different fishes, including whitefishes, makes migrations along the arctic sea shores.

In the southern mountainous part of the Ob river basin (Teletskoye Lake), two endemic forms/species of whitefishes were previously described (Bochkarev et al., 2018). These whitefish have different numbers of gill rakers on the first gill arch but shared the same mtDNA haplotypes (based on ND1 gene) (Bochkarev at al. 2011).

In the southern mountainous part of the Yenisei river basin (namely in the Karakul, Borzu-Khol, Noyon-Khol, Kadysh, Todzha and Dodot lakes), several forms/species of *C. l. pidschian* were described in the middle of the last century (Lobovikova 1959; Gundrizer 1978). The studies on whitefishes from the upper and middle reaches of the Yenisei River basin revealed that their populations from geographically distant/peripheral water bodies (in a gradient towards the main river course) have a reduced number of perforated scales in the lateral line compared to those populations from the main course of the river. Moreover, the variability in mitochondrial DNA (based on the ND1 gene) shows a high level of genetic differentiation of the whitefishes from this region (Bochkarev at al. 2011).

In the southern mountainous part of the Lena river basin (Baunt and Dorong lakes), up to 8 morphologically different endemic forms/species of whitefishes were described (see Fig. 1) (Kirillov 1972; Scryabin 1977; Bochkarev et al. 2013). Most of the coregonids from the Baunt lake system are lacustrine spring spawning fishes, whereas lacustrine-riverine populations belong to autumn spawning whitefishes, which are morphologically similar to whitefishes from the downstream area of the Lena River basin. All known whitefish forms/species from the Baunt lake system have a *pidschian*-like mtDNA highly similar to mtDNA of whitefishes from the main course of Lena River. They all have a reduced number of perforated scales in the lateral line and low (or medium) number of gill rakers in the first branchial arch (sparsely-rakered whitefish) (Bochkarev et al. 2013). We believe that the majority of these forms/species were formed due to hybridization between whitefishes from the Lena River basin and the Baunt Lake system (earlier belonging to the Baikal Lake basin).

We believe that these differences in morphological features and similarity in mtDNA may suggest a secondary introgression among whitefish populations in these regions (Bochkarev et al. 2011, 2018).

