Genetic differentiation of *Coregonus muksun* in natural populations and broodstocks of fish rearing farms “Forvat” and “Sobsky” in relation to the problem of restoring the species population in Western Siberia

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Abstract. With regard to the problem of restoring the muksun (*Coregonus muksun*) population in Western Siberia by means of artificial regeneration, the genetic differentiation of natural populations and broodstocks of fish rearing farms “Forvat” and “Sobsky” was studied. Eighty-four sequences of mtDNA (3,006 bp) containing the highly polymorphic genes ND1 and ND2 were analysed. The differences between the populations of Western and Eastern Siberia were revealed. These findings must be taken into account when attempting to restore the species population in each particular region. The genetic closeness of the muksun from fish rearing farms “Forvat” and “Sobsky” with muksun from the Ob-Taz basin gives grounds for using these broodstocks for seeding the Ob and Taz rivers.

1. Introduction
The conservation of biological diversity and the restoration of valuable commercial fish species populations are of great ecosystem and socio-economic importance. The endemic Siberian muksun (*Coregonus muksun*), widespread from the Kara river in the west to the Kolyma river in the east, is one such species. Since the end of the 20th century, there has been a widespread decline in muksun population, caused mainly by increasing anthropogenic impact. The near complete destruction of the muksun in the Ob and Taz rivers and the increasing anthropogenic load on the ecosystems of the Gulf of Ob determine the need for urgent implementation of fish-breeding measures in Western Siberia, including the stocking of fish juveniles produced in fish hatcheries. However, in order to preserve both particular populations and the species as a whole, knowledge of the intraspecific genetic diversity of muksun and an assessment of the genetic identity of natural populations and broodstock of fish farms are required.

Aim of the work: the analysis of genetic differentiation of natural populations of muksun and broodstocks of fish rearing farms “Forvat” and “Sobsky”.

2. Materials and methods
The genetic analysis was carried out based on the data of mtDNA sequences (3,006 bp) of 84 muksun specimens from natural populations of Western and Eastern Siberia (figure 1) and broodstocks of fish farms “Forvat” and “Sobsky”.

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Figure 1. Range of muksun and sampling points.

The source of the muksun spawn for the “Forvat” broodstock rearing was the Ob population. For broodstocks of “Sobsky” the spawn from the “Forvat” fish rearing farm was partly used.

PCR reactions were carried out in 20 μl volumes including 1 μl of each 2 pM primer, 2 μl 10xAS Buffer (MgCl₂), 0.5 μl of 10 mM dNTP, 1 μl of 50 mM MgCl₂, 0.4 μl of Taq 5 U/μl and 1 μl of DNA (stock). Three pairs of primers, used in the study (table 1), were designed from C. muksun and C. peled mitochondrial sequences (KT824877 [1] and KM361633 [2]) using Pairwise – BLAST (National Centre for Biotechnology Information).

Table 1. Primer pairs used in the study.

| Primer | Sequence, 5'-3' | PCR thermal cycling program | Product length, bp | Product |
|--------|-----------------|----------------------------|--------------------|---------|
| Cm5    | F: CGACCTCGATGGATGCAGGACG R: AAGGGCGAAGGCTCTCGG | 5 min – 95°C; 15 sec – 95°C, 15 sec – 59°C, 80 sec – 72°C for 25 cycles; 10 min – 72°C | 1,015 | tRNA-Leu, ND1 |
| Cm6    | F: CCTTTGACCTCACAGAGGGG R: CAGATGTTGGTCTGGTAACTCC | 15 sec – 95°C, 15 sec – 59°C, 80 sec – 72°C | 1,110 | ND1, tRNA-Ile, tRNA-Glu, tRNA-Met, ND2 |
| Cm7    | F: GTAGCCCACCGTTAACTCCTC R: CGACTATCCGGCTCAGGC | for 25 cycles; 10 min – 72°C | 1,096 | ND2, tRNA-Trp, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, COI |

