# NATIVE VENDACE *(COREGONUS ALBULA)* AND ALIEN PELED *(C. PELED)*: GENETIC COMPARISON AND INTROGRESSIVE HYBRIDIZATION

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Non-native species of the Coregonidae were introduced in many lakes of the Northern Europe. At the same time it is known that very common process for this group of fish is hybridization. The aim of this study was to analyse the genetic relationships between two close species, vendace *Coregonus albula* and peled *C. peled*, because these data can be useful for understanding the origin and taxonomical status of these species as well as monitoring of interspecific hybridization.

Samples of the vendace and peled were collected from 2003 to 2011. Genomic DNA was extracted using the phenol-chloroform extraction protocol or the Diatom®DNA Prep 100 ("IzoGen", Moscow). We performed PCR-RFLP analysis of the mitochondrion *ND*-1 fragment (about 2050 bp). Also a fragment of the ND-1 gene (300 bp) was sequenced.

The sequences of the studied fragment of all specimens of the peled entirely coincided with the *E*-1 variant of the vendace, although we studied the mtDNAs of fishes from two local populations and analysed samples belonging to two different composite haplotypes from each population. At the same time we found specimens with peled's type composite haplotype in vendace's population from Rybinsk reservoir (upper reaches of Volga River) although peled was not observed in catches after its introductions in the middle of the 20th century.

The obtained data allow us to discuss different models of the relationships between two species. First, vendace and peled may be assumed to be ecological forms of the same species. Second, the data may also be interpreted as a consequence of the introgressive hybridization between vendace and peled. The possibility of this case was observed in the population of Rybinsk reservoir. Third alternative is that peled and vendace originated from a common ancestor and have diverged recently.

Key words: vendace, peled, mitochondrial DNA (mtDNA), introgressive hybridization, phylogeny

## INTRODUCTION

Anthropogenic activity significantly changes the freshwater ecosystems. One of the directions of this activity is introduction of alien fish species. In Northern Europe, where water bodies are not characterized by very exceptional biodiversity, the goals of many introductions are to increase stocks of high-quality commercial species and/or to improve sport fisheries, artisanal fisheries and aquaculture (Lévêque, 1996). One of the most commercially important group of northern fish is family Coregonidae, non-native coregonid species were introduced in many lakes of Northern Europe. In many cases,

peled (*Coregonus peled*) is moved into water bodies since it is characterized by good and fast growth and high resistance to adverse environmental conditions (Zvereva et al., 1953; Peljad'..., 1989; Kudersky, 2001; Andriyasheva, 2011).

In Russia numerous introductions of coregonidae were carried out during the 20th century (Kudersky, 2001). One of the largest water bodies of Northern Russia, where different coregonid species including peled were imported, is Rybinsk reservoir in upper reaches of Volga River (Table 1, Figure 1). However, these introductions had not any successful effects. At present family Coregonidae is only represented in reservoir by vendace.

Native vendace's population has been detected in Rybinsk reservoir since 1943. It should be noted, that vendace had not been the typical species for Volga River before the reservoir was created in 1941. The appearance of vendace in Rybinsk reservoir was connected with its migrations from Lake Beloye in upper Volga basin. This assumption was done by the comparison of the morphological features of vendace inhabiting Lake Beloye and Rybinsk reservoir (Vasil'ev, 1952).

The introduction of new species directly affects the structure and function of communities. In addition, one of the important results of the introduction and acclimatization is hybridization (Lévêque, 1996; Luczynski et al., 1999; Biologicheskie invazii..., 2004; Ilmast & Sterligova, 2004). It is known that hybridization is very common process for coregonid species, and events of the appearance of their hybrids are very often detected by morphological and molecular markers (Zvereva et al., 1953; Vuorinen, 1988; Šlechtová et al., 1992; Kohlmann et al., 2007). At the same time, the fertile inter- and intraspecies hybrids are direct threat for native species and biodiversity of the freshwater ecosystems. Moreover, hybrids turned out to grow at the rate lower than that

typical of parental species (Lévêque, 1996; Luczynski et al., 1999).