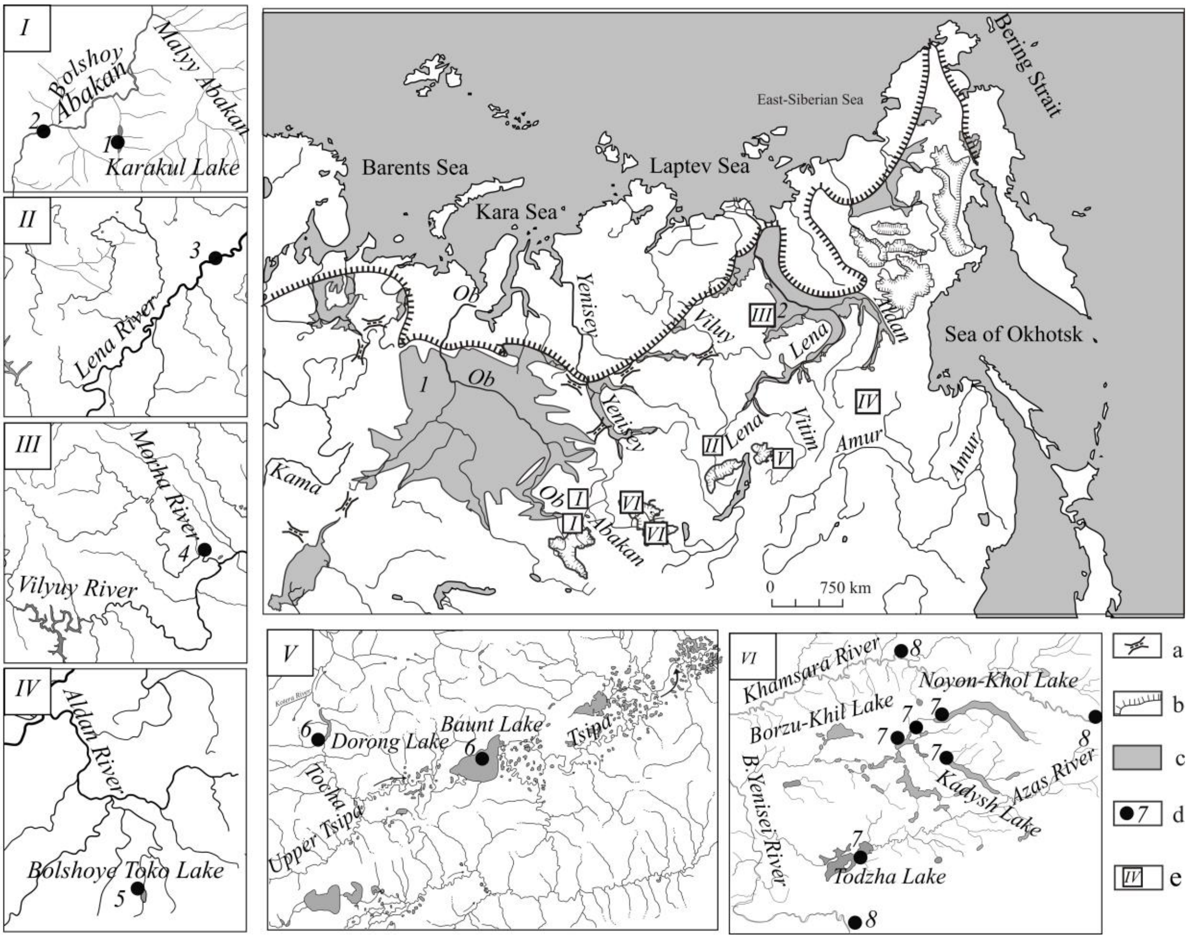

Fig. 1. The main elements of the transcontinental system of the snowmelt runoff of the last glaciation were put on the modern map of the Siberian river basins (according to Grosswald, Kotlyakov 1989). a – spillway ducts, b – glacier borders, c – the boundary of the lake systems, d – sampling sites, e – sampling areas. The roman and arabic numbers indicate the region and particular river/lake where samples were collected: I – Altai-Sayan region (1– Abakan River; 2 – Karakul Lake); II – the upper stream of the Lena River basin (3 – upstream of the Lena River); III – the middle stream of the Lena River basin (4 – Markha River); IV – the middle stream of the Lena river region (5 –Bolshoye Toko Lake, the Aldan River basin); V – the upper stream of the Lena River basin (6 – Baunt and Dorong lakes, Vitim River basin); VI – Yenisei river region (7 – Noyon-Khol, Dodot, Kadysh, Todzha, and Borzu-Khol lakes, the Bolshoi Yenisei River basin; 8 – the Khamsara and Bolshoi Yenisei river basins).

When studying the morphological and genetic diversity of whitefish forms/species *C. l. pidschian* and the possible routes of their distribution, it is impossible to ignore the most recent significant geological events that differentially affected the ecosystems of large Siberian rivers situated at large distances from each other. It is thought that the last glaciation was one of the most extensive ones in the geological history of Siberia (Grosswald 1965). However, the time and mass (size) of glaciations are still being discussed (Mangerud et al. 2004; Astakhov et al 2016).

In the upper streams of large Siberian rivers the most extensive glaciations were in the Altai mountain region where the Chulyshman glacier went down into the basin of the Biya River (Ob River basin) (Gundrizer 1978; Vysotsky 2001). Further to the east, another glacier of similar height existed in the upper reaches of the Bolshoi Yenisei River (Grosswald 1965; Arzhannikov et al. 2010). But eastwards, in the Lena River basin, a continuous glacial cover was not observed (Grosswald and Rudoy 1996; Matz et al. 2001). In general, there were up to five large ancient water bodies in the Baikal Rift Zone existing since the Neogene (Martinson 1955, 1967; Dmitriev 1968). Small valley glaciers in the Vitim river basin (one of the large tributaries of the Lena River) as well as in the valley of its own tributaries (Tsipa and Tocha rivers) have formed periglacial, large, freshwater lakes (Grosswald and Kotlyakov 1989) (Fig. 1).

In the middle streams of the same Siberian Rivers, a single sea glacier dammed the courses of the rivers due to huge periglacial reservoirs that were formed. One such large-scale event happened about 35,700 ± 1000 years ago and led to a substantial increase of the water level in the middle reaches of the rivers and ultimately to the appearance of large periglacial water bodies (Grosswald and Kotlyakov 1989; Grosswald 1999; Mangerud et al. 2004; Volkov and Kazmin 2007). Additionally, the Obskoye, Yeniseiskoye, Lena-Vilyuiskoye and Vitimskoye lakes are the result of such events and they were connected by a network of channels and spillways (Fig. 1). Perhaps, all these water bodies were refugia for whitefishes during different periods of the Pleistocene. Due to the difficulty in precisely reconstructing the geological history of the studied area, the routes of migrations inside this region as well as among different regions of Eurasia of different forms/species of whitefishes, is a very complicated issue. It has been shown that the postglacial distribution of whitefishes originated from the Altai refugia along the Ob River and further along the Arctic coast in both western and eastern directions (Bochkarev et al 2018; 2020; 2021). It is obvious that, besides whitefishes from the Altai refugia, the whitefishes from arctic water bodies of Siberia (*C.*