Sequence reactions were performed using BrilliantDye Terminator Cycle Sequencing with the same primers. Sequence products were analysed in the Centre of Collective Use IPAE UrRAS. The chromatograms were analysed using the BioEdit v7.2.0 [4]. Sequence alignment and genetic distances calculation were carried out in the Mega v6 [3]. Mitochondrial markers polymorphism was assessed in the Arlequin v 3.1 [5] and DnaSP v.5.10 [6]. Construction of phylogenetic tree was carried out in the MrBayes v3.2.2 [7]. A complex approach with the choice of the model for each of the three codon positions separately was used. For all three codon positions, it was the GTR+G model. The optimum model of nucleotide sequence evolution was selected using the MrModeltest 2.3 [8]. In the Bayesian inference of phylogenetic trees, two parallel analyses consisting of four Markov chains, each for 10,000,000 cycles, were run simultaneously, with sampling every 500th cycles and removing the first 5,001 cycles as the burn-in stage.
3. Results

For 84 sequenced mtDNA fragments (3,006 bp) including the ND1 and ND2 genes as well as the complete tRNA-Leu, tRNA-Ile, tRNA-Gln, tRNA-Met, tRNA-Trp, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr sequences and a partial sequence of COI, 56 haplotypes were described (GB accession numbers: MZ711093-MZ711148).

Most haplotypes were unique. Two haplotypes were common for the Ob population and broodstocks. One haplotype was observed in brood stocks of both “Forvat” and “Sobsky” fish farms. The indices of genetic diversity calculated for the most representative samples were higher in natural populations than in broodstocks (table 2). The highest values of the indices were found in the populations of Eastern Siberia’s rivers, where the decrease in the muksun population was not as critical as in Western Siberia. The genetic variability of muksun from the Ob-Taz river basin is comparable to that observed in the broodstock of fish farms.

Table 2. Polymorphism of mtDNA sequences (3,006 bp) of muksun in natural populations and broodstocks of “Forvat” and “Sobsky” fish farms.

| Samples                  | Sample size | Number of polymorphic sites | Number of haplotypes | $h^a ± SD^b$ | $\pi^c (\times 100) ± SD$ | $\kappa^d$ |
|--------------------------|-------------|-----------------------------|----------------------|--------------|---------------------------|-------------|
| Natural populations      | 46          | 97                          | 38                   | 0.988 ± 0.008| 0.306 ± 0.031             | 9.19        |
| Ob-Taz river basin       | 19          | 21                          | 13                   | 0.942 ± 0.014| 0.127 ± 0.012             | 3.81        |
| Lena                     | 9           | 34                          | 8                    | 0.972 ± 0.064| 0.336 ± 0.076             | 10.11       |
| Indigirka                | 14          | 49                          | 13                   | 0.989 ± 0.031| 0.397 ± 0.038             | 11.92       |
| Broodstocks              | 38          | 31                          | 20                   | 0.933 ± 0.025| 0.167 ± 0.011             | 5.02        |
| “Sobsky-1”               | 8           | 10                          | 5                    | 0.857 ± 0.108| 0.121 ± 0.019             | 3.64        |
| “Sobsky-2”               | 8           | 13                          | 5                    | 0.857 ± 0.108| 0.154 ± 0.036             | 4.64        |
| “Forvat”                 | 22          | 19                          | 12                   | 0.861 ± 0.065| 0.149 ± 0.016             | 4.49        |

* $h$ – haplotype diversity;  
* $SD$ – standard deviation;  
* $\pi$ – nucleotide diversity;  
* $\kappa$ – average number of nucleotide differences.

The phylogenetic reconstruction (figure 2) revealed the intraspecific genetic differentiation of muksun from Western and Eastern Siberia. There were no differences between the populations within the regions.

Haplotypes of individuals from broodstocks did not form statistically supported groups and were closest to muksun from the Ob-Taz river basin. The analysis of genetic distances confirmed the results of phylogenetic reconstruction (table 3).