However, we can consider the ecosystems inhabited by close native and alien species as a model for understanding phylogenetic relationships of these species. Furthermore we can understand some mechanisms, its origin and speciation process. Based on these data, we can more successfully manage the freshwater recourses and preserve more important artificial species and endangered endemic species.

Sustainable management of commercial fisheries as well as the conservation of still existing pure native populations require their genetic characterization (Kohlmann et al., 2007). Thus, the aim of this study was to analyse the genetic relationships between close species, vendace (*Coregonus albula*) and peled (*C. peled*), because these data can be useful for understanding the origin and taxonomical status of these species as well as monitoring of interspecific hybridization.

**MATERIALS AND METHODS**

***Collection of materials.*** The vendace from natural populations were caught with a gill net or trawl in 2002-2009 (Table 2, Figure 1). The vendace's population from Rybinsk reservoir was presented by 47 specimens sampled in different localities of the reservoir (near countries Vsehsvyatskoye (38°33'N, 58°23'E); Kamenka (38°44'N, 58°31'E); Gorelovo (38°71'N, 58°14'E); Brejtovo (37°53'N, 58°18'E)). The genetic polymorphism of reservoir's population was only investigated by PCR-RFLP analysis of the mitochondrial ND-1 fragment.

For several specimens of vendace from different freshwater bodies of Northern Russia sequencing of a part of mitochondrial ND-1 gene was performed. All these individuals were characterized by the presence of the composite haplotype E which according to the previous results of PCR–RFLP analysis of complete ND-1 fragment, was the most widespread in vendace populations, was found

in least cisco populations, and was prevailing in peled (Borovikova, 2009). These specimens were sampled from Onega Lake and its sub-catchment (4 individuals were typical form of vendace, and 1 specimen was large vendace's form from Onega Lake, or kilets) and from two lakes of Volga River basin (3 individuals) (Table 2). Specimens of peled were collected in the Federal Centre for Fish Genetics and Selection (the town Ropsha, Leningrad region, Russia) in 2003 and 2011. Peled was transported into this Centre from Lake Endyr' (the Ob River basin) and have been artificially bred beginning from the mid-1950-ies (Golovkov, 1956). For 72 peled specimens PCR-RFLP analysis was performed. Additionally we analysed nucleotide sequence of two specimens, that were carriers of E mitochondrial ND-1 fragment composite haplotype, and of one specimen, that was carrier of PE composite haplotype, which is more characteristic for peled and has never been found in vendace populations before. Nucleotide sequences were also analysed for two peled specimens from some water body of Yenisei River basin (Table 2, Figure 1). In all cases for mtDNA analysis the liver or white muscle tissue, or adipose fin samples were fixed with 96% ethanol (1:5).

**Morphological analysis** was carried out only for 11 mature specimens from different localities of Rybinsk reservoir (near countries Kamenka, Gorelovo, Brejtovo). Morphology was described according to the methodology previously used for coregonid from North Eastern Europe (Pravdin, 1966).

**PCR–RFLP analysis of mitochondrial ND-1 fragment.** Total cell DNA was isolated from ethanol-preserved specimens by the phenol-chloroform extraction protocol (Sambrook et al., 1989) or the DNA kit Diatom®DNAPrep100 (manufactured by "Izogen", Moscow, www.rugenlab.ru). For PCR-RFLP analysis the amplified mtDNA fragment of about 2050 bp including the gene that encodes subunit I of NADH – dehydrogenase complex (ND-1; hereafter referred to as ND–1 fragment) was