l. n. *glacialis*) had to also be settled the lakes of North-West Europe that opened in the postglacial period (Østbye et al 2005; Bochkarev et al 2018; 2020; 2021). Thus, it has been shown that there were three whitefish lineages that migrated from postglacial North European, South European, and Siberian water bodies (Østbye et al 2005). However, the previous studies did not include phylogenetic information about whitefishes from a number of refugia across the greater part of Eurasia. Such information may significantly alter our understanding of the process of whitefish dispersal both within Eurasian and between continents during glacial and postglacial times.

In the present work we performed a comparative genetic analysis of some populations of the *pidschian*-like whitefishes inhabiting the largest Central (Yenisei River) and East (Lena River) Siberia region in order to: 1) study the possibility of their migration among the studied water bodies of Siberia; 2) test the hypothesis of an exchange of haplotypes among different whitefish populations through the transcontinental system of snowmelt water bodies in the Late Pleistocene.

Materials and methods

Whitefish populations and sampling

The analysis includes samples of *pidschian*-like whitefishes from two largest river basins of Russia, namely the Yenisei and Lena rivers. The sampling sites with coordinates (in decimal degrees), codes and sample sizes are shown in Table 1. The Yenisei group consists of the whitefish populations from the Bolshoi Yenisei, Bolshoi Abakan, Khamsara rivers, the Karakul, Noyon-Khol, Borzu-Khol, Kadysh and Todzha lakes. The Lena group includes the whitefish populations from the Markha River, Lake Bolshoye Toko. The Baunt and Dorong lakes of the Baunt lake system are situated in the upper stream of Vitim river. The Vitim River, a large tributary of the Lena River, flows from the Vitim Plateau (Fig. 1). The upper reaches of the Yenisei River are situated in the Altai-Sayan Mountains; the Lena River flows from the western slope of the Baikal Mountains. The Vitim River, a large tributary of the Lena River, flows from the Vitim Plateau. Lake Bolshoye

Toko is situated in the Aldan Plateau. The Markha River is a large tributary of the Vilyui River, which flows into the middle reaches of the Lena River (Kirillov 1972; Gundrizer 1978).

Different forms of sparsely-rakered whitefish *C. l. pidschian*, with different numbers of perforated scales, inhabit both lakes and rivers situated in the upstream reaches of the Yenisei River (Table 1). All whitefish populations from the upper and middle reach of the Yenisei River are autumn-spawning benthophagous forms/species (Skryabin 1978; Bochkarev et al. 2011). Structuring of populations of whitefishes from the Bolshoi Yenisei and Bolshoi Abakan river basins by morphological and ecological characteristics were presented in a previous study (Bochkarev et al. 2011). The whitefish populations from the Markha River and Lake Bolshoye Toko are *C. l. pidschian* benthophages, sparsely-rakered and with low numbers of perforated scales. In the basin of the Vitim River of the Baunt Lake system whitefishes are represented by two forms/species: the sparsely and densely rakered (Bochkarev et al. 2013). Here we consider the *C. l. pidschian* fishes inhabiting the water bodies of Siberia as complex with ambiguous taxonomic structure.

In this study all whitefishes were subdivided into two main groups according to geographical principle. The first group (Yenisei basin) consists of lacustrine whitefishes from Lake Karakul and the Todzha lake system, and the riverine whitefishes from the Abakan, Bolshoi Yenisei and Khamsara rivers. The second main group comprised the whitefishes from the Lena River basin, namely from the middle reaches of the Lena River, the Markha River and Lake Bolshoye Toko, and the combined whitefish samples from the Baunt lake system (the Baunt and Dorong lakes) situated in the Vitim River basin (right reach of the Lena River) (Fig. 1). Moreover, all studied whitefishes were structured based on ecological characters of the water bodies comprising their habitat. In the Yenisei group we have formed four haplogroups: 1) whitefishes from Karakul lake, then 2) Abakan river 3) five lakes of Todzha lake system and 4) lacustrine whitefishes from Bolshoi Yenisei and Khamsara rivers. In the Lena group we have also formed four haplogroups geographically very distinct from each other: 1) upper stream of Lena river 2) Markha river 3) Bolshoye Toko lake 4) Baunt and Dorong lakes. The fishes were caught in fixed gill nets with mesh sizes of 10-35 mm at shallow depths. The whitefish forms were identified according to previous descriptions (Ioganzen and Moiseev 1955; Kirillov 1972; Scryabin 1977; Gundrizer 1978; Karasev 1987). All fishes were photographed immediately after being caught from a lateral perspective with a digital camera Nikon D60. The number of gill rakers and the lateral line scale numbers were also counted.

