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BRIEF COMMUNICATIONS

Detection of the Mitochondrial DNA Haplotype Characteristic of the Least Cisco (*Coregonus sardinella*, Valenciennes, 1848) in the Vendace (*C. albula*, Linnaeus, 1758) Population of Vodlozero (the Baltic Sea Basin)

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Abstract—Analysis of the nucleotide sequence of the *ND-1* gene fragment of mitochondrial DNA in the vendace population in Lake Vodlozero (the eastern part of the Baltic Sea basin) revealed a sequence variant that is closely related to that of the least cisco of Siberia (the Indigirka River). Thus, together with the results of morphological and allozyme analysis of this population performed earlier, the results obtained in this study are suggestive of the expansion of the least cisco to the Baltic Sea basin during the last glaciation.

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INTRODUCTION

It is well known that a number of fish of the Siberian origin inhabit the basins of the White and Barents seas. These species have immigrated into these water bodies apparently soon after the glacier retreat (Kuderskii, 1987). These data were corroborated and supplemented by the results of molecular-genetic studies (Makhrov, Bolotov, 2006).

In particular, the results of morphological studies showed that cisco populations in the Pechora River basin occupy an intermediate position between the vendace and least cisco. Some populations of this basin are more similar to the first species, whereas others share more similarity with the second one (Tanfil'ev, 1896; Pokrovskii, 1967; Solovkina, 1974; Reshetnikov, 1980; Tumanov, 2002). It was suggested that *CK-1,2** genes, which encode creatine kinase, can be used for identification of these two species (Perelygin, 1992). Tests of fish using these gene loci showed that the allele frequencies in cisco samples from the Pechora River basin are also intermediate (Sendek, 1998).

Recent studies showed that *CK-1,2** alleles characteristic of the least cisco are also present at a sufficiently high frequency in the populations of Lake Beloe in the Volga River basin (Makhrov et al., 2003) as well as Lake Vodlozero in the Baltic Sea basin (Borovikova et al., 2006). We detected the mitochondrial DNA (mtDNA) haplotype characteristic of the least cisco in the sample from Vodlozero. However, it should be noted that, in the above-cited work, we used restriction fragment length polymorphism (RFLP) analysis, which does not provide comprehensive information on the nucleotide sequence. Since the haplotype characteristic of the least cisco differed from the wide-spread haplotype of vendace by the absence of only one restriction site (for restriction endonuclease Rsa I), it could not be ruled out that this haplotype appeared in the Vodlozero cisco population as a result of a single reverse mutation.

The goal of this study was to confirm that a gene flow from the Siberia to the population of the modern lake Vodlozero indeed existed in the past. For this purpose, we analyzed data on the complete nucleotide sequence of an mtDNA fragment in fish of this population.

MATERIALS AND METHODS

Lake Vodlozero, which is located in the southeast of Karelia, belongs to the Lake Onega basin. This is one of the easternmost water bodies of the Baltic Sea basin (Fig. 1). Lake Vodlozero has a rich and peculiar ichthyofauna (Petrova, Kuderskii, 2006).

The material for this study was collected in August 2005 near the village of Kuganavolok (62°24′ N, 36°88′ E). Fish was caught with gill nets. For mtDNA polymorphism analysis, liver, muscle, adipose fin tissues were fixed in 96% ethyl alcohol (1 : 5). Sequencing was performed with three samples of cisco DNA, two of which belonged to the composite haplotype, which is widespread in vendace, and one belonged to the haplotype characteristic of the least cisco.