used. The amplification was performed using the primers developed by M. Cronin et al. (1993): 5'ACCCCGCCTGTTTACCAAAAACAT3' (LGL381, forward) and 5'GGTTCATTAGTGAGGGAAGG3' (LGL563, reverse). Synthesis of fragments (polymerase chain reaction, PCR) was conducted in 25 µL of 1X amplification buffer from "Bion-m" (Moscow). The amplification mix contained 100-300 ng of total cell DNA, 10-15 pmol of each of the two primers, 200 nmol dNTPs, and 0.5 – 1 units of *Taq* polymerase ("Bion-m", Moscow or "Fermentas", Lithuania). A small amount of mineral oil was added on the surface during PCR to avoid evaporation. Amplification program of *ND-1* fragment included initial DNA denaturation step at 95°C for 5 mins; 32 cycles of fragment synthesis at 95°C for 1 min, +53°C for 50 sec, 72°C for 1 min 45 sec, and final elongation at 72°C for 5 mins. The amplified fragments of mtDNA were investigated by means of 14 restriction endonucleases (*Ase*I, *Bst*NI, *Bst*UI, *Dde*I, *Dpn*II, *Hae*III, *Hha*I, *Hinc*II, *Hinf*I, *Hph*I, *Msp*I, *Nci*I, *Rsa*I, *Taq*I). Endonuclease treatment of samples was carried out for 3 – 4 hours at the temperature recommended by manufacturers. Fragment length analysis of the cut amplification products were performed in 2% gel for all endonucleases except *Bst*NI. For the latter, the fragment lengths were analyzed in 1.3% agarose gel. To reveal polymorphisms of low-weight molecular fragments, the products of cutting *ND-1* fragment by *Dde*I and *Hae*III were also run in 11% polyacrylamide gel with TBE buffer. After electrophoresis agarose and polyacrylamide gels were stained with ethidium bromide. Composite haplotypes were created for each sample and labeled following E. Borovikova (2009) and (Borovikova & Makhrov, 2009).

**Sequencing of a fragment of the ND-1 mitochondrial gene.** We analysed the nucleotide sequences of a part of mitochondrial ND-1 gene (301 bp). One of the mtDNA regions contained in amplified fragments was sequenced at the EuroGene Laboratory (Moscow, Russia) by means of a

MegaBACE-500 automated sequencer with an electrophoresis chamber (48 capillaries) using the DYEnamic ET, Dye Terminator Cycle Sequencing KIT for Mega BACE DNA Analysis System. For sequencing, we developed the following primers:

F2-1, forward: 5'–CTTGGCTCAGGCTGGGCCTCT–3'

R2, reverse: 5'–GCGCAAGGTGTCATAGGA–3'.

Subsequently, we analysed nucleotide sequences read from both primers for all samples. The sequences described for peled were deposited in the data base NCBI (Acc. Nos. EU339166-EU339170). For comparison we also used sequences of vendace that were deposited in NCBI by the authors previously (EU339159, EU339160, EU339162-EU339165, EU339171, EU339172).

***Analysis of the PCR-RFLP and sequencing data.*** We used the SeqMan 4.00 software (DNASTAR. Inc.) for viewing chromatograms with the results of sequencing and reading the nucleotide sequences. The ChromasPro Version 1.2 software (Technelysium Pty Ltd) was used to search for restriction sites. The ND-1 sequence polymorphisms, genetic distances between haplotypes and their phylogenetic relationships were investigated using MEGA version 4.0 (Tamura et al., 2007) and Arlequin version 3.01. (Excoffier et al., 2006).

## RESULTS

***Comparison of the morphological features of vendace from Rybinsk reservoir and peled.*** We only analyzed diagnostic morphological characteristics for two species because many of the morphological features are very variable and depend on environmental conditions. Thus, the information about the number of vertebrae, gill-raker number and mouth opening position is presented in Table 3. According to revealed morphological data the coregonid fish from Rybinsk reservoir is typical vendace.

***Genetic comparison of the vendace and peled population polymorphism.*** The *ND-1* gene fragment of peled showed lower variability in comparison to vendace from Rybinsk reservoir: we only revealed three composite haplotypes (Table 4). The most frequent haplotype was E. It was expressed by 68.06% of examined peled individuals; one specimen was carrier private haplotype (1.4%) and the rest of the peled specimens were carriers of composite haplotype PE (30.55%). The difference among common haplotype E and PE is one nucleotide substitute that, however, resulted by change of restriction sites for two enzymes *Bsp*1286I and *Dpn*II (Figure 2). At the same time, for samples of vendace from Rybinsk reservoir altogether eleven composite haplotypes were described. Six of these eleven haplotypes were private. The most frequent haplotype in reservoir's population of vendace was E: frequency of this haplotype was 58%. In addition, characteristic haplotype of peled PE was found in vendace's population of the reservoir with frequency 6% (all of these specimens were caught near Vsehsvyatskoye). Table 4 presents nucleotide variation ($\pi$) and haplotype diversity index (H) for peled and vendace populations.