DNA extraction and amplification of the mitochondrial gene

Immediately after the fishes were caught, samples for genetic analysis (liver or muscle) were collected, placed in 96% ethanol and then stored at -20 ˚C. Total DNA was extracted by the phenol-chloroform method (Sambrook et al. 1989) and was stored at 4 ˚C in 1× TE buffer (pH = 8.0). Amplification of the mitochondrial DNA fragment (the 16S-ND1 genes) was carried out with polymerase chain reactions using two external primers, LGL381 and LGL563 (Cronin et al. 1993; Politov et al. 2004) and three pairs of internal primers (Bochkarev et al 2011). The reaction mixture and PCR conditions were according to the protocol described in Bochkarev et al. (2011). The amplified products were purified using the kit by BIOSILICA (Novosibirsk, Russia, http://biosilica.ru/), and both product strands were sequenced on an ABI 3130xl automated capillary sequencer (Applied Biosystems) with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit 3.1 at the SB RAS Genomics Core Facility (Novosibirsk, Russia, http://sequest.niboch.nsc.ru). The DNA sequences were aligned using the CLUSTALW algorithm and then manually edited. The general assembly of the fragment was performed on the basis of mtDNA AB034824 (Miya, Nishida, 2000). The newly obtained nucleotide sequences of the with a total length фрагмента 16S rRNA (partial), tRNA-Leu (full), NADH dehydrogenese subunit 1 (full), tRNA-Ile (full) and tRNA-Gln (partial) 1929 bp were deposited into the GenBank database (Acc. No. see Table 1).

Haplotype distribution

To visualize the haplotype distribution among studied whitefishes the median joining (MJ) network (Bandelt et al. 1999) was constructed using Network v. 4.5 (www.fluxusengineering.com). Genetic landscape diagrams were plotted in concordance with sampling sites.

Genetic polymorphism and population structure

Since the nucleotide and haplotype diversity of the regions corresponding to the 16S RNA was relatively low (Table 1), only the fragments corresponding to the ND1 gene were used for haplotype analysis and subsequent calculations. This made it possible to simplify the calculations without noticeable damage to biologically significant conclusions. Moreover, the consideration and analysis of the haplotypes of the protein-coding DNA sequences separately from the haplotypes of the rRNA gene, would support obtaining more correct biologically significant conclusions.

For the analysis of genetic polymorphisms the following parameters of the ND1 gene were calculated: number of polymorphic sites (S), number of haplotypes (h), haplotype diversity (H_d), nucleotide diversity (π). The calculations were done using DnaSP v. 5.10 (Librado and Rozas, 2009). A hierarchical analysis of molecular variance AMOVA for the population and groups were performed using Arlequin v. 3.5.2.2 (Excoffier and Lischer, 2010). Two AMOVAs were carried out to examine patterns of genetic differentiation into (1) the “among major basins” (Yenisei and Lena), “among population groups within major basins” and “within population groups” components; (2) the “among population group” and “within population groups” components. The significance of the Φ-statistic parameters was assessed by permutation tests with 10 000 replicates as implemented in Arlequin v. 3.5.2.2.

Genetic differentiation and demography

The genetic distances between geographical population groups of *C. l. pidschian* using the Kimura 2-parameter model were estimated in MEGA v. 5 (Tamura et al. 2011). To assess genetic differentiation among population groups, pairwise F_{ST} values were calculated with Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010). The neutrality tests of Fu’s F_s (Fu 1997) and Tajima’s D (Tajima

1989) were calculated for different population groups with Arlequin v. 3.5.2.2 to investigate the demographic history and testing whether the sequences conformed to the expectations of neutrality. The significance of these tests was proven using the coalescent simulation with 1 000 permutations. Additionally, the demographic history of the population groups from the two major river basins was inferred from mismatch distribution (MMD) using Arlequin v. 3.5.2.2. In general, multimodal MMD testifies to a population of demographic equilibrium or constant size, whereas unimodal distribution suggests sudden or expanding population (Slatkin and Hudson 1991; Rogers and Harpending 1992; Ray et al. 2003; Excoffier 2004).

Isolation by distance

The correlation between the matrices of genetic (F_{ST}) and logarithmically transformed geographical distances was estimated using the Mantel test (using 100 000 permutations) as implemented in MantelTester v. 1.1.2 (http://manteltester.berlios.de).