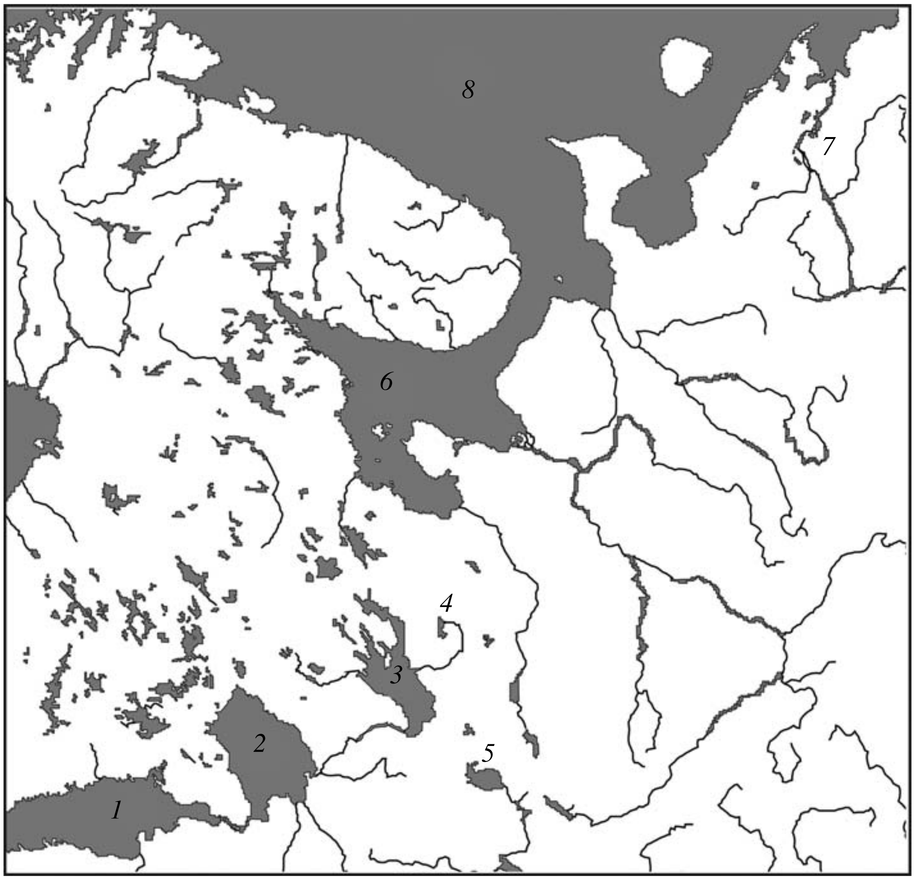

Fig. 1. European north of Russia. Designations: *1*, Gulf of Finland of the Baltic Sea; *2*, Lake Ladoga; *3*, Lake Onega; *4*, Lake Vodlozero; *5*, Lake Beloe; *6*, White Sea; *7*, Pechora River; *8*, Barents Sea.

DNA was isolated by the standard phenol–chloroform extraction method (Sambrook et al., 1989). The nucleotide sequence was determined of the 300-bp fragment of the mtDNA *ND-1* gene, which encodes subunit 1 of the NADH dehydrogenase complex and which is polymorphic in ciscoes. Sequencing was performed using the full-length *ND-1* region of the 300-bp obtained using the primers developed by Cronin et al. (1993): 5'-ACCCCGCCTGTTTACCAAAAACAT-3' (LGL381, forward) and 5'-GGTTCATTAGTGAGGGAAGG-3' (LGL563, reverse).

The *ND-1* region was amplified by polymerase chain reaction (PCR). The reaction was performed in 25 μl of amplification buffer (Bionem, Moscow) containing 10 mM Tris-HCl (pH 8.8), 50 μmol KCl, 2.5 μmol $MgCl_2$, and 0.08% Nonidet P40. The amplification mixture contained 100–300 mg of total cell DNA, 10–15 pmol of each of the two primers, 200 nmol of each of the four deoxyribonucleotides, and 0.5–1 units of Taq polymerase (Bionem).

Amplification included the initial denaturation of DNA at 95°C for 5 min (1×); fragment synthesis at 95°C for 1 min, at 53°C for 50 s, and at 72°C for 1 min 45 s (32×); and final elongation at 72°C for 5 min. The amplification product was quantitated by electrophoresis in 1% agarose gel with subsequent visualization of the PCR fragments under UV light after staining with ethidium bromide. The size of the relevant fragment was determined using 1-kb DNA Ladder (Fermentas, Lithuania).

The nucleotide sequence of the 300-bp *ND-1* fragment was determined on the basis of the EvroGen laboratory (Moscow). Sequencing was performed using the specially developed primers 5'-CTTGGCTCAGGCTGGGCCTCT-3' (F2-1, forward) and 5'-GCGCAAGGTGTCATAGGA-3' (R2, reverse).

In further experiments, we analyzed the *ND-1* gene fragment whose sequence was read from both primers for all samples.

The chromatograms with the results of sequencing were viewed and the nucleotide sequences were read using the SeqMan 4.00 software (DNASTAR Inc.).

The sequences of the *ND-1* gene fragment of the Vodlozero cisco mtDNA were deposited in GenBank (Acc. Nos. EU339171–EU339173). For a comparison, we also used the sequences for the following species, which were extracted from GenBank: *C. lavaretus* (Acc. No. NC002646), *C. albula* (Acc. Nos. EU256379, EU339159–EU339165, EU339174, DQ399869, and DQ399870), and *C. sardinella* (Acc. Nos. EU339150–EU339158).