***Analysis of the*** **ND-1** ***gene fragment's nucleotide sequences*** (300 bp) for vendace with the same composite haplotype E, but from different populations, demonstrated the presence of polymorphism that had not been detected by RFLP analysis. We found four types of sequences: *E*-1 (EU339164, EU339165), *E*-2 (EU339162), *E*-3 (EU339159, EU339163, EU339171, EU339172) and *E*-4 (EU339160). These variants differ from one another in one to three nucleotide substitutions within the haplotype E. The close relationships of vendace and peled were revealed by comparison nucleotide sequences. Thus, the sequences of the studied mtDNA fragment in all the five specimens of peled entirely coincided with that of the *E*-1 variant in the vendace, although we studied the mtDNAs of fish from two local populations and analysed samples belonging to two different composite
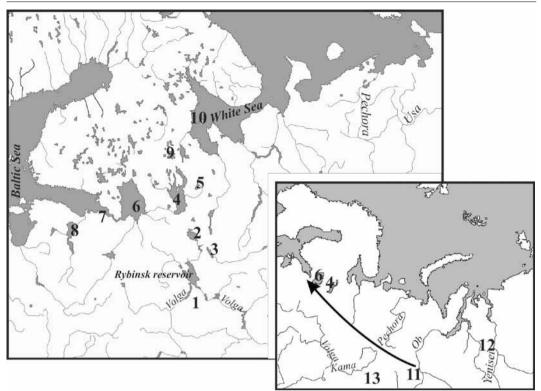
Figure 1. The map of Northwestern Russia and Western Siberia with the mine water bodies discussed in the study. 1. Lake Plescheevo; 2. Lake Beloye; 3. Lake Kubenskoye; 4. Lake Onega; 5. Lake Vodlozero; 6. Lake Ladoga; 7. the Federal Centre for Fish Genetics and Selection (the town Ropsha, Leningrad region); 8. Lake Peipus; 9. Lake Segozero; 10. Solovetsky Archipelago (Lake Goreloye); 11. Lake Endyr'; 12. some water body in Yenisei River basin; 13. Lake Uvil'dy, South Urals (55°31N, 60°30'E). Arrow indicates the transportations of the peled from Lake Endyr' to the Federal Centre for Fish Genetics and Selection in Ropsha.

haplotypes, E and PE, from each population. It should be mentioned that these composite haplotypes differ in a region outside the sequenced fragment of the ND-1 gene (Figure 2).

## DISCUSSION

***Was there introgressive hybridisation in vendace population of the Rybinsk reservoir?*** Numerous introductions of peled into Rybinsk reservoir had not any successful effects: this species was not revealed in the catches by morphological criteria. At the same time we found specimens with peled composite haplotype, PE, in vendace population from

