Сибирский экологический журнал, 2 (2016) 202–207

УДК 575.174.5

DOI 10.15372/SEJ20160204

Coregonus peled (Gmelin) transplanted into Ulaagchny Khar Lake (Western Mongolia) showed no evidence of hybridization with other introduced *Coregonus* species

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Статья поступила 15.07.2015

Принята к печати 20.10.2015

ABSTRACT

Coregonus peled (Gmelin) (Teleostei: Salmoniformes: Coregonidae), which is considered an important object of coldwater aquaculture, had been successfully introduced into an enclosed Western Mongolian lake Ulaagchny Khar in the early 1980s. At the same time larvae of two other *Coregonus* species – Baikal omul *C. migratorius* (Georgi) and least cisco *C. sardinella* Valenciennes – had also been released into the lake. Baikal omul was then reported as a naturalized species. This might have caused interspecific hybridization and gene introgression. Identification of coregonids by morphology can be problematic, so to determine which species was dominant in the lake (we presumed it was peled) and if its gene pool was affected by other introduced *Coregonus* species we sampled 40 individuals and analyzed them by sequencing a fragment of mtDNA *cyt b* and by allozyme electrophoresis. The analysis showed that all the fish belonged to *C. peled* with no evidence of admixture from other coregonid species. Taking into account mass release of both species in 1980s, it is evident that naturalization of peled in the lake was much more successful than that of Baikal omul.

Key words: genetic identification, *Coregonus*, peled, introduction, hybridization, mitochondrial DNA, allozymes.

Coregonus peled (Gmelin) is characterized by fast growth and is considered as an important object of coldwater aquaculture in Europe and Siberia. Peled is one of the traditional reared coregonid species that are used for introduction both within and far outside their natural range. Oligotrophic lakes of Mongolia are prospective watersheds for acclimatization of coregonids, and many examples of successful introduction are known. Baikal omul was naturalized in Lake Hovsgol, peled was introduced into lakes of Altai and Khangai regions.

The enclosed Ulaagchny Khar Lake is located at the altitude of 1980 m a.s.l. in western Mongolia (Dzavhan aimak) on the Hangayn Plateau and is surrounded by Bor Khar sands. The lake is 32 км long and has a maximal width of 7 km and a maximal depth of 50 m. The total water area of the lake is 89 km^2, water volume constitutes 1.7 km^3 and drainage area is 1450 km^2 [Tserensodmon, 1970; Ayuushsuren, Dulmaa, 2012].

Conditions in Ulaagchny Khar are favorable for predominantly planktivorous peled since it is a freshwater lake (which is rare in this region) rich in zooplankton: mostly *Rotatoria*, *Cladocera* and *Copepoda* [Dulmaa, 2007; Ayushsuren, Dulmaa, 2012]. Lake Ulaagchny Khar and two other lakes located nearby – Zhaahan and Baga Nuur – belong to closed drainage area of western Mongolia and lacked any indigenous fish. In 1980 and 1982 peled larvae were released into these three lakes. Peled has been reported to adapt well to the conditions of Mongolian lakes reaching age 10+ and weight up to 4500 g, its growth rate exceeding that of the populations within natural range [Dulmaa, 1999].

Several other introductions of coregonid species were made in 1980s. Baikal omul (*C. migratorius* Georgi) and least cisco (*C. sardinella* Valenciennes) larvae were introduced most extensively [Dulmaa, 1999]. Taking into account the ability of *Coregonus* species to interbreed in sympatry zones, one could expect some gene introgression in such relatively small lake as Ulaagchny Khar. In 1998 morphological analysis showed the presence of hybrids in the lake [Dulmaa et al., 1998]. Molecular markers of nuclear localization such as allozymes proved to be effective in genetic identification of hybrids among *Coregonus* species [Reist et al., 1992; Politov et al., 2000, 2002] while maternally inherited mitochondrial DNA (mtDNA) showed the direction of hybridization. The aim of this study was to identify by means of the combined use of both nuclear and mitochondrial genetic markers which *Coregonus* species are now inhabiting lake Ulaagchny Khar, to check if subsequent introductions lead to hybridization and to trace the possible influence of gene pools of Baikal omul and Siberian whitefish on the genetic composition of the introduced peled population which now dominates the lacustrine ecosystem.