Results

Haplotype distribution

A median-joining haplotype network was reconstructed to examine the genealogical relationships among whitefish haplotypes from the Yenisei and Lena river basins. This analysis showed that the whitefish haplotypes could be subdivided into two-three separate groups, which mainly corresponded to the "main river basins" from the Yenisei and Lena rivers. The connection between two "major groups" is through the haplotype H42 and spanning six to eight mutations (Fig. 2).

The Yenisei haplogroup consisted of two star-like structures connected to each other through four mutations. All these subgroups include several haplotypes spaced apart from a central haplotype spanning one or two mutations. Remarkably, most ND1-haplotypes are not shared between the two major riverine basins. Nevertheless, several haplotypes from the Abakan River basin are closely related to two haplotypes from the Lena River basin (H10, 11). The Lena haplogroup consisted of multiple star-like structures spaced apart from the central haplotype (H 42) spanning one to three mutations. The whitefish haplotypes from the different water bodies of the Lena River basin were mixed. In contrast, the whitefish haplotypes from Lake Bolshoye Toko separated into a distinct group (H29, 35). The haplotypes of endemic whitefish from the Baunt Lake system were split into two branches with a central haplotype H53, and distant from the central haplotype by 1-2 mutations (H50, 51, 54-58, 62, 63) and (H29,49,52,59-61). The second group consists of the haplotypes of the whitefishes from the Markha River (central – H29), (spanning H29, 32). The remaining whitefish haplogroups from the Markha River were closely related to the Yenisei haplogroup (H30, 31, 33).

Genetic polymorphism and population structure

The study of nucleotide sequence polymorphism of the ND1 gene was conducted on 132 specimens obtained from 14 water bodies of Siberia. Populations were joined into eight population groups based on geographical and ecological criteria. In total, 50 haplotypes (based on ND1) were detected in studied whitefish populations (Table 2). High genetic variability (H_d and π values more than 0.722 and 0.00136 respectively) was detected for all whitefish populations, with the exception of whitefish from Lake Karakul (H_d = 0.182, π = 0.00019), situated in the Yenisei River basin that was inhabited by whitefish relatively recently. In general, the general genetic characteristics in haplogroups of whitefish from Yenisei river basin a bit lower than in haplogroups of whitefish from Lena river basin. This is especially noticeable for whitefish inhabit Baunt lakes (Dorong and Baunt lakes), where two phylogenetic lineages are hybridized (H_d = 0.962, π = 0.0371).

The AMOVA analysis using the unstructured data set showed significantly high molecular variance for both the “among groups” and “within groups” components, accounting for 54.03 % and 45.66%, respectively, and 0.33% among populations (Table 3). When we considered the hierarchical level “main river basins”, the AMOVA assigned a large portion of molecular variance to the “among main river basins” level (50.08%) that was similar to that given to the “among groups” component by the two-level analysis; the “among groups within main river basins” and “within groups" levels accounted for 14.23% and 35.69%, respectively (Table 4). All Φ-statistics parameters associated to each level were highly significant.

Genetic differentiation and demography

Pairwise F_{ST} values calculated between 8 population groups indicated the occurrence of a high degree of genetic divergence between groups belonging to different riverine basins, up to 0.530 (Table 5). The F_{ST} values under pairwise comparison of the whitefish populations from the Yenisei and Lena river basins too, were mostly high and reliable (0.01-0.69). Therefore, there is isolation between the populations of the two river basins, which was confirmed by the Mantel test (r = 0.23, P < 0.001). All whitefish population groups are characterized by negative and insignificant values of Tajima's *D*, although for the generalized sample these values were significant (Table 6). A neutrality test using Fu's F_s also showed negative values, but, on the contrary, they were significant for many of the whitefish population groups as well as for the generalized samples. Negative Tajima's *D* and Fu's F_s values were registered for both "main river basins", but only the latter were significant. The shape of the mismatch distribution for the united whitefish sample was bimodal (and suitable for both demographic and spatial expansion models), but the model of spatial expansion is apparently more plausible (SSD_{obs} = 0.00309, Table 6, Figs. 3a, 3b).