Rybinsk reservoir. It should be noted that the characteristic peled haplotype (PE) and its derivatives were not observed before in vendace populations of Northwestern Russia. Even in population of vendace from Lake Krasnoye of Solovetsky Island, where introductions of peled were carried out (Dvoryankin, 2005), we detected neither PE haplotype nor its derivatives (Borovikova, 2009). Detection of the peled haplotype in vendace population of Rybinsk reservoir allows assuming two hypotheses. The first hypothesis is - peled coexists with vendace in reservoir; feeding, growing and maturating of vendace and peled happen in the same localities of
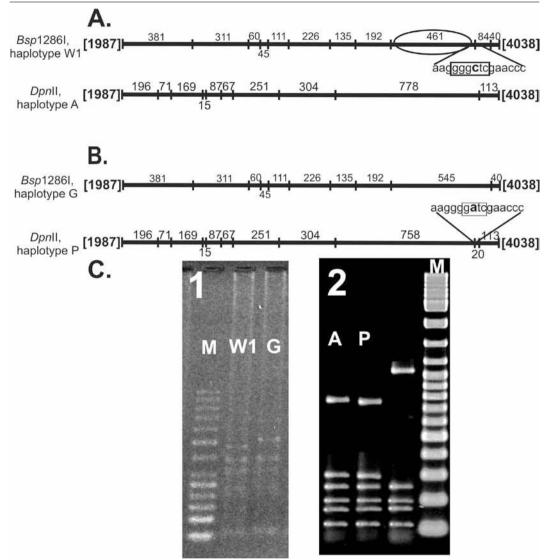
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Figure 2. Restriction maps of the mitochondrial *ND-1* fragment (2050 bp) for *Bsp*1286I and *Dpn*II endonucleases. **A.** The combination of haplotype W1 for *Bsp*1286I with haplotype A for *Dpn*II is characteristic for composite haplotype E: recognition site for *Bsp*1286I obtains ("gdgchc"), but site for *Dpn*II ("gatc") does not exist. **B.** The combination of haplotype G for *Bsp*1286I and haplotype P for *Dpn*II is characteristic for composite haplotype PE. If in position 3907bp of the complete sequence of mitochondrial genome of *Coregonus lavaretus* (Acc. № NC_002646.1) C is substituted by A, recognition site for *Bsp*1286I disappears, but site for *Dpn*II appears. **C.** Electrophoregrams of the cut amplification products in agarose gels: 1. *Bsp*1286I, 2. *Dpn*II. **Notation:** in square brackets – positions of the complete genome of *C. lavaretus* (Acc. № NC_002646.1); numbers near the segments denote its length in base pair (for estimation of the segments length we used the complete genome of *C. lavaretus* (Acc. № NC_002646.1); squares mark the restriction sites; M – ladder (on the left gel GeneRuler™50bp DNALadder, "Fermentas"; on the right gel GeneRuler™ DNALadderMix, "Fermentas"); W1, G, A, P – denote haplotypes for digests by *Bsp*1286I and *Dpn*II enzymes. Ellipse marks sequenced region of *ND-1* fragment.

Table 1.
Coregonid species and forms used for introductions in Rybinsk reservoir.

| The introdused species and forms | Years of introductions | Quantity of introduced eggs | Donor localities |
|---|---|---|---|
| *Coregonus albula* | 1952 | ? | ? |
| Ripus - a large form of vendace of Lake Ladoga | 1944-1949 | 20.000.000 | Lake Ladoga |
| | 1955 | 120.000 | |
| Hybrid "ripus x whitefish" or "ural'sky ripus" | 1944-1949 | ? | Lake Uvil'dy, South Urals |
| *C. peled* | 1969-1985 | ? | Different fish hatcheries, that received eggs of peled from the Federal Centre for Fish Genetics and Selection (Ropsha town). In Centre eyed eggs of peled from Lake Endyr' (western Siberia) were transported in the 1950s. |
| *C. lavaretus* | 1944-1949 | 52.000 | Lake Ladoga |
| | 1955 | 1.110.000 | Lake Peipus |
| | | 805.000 | Lake Ladoga |
| *C. lavaretus nelmuschka*, dwarf form of whitefish of Lake Kubenskoye | 1956 | ? | Lake Kubenskoye |

Table 2.

Summary of the populations sampled in the study.
**Notation:** MT — PCR–RFLP analysis of mtDNA, S – sequencing. For peled in round brackets the quantity of the specimens with different composite haplotype are presented: **bold** type denotes specimens characterized by haplotype E, *italic* – by haplotype PE.

| Species | Localities | | Coordinates | | Year sampled | Sample size for molecular-genetic analysis | |
|---|---|---|---|---|---|---|---|
| | Basin | Freshwater body | Latitude | Longitude | | MT | S |
| *C. albula* | Onega Lake | Lake Onega, typical small form | - | - | 2002 | - | 2 |
| | | Lake Onega, large form, or kilets | - | - | 2004-2005 | - | 1 |
| | | Lake Vodlozero | 62º30'N | 36º90'E | 2005 | - | 2 |
| | Volga River | Lake Beloye | 60º12'N | 37º62'E | 2002 | - | 1 |
| | | Rybinsk reservoir (different localities) | 37º52'N -38º44'N | 58º14'E-58º31'E | 2009 | 47 | - |
| | | Lake Plescheevo | 56º80'N | 38º80'E | 2005 | - | 2 |
| *C. peled* | Yenisei River basin | | 65º47'N | 87º56'E | 1998 | - | 2(**1**+*1*) |
| | Ob River | Lake Endyr' | 61º63'N | 67º19'E | 2003 | 15 | 3(**2**+*1*) |
| | | | | | 2011 | 57 | - |