Table 3. The genetic distances – $d \times 100$ (above the diagonal) and standard deviation – $SD \times 100$ (under the diagonal) between the muksun populations for analysed mtDNA fragment (3,006 bp).

|          | Ob-Taz | Lena   | Indigirka | Baidarata | Yana | Yenisei | “Sobsky-1” | “Sobsky-2” | “Forvat” |
|----------|--------|--------|-----------|-----------|------|---------|------------|------------|----------|
| Ob-Taz   | 0.274  | 0.312  | 0.257     | 0.231     | 0.490| 0.132   | 0.184      | 0.182      |          |
| Lena     | 0.044  | 0.413  | 0.380     | 0.377     | 0.573| 0.283   | 0.344      | 0.319      |          |
| Indigirka| 0.049  | 0.061  | 0.344     | 0.419     | 0.469| 0.320   | 0.378      | 0.360      |          |
| Baidarata| 0.061  | 0.072  | 0.063     | 0.369     | 0.319| 0.252   | 0.319      | 0.312      |          |
| Yana     | 0.079  | 0.097  | 0.093     | 0.102     | 0.607| 0.242   | 0.301      | 0.287      |          |
| Yenisei  | 0.122  | 0.120  | 0.098     | 0.089     | 0.152| 0.497   | 0.540      | 0.546      |          |
| “Sobsky-1”| 0.032  | 0.048  | 0.054     | 0.059     | 0.082| 0.122   | 0.188      | 0.180      |          |
| “Sobsky-2”| 0.044  | 0.057  | 0.056     | 0.068     | 0.091| 0.122   | 0.046      | 0.247      |          |
| “Forvat” | 0.035  | 0.050  | 0.056     | 0.068     | 0.085| 0.128   | 0.042      | 0.050      |          |
Figure 2. Phylogenetic tree reconstructed by Bayesian analysis for 56 haplotypes of the mtDNA fragment (3,006 bp) including the ND1 and ND2 genes.

Significant differences were observed between the muksun populations from Western and Eastern Siberia. The most differentiated was muksun from the Indigirka river. Broodstocks were relatively close to each other and the population from the Ob-Taz river basin.

According to the obtained results, muksun broodstocks of “Format” and “Sobsky” fish rearing farms have kept a close genetic relationship with the maternal population from Western Siberia.
4. Conclusion
The analysis of intraspecific genetic differentiation of muksun using mtDNA fragments of 3,006 bp length, including the highly polymorphic ND1 and ND2 genes, revealed differences between the Western and Eastern Siberia populations. The same interpopulation differences were also observed when using other genetic markers [9-10].

This result must be taken into account while restoring the number of the species in each specific region. Phylogenetic reconstruction, analysis of genetic diversity indices, and genetic distances indicated the proximity of muksun from the “Forvat” and “Sobsky” fish farms to the population of the Ob-Taz river basin. Thus, broodstocks of these fish rearing farms can be recommended for stocking the Ob and Taz river systems without the risk of disrupting the intraspecific genetic structure formed by evolution.

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References
[1] Xu G F, Zhen-Bo M, Qi-Rui H and Han Y 2017 Complete mitochondrial genome of Coregonus muksun Mitochondrial DNA 28 9–10
[2] Xue S Q and Sun Z W 2016 Complete mitochondrial genome of Coregonus peled Mitochondrial DNA 27 2102–3
[3] Tamura K, Stecher G, Peterson D, Filipski A and Kumar S 2013 MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. 30 2725–9
[4] Hall T, Biosciences I and Carlsbad C 2011 BioEdit: an important software for molecular biology GERF Bull. Biosci. 2 60–1
[5] Excoffier L, Laval G and Schneider S 2005 An integrated software package for population genetics data analysis Evol. Bioinformat. Online 4 47–50
[6] Librado P and Rozas J 2009 DnaSP v5: A software for comprehensive analysis of DNA polymorphism data Bioinformatics 25 1451–2
[7] Ronquist F and Huelsenbeck J P 2003 MrBayes3: Bayesian phylogenetic inference under mixed models Bioinformatics 19 1572–4
[8] Nylander J A A 2004 MrModeltest v2. Program distributed by the author. Evol. Biol.
[9] Baldina S N, Gordon N Y and Politov D V 2008 Mitochondrial DNA genetic differentiation of the Coregonus muksun (Pallas) and related Siberian species of Coregonus (Coregonidae, Salmoniformes) Rus. J. Genetics 44 777–85
[10] Politov V D 2017 Coregonids of Russia: Evolutionary genetic approach in assessment of the current state of biodiversity Fundam. Appl. Limnol. 189/3 181–92