Discussion

Whitefish distribution and population structure

The *pidschian*-like whitefishes are widespread over the area of Southern Siberia, spanning from the Ob River basin in the West to the Lena River basin in the East. However, there are only few suitable habitats for whitefishes. Predominantly, the whitefish populations inhabit large and deep lakes, or lake-shaped opening of the upper reaches of large rivers. In the course of small rivers whitefishes are absent, or their number is extremely low. Water bodies of Siberia are primarily inhabited by low-scale and sparsely-rakered whitefishes (*Sp.br.* = 17-27). There are not many medium raked populations. One population of medium-rakered whitefishes (*Sp.br.* = 30-40) inhabits Lake Teletskoye. The medium-rakered whitefishes are not found in the Yenisei River basin. In the Lena River basin, medium and densely-rakered whitefishes inhabit the Baunt lake system. Thus similar forms/species based on ecological features of whitefishes inhabit both of the studied river basins.

Changes in the hydrological network of Siberia caused by paleogeologic events in the Late Pleistocene strongly affected the distribution of different forms/species of whitefishes. It is assumed that the raising of the Ikatsky and Southern-Muysky ridges resulted in a redirection of the Tsipa River (the Baunt lake system belongs to the Vitim river basin) from its original direction to Lake Baikal, and changed its flow towards the Lena river basin. According to contemporary data this event happened rather recently (~ 0.4-0.15 million years ago) (Karasev 1989; Smirnov et al. 2009). As a result the whitefish populations from the Lena River invaded the Baunt lake system where supposedly a hybrid population evolved. Initially, the hybridization affected all populations from the main drainage of the lakes (the Baunt and Dorong lakes). Obviously, migrants had far less impact on the whitefish populations from remote water bodies situated in upstream reaches of the mountain rivers with numerous rapids (Fig. 1).

The hierarchical AMOVA analysis on the level “main river basin” showed significant decrease of population genetic variability from 50.08 % to 14.23 % and showed that a large part of molecular variance is accounted for the “among main basins” component. In other words, the whitefish populations really could be grouped into two large population groups according to the river basins. Nevertheless, within population genetic variability remains similar in both the unstructured dataset and at the level “two main basins”. It could be evidence for secondary intergradation of the whitefish populations from the two main riverine basins in the recent past, however, this requires additional research.

Our analysis showed that the haplotypes from the Abakan River basin (Yenisei River basin) were the only ones shared among the Yenisei and Lena river basins. Despite this, most of the whitefish haplotypes from the Abakan River basin belong to the Yenisei haplogroup. The presence in the haplogroup I of the haplotypes (H4, H8, H9, and H12, 13) suggests an extended dispersion of this whitefish group in the recent past. On the other hand, the presence of the whitefish haplotypes (H10, 11) from the Abakan River basin (Yenisei haplogroup) in the Lena haplogroups, which are tightly bound with the haplotypes from the upstream reaches of the Lena River and Lake Bolshoye Toko (Lena River basin), with central haplotype H42, gives evidence of their common origin and whitefish migration from an eastern towards a western direction (Fig. 1).

Based on many minor haplotypes of the second and third orders it therefore may be concluded that the populations composing this structure are ancient, and, probably, were not influenced very much by the last glaciations. The star-shaped structures with a number of minor haplotypes clearly indicate a recent bottleneck effect for the whitefish populations from the mountain lakes of the Yenisei River basin. This is also relates to the haplogroups of whitefishes from Lena river basin and we assume that their structure is more complicated than then the structure of whitefishes from the Yenisei River basin.

The F_{ST} values between two “main river basins” were high and significant (0.51), whereas in contrast, this index under pairwise comparison of population groups varied from low (0.01) to high (0.69) values. Hence, this index points to a long-term genetic isolation of two large population groups belonging to the Yenisei and Lena river basins, but there was gene flow between some populations within these large groups. The gene flow is negligible between the whitefish population from Lake Karakul and other populations from the Yenisei River basin, except for the whitefish population from the Abakan River. Results of the Mantel test indicate the isolation by distance for the studied whitefish populations, with the matrices of between populations F_{ST}-values and geographical distances being significantly correlated (r = -1.000, $p < 0.001$). In most cases, the divergence of the mitochondrial lineages can be explained by geographical isolation of the populations.

Demographic history

The negative, although insignificant, values of the Tajima's *D* and Fu's F_s neutrality tests for the whitefish population indicate deviation from mutation-drift equilibrium. Nevertheless, the high negative Fu's F_s values for two "main river basins" are significant and clearly show the recent expansion of the populations. Probably, this process is more intensive in the whitefish populations from the Lena River basin.