Table 3.
Some morphological traits of vendace from Rybinsk reservoir.
We only focused on the features that are often used as diagnostic for species and forms of coregonid fishes. For comparison we include data about different populations and species of family Coregonidae. **Notation:** lim — trait variation limits; M — mean. * denotes populations and species from which introductions were carried out in Rybinsk reservoir; ** - the water body from which vendace invaded the Rybinsk reservoir after its creation by oneself (according to L.Vasil'ev (1952)).

| Species and forms | Localities | | Number of vertebrae | | Gill-raker number | | Length of upper jaw as % of length of lower jaw | | References |
|---|---|---|---|---|---|---|---|---|---|
| | Basin | Water bodies | lim | M | lim | M | lim | M | |
| C. albula | species diagnose | | 54-59 | 55-56 | 35-58 | 40-44 | - | - | Atlas…2003 |
| | White Sea | Lake Goreloye, Solovetsky Archipelago | 56-59 | 57.45 | 35-48 | 43.5 | 52.4-92.9 | 71.7 | our data |
| | Volga River | Lake Beloye** | 53-56 | 54.37 | 36-44 | 39.18 | - | 73.5 | Dryagin 1933 |
| | | | 54-56 | 55.1 | 35-42 | 38.2 | 70.0-88.0 | 75.0 | our data |
| | | Rybinsk reservoir | 53-57 | 54.5 | 36-41 | 38.5 | - | - | Vasil'ev 1952 |
| | | | **54-56** | **54.56** | **40-43** | **41.4** | **68.2-83.3** | **76.8** | **our data** |
| | | Lake Plescheevo | 50-59 | 53.36 | - | - | 60.0-88.9 | 72.9 | our data |
| | Baltic Sea | Lake Vodlozero | 52-56 | 54.72 | 29-42 | 36.23 | 67.0-90.0 | 79.0 | our data |
| | | Lake Segozero | 51-57 | 54.18 | 41-50 | 45.25 | 66.7-80.0 | 75.5 | our data |
| Large form of vendace from Lake Ladoga, or ripus | | Lake Ladoga* | 54-59 | 56.61 | 43-56 | 49.36 | - | 63.9 | Pravdin et al. 1937; Pravdin 1939 |
| C. peled* | species diagnose | | 57-63 | - | 46-69 | - | - | - | Peljad'..1989 |
| | Pechora River | | - | - | 52-63 | 57.4 | - | 62.0 | Zvereva et al. 1953 |
| C. sardinella* | species diagnose | | 57-64 | more than 60 | 35-56 | - | - | - | Atlas…2003 |
| | Yenisei creek, near Sosnovaya River | | 54-62 | 59.77 | 38-45 | 41.69 | - | 73.0 | Bobrova 1958 |
| C. lavaretus | species diagnose | | 58-65 | - | 15-64 | - | - | - | Reshetnikov 1980 |
| | Baltic Sea | Lake Segozero | 55-60 | 57.7 | 32-19 | 25.15 | 63.6-82.4 | 72.7 | our data |
| | White Sea | Lake Kubenskoye* | 55-62 | 57.69 | 24-31 | 27.15 | 58.3-136.4 | 76.1 | our data |
| C. lavaretus pidschian | Arctic Ocean | Usa River | - | 60.96 | - | 22.22 | - | 81.1 | Protopopov 1983 |

Table 4.