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                             10                  20                  30                  40                  50                  60                  70                  80
C.lavaretus TTTGACCTCACAGAGGGGGA ATCAGAACTAGTCTCTGGGT TTAATGTAGAATACGCCGGA GGACCCTTCGCCCTTTTTTT
        E-3 .................... ...G................ ..........G......... ....................
        S-1 .................... ...G................ .................... ....................
        S-2 .................... ...G................ .................... ....................
                             90                 100                 110                 120                 130                 140                 150                 160
C.lavaretus TCTGGCGGAGTATGCCAACA TCCTCCTCATAAATACGCTC TCAACCATCCTATTTCTGGG GGCATCACATATCCCCGCCT
        E-3 .................... ....T............... .................... ....................
        S-1 .................... ....T............... .................... ....................
        S-2 .................... ....T............... .................... ....................
                            170                 180                 190                 200                 210                 220                 230                 240
C.lavaretus TTCCCGAACTAACAGCCATA AATCTAATAACAAAAGCCGC CCTACTATCCGTAGTCTTTT TATGAGTGCGAGCCTCATAC
        E-3 .C.................. .................... .................... .........A.........T
        S-1 .C.................. .................... .................... .........A..........
        S-2 .C.................. .................... .................... .........A..........
                            250                 260                 270                 280                 290                 300
C.lavaretus CCCCGCTTTCGGTACGACCA GCTCATACACCTTGTTTGAA AAAGCTTCCTACCTATAACC
        E-3 .............T...... .................... ..........G.........
        S-1 .............T...... .................... ..........G.........
        S-2 .....A.......T...... .................... ..........G.........
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Fig. 2. Mitochondrial DNA sequence variants of the *ND-1* gene fragment of vendace and least cisco compared to the whitefish, whose mitochondrial genome was sequenced completely (GenBank Acc. No. NC002646). Designations: E-3, S-2, mtDNA sequence variants of the Vodlozero cisco population; S-1, mtDNA sequence variant of the least cisco from the Indigirka River (GenBank Acc. No. EU339155); *10–300*, nucleotide positions. Points indicate the nucleotides coinciding with those in the whitefish nucleotide sequence.

RESULTS AND DISCUSSION

As expected, sequencing of the mtDNA fragment revealed two variants of the studied sequence—E-3 and S-2 (Fig. 2). Variant E-3 was found in both studied individuals whose haplotype is characteristic for vendace from the European water bodies, whereas variant S-2 was found in the cisco that had the Siberian composite haplotype.

Variant E-3, found in two Vodlozero ciscoes, is not unique but has a limited distribution. For instance, this variant is present in the ciscoes populations of Lake Onega, Pechora River, and water bodies of western Yamal Peninsula (Acc. Nos. EU339150–EU339153, EU339159, EU339163, and EU339156). Variant E-3 is characterized by the substitution of cytosine with thymine at position 240 (which corresponds to position 3690 in the full-length mtDNA of *C. lavaretus,* Acc. No. NC002646). This sequence variant was not found in water bodies located to the west of Lake Onega (Kohlmann et al., 2007; Borovikova, Artamonova, unpublished data).

We found that variant S-2 differed from variant E-3 by three nucleotides (substitutions at positions 51, 240, and 246 (Fig. 2). At the same time, variant S-2 differed from the nucleotide sequence of the same fragment of the *ND-1* gene of cisco from the Indigirka River (Acc. No. EU339155) by only one nucleotide at position 246. These findings confirmed that, indeed, variant S-2 is a derivative of the Siberian variant, differing from the latter by only one mutation, rather than a derivative of the European variant.

Judging by the data that are available at GenBank and published by Kohlmann et al. (2007), this sequence variant was not found in other studied cisco populations of the eastern and southern parts of the Baltic Sea basin. However, it should be noted that RFLP analysis of the *ND-3* region and the control region of mtDNA of the cisco of Lake Breiter Luzin (Germany, the North Sea basin) revealed in this population a haplotype that is more similar to the haplotypes of *C. sardinella* from North America than the haplotypes of *C. albula* (Schulz et al., 2006).

It should be also noted that the possibility of penetration of the least cisco to the basins of the Baltic and Caspian Seas has been discussed for a long time. On the basis of results of morphological analysis, a number of researchers in the first half of the 20th century regarded some cisco populations from the specified basins as the least cisco subspecies. In particular, the cisco of Lake Beloe (the Volga River basin) was described as the subspecies *C. sardinella vessicus* (Dryagin, 1933). Pokrovskii (1967) classified the cisco of Lake Vodlozero as the subspecies *C. sardinella maris-albi.* However, it should be taken into account that no clear hiatus between the least cisco and vendace was found in the past decades on the basis of morphological criteria (Pokrovskii, 1967; Dryagin et al., 1969).

Later, Reshetnikov (1980) suggested the morphological characteristics that can be used to discriminate between the two cisco species. On the basis of these diagnostic traits, all ciscoes of the North-West of Russia belong to the species *C. albula* (Reshetnikov, 1980). However, the morphological analysis of the cisco of Lake Vodlozero (Borovikova et al., 2006) showed that, judging by one of the traits suggested by Reshetnikov, some ciscoes corresponded to the least cisco.

Thus, analysis of data using three groups of markers (morphology, allozymes, and mtDNA) suggests that the least cisco contributed to the formation of the cisco population of Lake Vodlozero. The fact of distribution of the Siberian form so far to the west is of great interest in terms of zoogeography.

Paleogeographic reconstructions indicate the existence of intricate relationships between the near-glacial water bodies in the European North during the last glaciation (Kvasov, 1975, 1976; Mangerud et al., 2004;

Svendsen et al., 2004). The complex network of near-glacial water bodies provided different fish species with an opportunity to radiate and maintain contact. A possible way of the least cisco radiation over European water bodies was discussed by Kuderskii (1977).

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