The mismatch distribution analysis revealed a bimodal pattern for the joined sample of all whitefish populations from the two studied riverine basins. The sum of square deviation (SSD) and raggedness index (*r*) is better correlated with the spatial expansion model. The observed bimodal pattern also could suggest the whitefish populations' subdivision as shown by the existence of two well resolved haplogroups according to two main riverine basins. The negative values of the neutrality tests also suggest a spatial expansion for whitefishes from the Lena River basin. Also, a postglacial spatial expansion of the whitefishes from the Yenisei River basin is supported by a star-like haplotypes network with two predominant haplotypes, which are accompanied by a low frequency of peripheral haplotypes. The mismatch distribution for whitefish populations from the Yenisei and Lena river basins likely correspond to alternating periods of decrease and increase of the population group size. These genetic analyses confirmed our hypothesis regarding repeated events of connection between the two riverine basins and gene flow among different whitefish populations. Obviously, the area inhabited by *C. l. pidschian* was diminished to a few isolated populations during the glaciation periods.

The deep genetic structuring observed in *C. l. pidschian* in the Lena River basin in comparison with the Yenisei River basin is quite coherent with glaciation data in the mountain regions of Siberia. It is accepted that a very extensive ice sheet was in the Altai-Sayan mountain region, and it was gradually decreased in thickness from west to east (Grosswald and Rudoy 1996; Arzhannikov et al. 2000; Vysotskiy 2001; Volkov and Kazmin 2007). The thickness of the ice sheet in Central Altai and the Todzha Depression was about 1.5-2.0 km (Grosswald 1999). Thus, the numbers of refugia were apparently very low in this region. Since, we have found the small number of central haplotypes of *C. l. pidschian* in the Bolshoi Yenisei River basin.

In Lena river basin (Transbaikalia) there was a glaciation of valleys only in the mountain region that it had less impact on aquatic ecosystems (Martinson 1955, 1968; Dmitriev 1968; Matz et al. 2001). The extent of glaciation of Eastern Siberia, and Transbaikalia was low and had probably a minor influence on the whitefish populations inhabiting this region; but numerous refugia (Zagorskaya 1961; Karasev 1987) served ongoing differentiation of whitefishes.

Dispersion of *pidschian*-like whitefishes of Eurasia during last glacial and postglacial time

The most important study focused on the pathways of migration of pidschian-like whitefishes of Eurasia was published relatively recently (Østbye et al 2005). It was shown that the settling of postglacial lakes of North-West Europe was performed by three phylogenetic lineages of whitefishes e.g. a North European (I), South European (II), and Siberian (III) clades that were directed along the arctic coast and river courses that originated from the Alps towards the North Sea (II) (Fig. 4) (Østbye et al 2005). However, based on recent studies we may conclude that in Central and East Siberia there are at least two more forms/species of whitefishes from the Ob River basin (IV) and Lena River basin (VI) that were also colonized towards the western direction (Fig. 4). Moreover, dispersal of *C.l. pidschian* n. *glacialis* was also in the same western direction as the haplotypes mentioned by Østbye et al (2005), from the Pyasina, and Khatanga Rivers. To-date, analyses including the phylogenetic lineages of whitefishes from Siberian refugia has substantially improved the understanding of the general view of whitefish colonization of water bodies of Eurasia. However, the information about genetic diversity of whitefish forms/species from basins of the White, Barents, Bering and other northern seas is needed in order to analyze whitefish transcontinental dispersion. It should be noted there are significant differences in time of glaciations and area of glacial coverage in the north part of Eurasia between various studies (Mangerud 2004; Volkov and Kazmin 2007; Astakhov et al 2016). However, besides the mentioned differences, it has to be concluded that the dispersion of whitefishes could be realized via both the outer freshened edge of a glacier or the chain of lakes around the glacier (Sendek et al., 2013).