Haplotype and nucleotide diversity values in different population of vendace and peled. Notation: N – number of specimens; NH – number of composite haplotypes; NHP – number of private haplotypes (A – absolute number; F – frequency); PS – number of polymorphic sites; H – haplotype diversity; SD – standard deviation; π – nucleotide diversity.

| Species | Localities | N | NH | NHP | | PS | H±SD | π |
|---|---|---|---|---|---|---|---|---|
| | | | | A | F | | | |
| *C. albula* | Rybinsk reservoir | 47 | 11 | 6 | 0.29 | 12 | 0.66±0.08 | 0.0026 |
| | Lake Plescheevo | 60 | 16 | 12 | 0.40 | 14 | 0.70±0.06 | 0.0025 |
| | Lake Beloye | 30 | 3 | 2 | 0.10 | 2 | 0.19±0.28 | 0.0003 |
| | Lake Onega | 40 | 6 | 3 | 0.10 | 5 | 0.28±0.09 | 0.0005 |
| | Lake Vodlozero | 50 | 6 | 3 | 0.08 | 5 | 0.35±08 | 0.0005 |
| *C. peled* | The Federal Centre for Fish Genetics and Selection in Ropsha town (Lake Endyr' population) | 72 | 3 | 1 | 0.01 | 2 | 0.45±0.04 | 0.0018 |

reservoir, but reproduction of these species occurs separately. The second hypothesis suspects introgressive hybridization between species. According to the first hypothesis we must find the specimens with features of both peled and vendace in samples from Rybinsk reservoir. Diagnostic morphological criteria for vendace and peled are the number of vertebrae (54-59 and 57-63 for vendace and peled, correspondingly), gill-raker number (35-58 and 46-69 for vendace and peled, correspondingly) and characteristic body shape and mouth opening position (Peljad'..., 1989). The number of vertebrae and gill-raker number of vendace from Rybinsk reservoir have remained practically invariable since formation of its population (Table 3) and do not overstep the limits of these characteristics for typical vendace. Thus, these results are not absolutely consistent with the coexisting hypothesis: we do not find specimens with morphological features of peled. We focused more attention on meristic features because other morphological characteristics of the coregonid fishes are very plastic (Etheridge et al., 2012, and references in this article).

However, many authors pointed that even meristic morphological features could be also influenced by environmental factors. It is known, for example, that number of vertebrae could be varied by temperature during embryogenesis (Eckmann, 1987; Lindsey, 1988). Many of the head features could be changed by diet peculiarities of fish (Men'shikov, 1951; Meyer, 1987; Balon, 1989; Wimberger, 1991). Body shape and position of the fins likely indicate ability to long-term migration (Belyaeva, 1951; Burmakin, 1953). Thus, some special features of vendace from Rybinsk reservoir can be explained by exposure of environmental conditions. It is interesting, that mouth of Rybinsk reservoir vendace is located in a position closer to the end of the head in comparison with peled. It should be noted, that in literature the events are described when upper jaw of peled is very short (Table 3). On the contrary, molecular-genetic analysis is more powerful, and mtDNA polymorphism is considered as one of the best markers for phylogeny and phylogeography (Avise, 2000; Avise, 2004). At the same time, we did not find careful differentiation of peled and vendace's

ND-1 fragments. As a result, for specimens, that were characterized by E composite haplotype, we cannot say with certainty about their species belonging because this haplotype is typical both for peled as well as for vendace. Thus, analysis of the polymorphism of the ND-1 fragment is useful only on population level: if in population carriers of the peled composite haplotype PE are detected, we can refer specimens of this population to *C. peled*.

The second hypothesis about introgressive hybridization seems to be probable enough, because we did not find any specimens with peled morphology. It should be noted, however, that we did not find specimens with intermediate between morphological features of peled and vendace. At the same time, morphological examination of the general species' and hybrids' appearance is difficult and may be even more misleading (Luczynski et al., 1999). If the hybridisation in Rybinsk reservoir took place, peled specimens with composite haplotype PE was the maternal species. Indeed, it is known that males of the out-numbered species very often fertilize the female of non-dominant species (Hayden et al., 2010). Thus, population of vendace from Rybinsk reservoir is characterized by the mixed origin and may contain the hybrids of vendace and peled. Any solution needs further molecular-genetic studies including analysis of the nuclear markers' polymorphism.

***Phylogenetic relationships between peled and vendace.*** In general, all available data allow us to consider three alternative hypotheses, though not all of them are equally probable, on the origin of the peled and its phylogenetic relationships with the vendace. First, vendace and peled may be assumed to be ecological forms of the same species. This is confirmed by the finding that composite haplotype E is typical for peled as well as for vendace and the sequence of the studied mtDNA fragment of peled is identical to the *E*-1 variant of this fragment in vendace. This also agrees with the data on the sequence of

another highly polymorphic mtDNA region, namely, the control region or D-loop (Reist et al., 1998; Brzuzan & Ciesielski, 2002). In the cited studies, the number of nucleotide substitutions in the studied mtDNA sequences of vendace compared to those of peled (sequences 321 and 517 bp in length, respectively) varied from 0.6% (Reist et al., 1998) to 0.9% (Brzuzan & Ciesielski, 2002) (intraspecific polymorphism was not analysed in these studies). Comparison of the mtDNA sequences of *C. albula* and *C. peled* with those of *C. lavaretus* (Reist et al., 1998; Brzuzan & Ciesielski, 2002) showed considerably greater differences (3.7–4.4%). In addition to these data, analyses of the allozyme polymorphism characterizing the variation of the nuclear genome, revealed that *C. peled* has common alleles with *C. albula* for most loci; the species being very similar to one another in this respect (Bodaly et al., 1991; Politov et al., 2000; Sendek, 2000). Obviously, this also applies to combining the peled with the vendace into a single species only because they share a 300-bp mtDNA fragment, moreover, karyological differences between peled and vendace correspond to the interspecific level. According to most authors, *C. albula* has 80 chromosomes, whereas *C. peled* has 74–76 chromosomes (Kaidanova ,1988; Coj et al., 1996). Although the peled karyotype was also once reported to contain 80 chromosomes (Viktorovski, 1964) - that study used embryonic material, therefore, errors in species identification could not be excluded. On the other hand, the same data may be interpreted as evidence for a hybrid origin of peled; in this case, it has inherited mtDNA from vendace with composite haplotype E. Studies on other fishes (including salmonid fish, which are closely related to coregonid fish) have demonstrated that some interspecific hybrids carry mtDNA of only one of the parental species, while their nuclear genome has been inherited from the other species or is a combination of the genomes of both ancestors (Chan & Levin, 2005). The speciation of peled may have followed this scenario. However, on contrary to situation in Rybinsk

reservoir the maternal species during speciation of peled was vendace. Indeed, according to results of phylogeography studies the vendace had been spread from Siberia to Europe in the past (Borovikova, 2009; Borovikova & Makhrov, 2009). Thus, the vendace was invasion species for many water bodies. At the same time it is known that undirectional gene flow from introduced to native species or from abundant to rare species is typical (Rhymer & Simberloff, 1996; Fitzpatrick et al., 2010; Cabria et al., 2011; Kahilainen et al., 2011). Therefore peled as offspring of the hybridization has inherited mitochondrial DNA of vendace with common haplotype E. The third alternative is that peled and vendace originated from a common ancestor and have diverged recently. In this case, the low frequency of nucleotide substitutions in the mtDNA sequence of the peled may be accounted as a slow evolution of the mitochondrial genome or a strong selection for one mtDNA variant. If so, further studies on the nuclear genome of the peled may show that the evolution of some of its regions is considerably more rapid, and nuclear markers ensuring reliable differentiation between these species may be found. Whatever hypothesis may be confirmed by further studies, our data demonstrate that peled and vendace are at least phylogenetically related to a high degree.

## CONCLUSIONS

Hybridization is very common process for coregonid fishes and can result in different consequences. For example, the inter- and intraspecies hybrids may potentially reduce phenotypic adaptations and fitness in native species. At the same time, hybridization has very important role in speciation of coregonid fishes. Analysis of the molecular markers' polymorphism (mtDNA) has revealed probable existence of the introgressive hybridization between vendace and peled in Rybinsk reservoir (upper reaches of Volga River). In addition, according to molecular-genetic analysis of the mitochondrial *ND-1* fragment

vendace and peled are very close species. The data obtained in this study allow us to discuss different models of the phylogenetic relationships between vendace and peled. First, vendace and peled may be assumed to be ecological forms of the same species. Second, the data may also be interpreted as a consequence of the introgressive hybridization between vendace and peled. Third alternative is that peled and vendace originated from a common ancestor and have diverged recently.

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