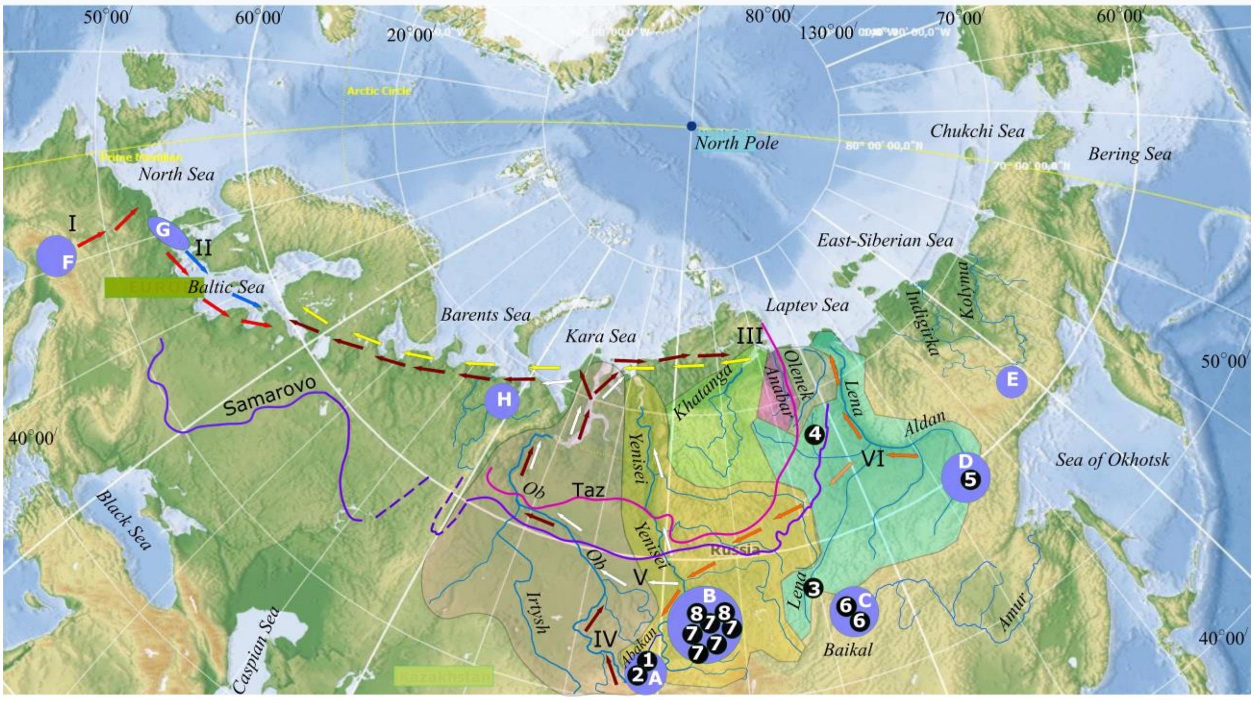

Figure 4. Scheme of known glaciations and postglaciation times of dispersions of *pidschian*-like whitefishes based on diversity of mtDNA. Modern borders of the main Siberian river basins are indicated in colors: Ob (brownish), Yenisei (yellow-sand), Lena (light blue), Anabar (pink), Khatanga (light green), Olenek (grey). Upper case letters indicate border of known modern refugia; sampling sites (the present study) are numbered 1-8; black line: the border of the last sea glacier (Volkov and Kazmin 2007; Astakhov et al 2016); Arrows designate the assumed migration paths of whitefishes during glaciations and postglacial periods (I, II, and III according to Østbye et al. 2005, IV – the present study; V and VI – Bochkarev et al. 2018, 2020). Red and violet lines designate the borders of Taz and Samarovo glaciations (Astakhov et al 2016).

Conclusions

The results of this study show that *pidschian*-like whitefish populations within the two large Siberian riverine basins, the Yenisei and the Lena rivers, can be subdivided into two separate haplogroups characterized by similar levels of genetic polymorphism. Some whitefish populations are characterized by a hybrid origin whereas perhaps other ones were recently originated from relatively low number of individuals (Karakul lake) and divergence was facilitated by the quite isolated nature of the water bodies where these whitefishes live (Bochkarev et al. 2018). The moderate and high values of the fixation indices of genetic diversity between *pidschian*-like whitefish populations demonstrate that in each basins the level of diversity was accumulated and the counter dispersal of whitefishes has happened and the exchange of genetic material during the period following the last Ice Age. It must be assumed that the gene flow between *pidschian*-like whitefish populations is currently limited and a diversification of forms is occurring within geographically isolated populations. These results confirm previous research regarding the events of hybridization between the whitefish forms/species during the last postglacial period (Mamontov 2000; Østbye et al. 2005; Ilmast et al. 2016; Bochkarev et al. 2018). It should be noted that, in contrast to European *pidschian*-like whitefishes, whose populations were probably eliminated by the Scandinavian glacier over large areas, from the White Sea Basin to the Ural Mountains, such significant glaciation was not observed in Southern Siberia. In this case, the colonization of Siberia water bodies by whitefishes originated from the nearest populations not affected by glaciations. As a result, in the Siberian water bodies there were not found such a large number of "ecological forms", in contrast to the water bodies of Fennoscandia, where at least three phylogenetic lines of whitefishes from the North Sea, Alpine, Ural (Lake Komi) and Siberian were hybridized among each other. In addition, the origin of Siberian whitefishes could be different as well (considering arctic and south Siberian refugia). Different numbers of hybridizing populations with their high morphological variability should lead to the emergence of an infinite number of ecological forms of the pidschian-like whitefishes in large European water bodies (Østbye et al. 2005, 2006; Kottelat and Freyhof 2007; Hudson et al. 2011).

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