MATERIALS AND METHODS

Fish was caught by gillnets (mesh size 20–32 mm) during the last week of August, 2013. All fish specimens had terminal mouth typical for both peled and Baikal omul. The habitus of the fish was more resemblant of peled than of omul or least cisco (Fig. 1). The number of furcated anal fin rays, that can be used to discriminate peled from Baikal omul [Berg, 1948], varied from 12 to 15. Therefore, for this trait the fish was also identified rather as *C. peled* than *C. migratorius* (which has 10–12 anal fin rays). However, lack of hiatus for this trait made this diagnosis not fully conclusive.

White skeletal muscle tissues were collected from 40 fish and stored in 1.5 ml Eppendorf microtubes at −20 °C in a portable field freezer for about one week. Then the samples were transported to the lab and transferred to the stationary deep freezer (−80 °C) where the

Fig. 1. A photograph of a fish (genus *Coregonus*) introduced into Ulaagchny Khar Lake (caught in 2013)

Enzyme systems studied, buffer systems used and scorable coding loci

Enzyme	Abbreviation	E. C. number	Buffer system	Scorable loci
Creatine kinase	*CK*	2.7.3.2	B	*CK-1,2*
				CK-3
Esterase D	*EST-D*	3.1.*.*	B	*EST-D*
Glucose-6-phosphate isomerase	*GPI-A*	5.3.1.9	B	*GPI-A2*
	GPI-B			*GPI-B1,2*
Glycerol-3-phosphate dehydrogenase	*G3PDH*	1.1.1.8	A1, A2	*G3PDH-1*
Isocitric dehydrogenase	*IDH*	1.1.1.42	A2	*IDH-1,2*
Lactate dehydrogenase	*LDH*	1.1.1.27	B	*LDH-A1,2*
				LDH-B1,2
Malate dehydrogenase	*sMDH-A*	1.1.1.37	A1	*sMDH-A1,2*
	sMDH-B			sMDH-B1,2
	mMDH			*mMDH*
Malate dehydrogenase ($NADP^+$) (malic enzyme)	*mME*	1.1.1.40	A1	*mMEP-1,2*
	sME			*sMEP-3,4*
6-Phosphogluconic dehydrogenase	*6-PGD*	1.1.1.44	A1	*6-PGD*
Phosphoglucomutase	*PGM*	5.4.2.2	B	*PGM-1*
				PGM-2
Superoxide dismutase	*mSOD*	1.15.1.1	B	*mSOD*
	sSOD			*sSOD*

N o t e. Buffer systems: A1 – Morpholine – citrate, pH 6.3, A2 – Morpholine – citrate, pH 8.0 [Clayton, Tretiak, 1971]; B – Tris – citrate, pH 8.5 (gel buffer) / LiOH – borate, pH 8.1 (electrode buffer).

tubes were kept until electrophoretic analysis. Duplicates of these specimens were fixed in 90 % ethanol. Aqueous extracts for electrophoresis were prepared by mechanical grinding of 500 mg of muscle tissue in a PIPES buffer [Aebersold et al. 1987]. After overnight extraction at +4 °C the extracts were centrifuged at 15 000 rpm, loaded onto horizontal gel blocks made of partly hydrolyzed 12.5 % starch and run at 180 V for about 4.5 h. The list of enzyme systems, e. c. codes, abbreviations, buffer systems used for electrophoretic separation of protein variants and loci scored are presented in Table. Loci nomenclature followed J. B. Shaklee et al. [1989]. Allelic variants, by-locus genotypes of the studied specimens were compared with the data available in our database on coregonid fish allozyme genotypes and published data [Bodaly et al., 1991, 1994; Sendek, 2002; Politov et al., 2000, 2002, 2007].

DNA sequences of the fragment of cytochrome *b* (*cyt b*) mitochondrial gene were analyzed. Total DNA was isolated from ethanol fixed skeletal muscle tissues using reagent kit Diatom™DNAPrep100 (Laboratories Isogen, Moscow). The c*yt b* fragment was amplified in polymerase chain reaction (PCR) using oligonucleotide primers *cytbF*: 5′-CATAATTCCTGCCCGGACTCTAACC-3′ and *cytbR*: 5′-TTTAACCTCCGATCTCCGGATTAC A-3′ [Crête-Lafrenière et al., 2012]. PCR cocktails contained 2,5 μl of 10 × PCR buffer (“Fermentas”, Lithuania: 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 25 mM $MgCl_2$, 0.8 % Nonidet P40); 200 μM of each of the four dNTP; 3.2 pmol of each of the primers; 0.9 U of *Taq*-polymerase (“Bionem”, Moscow) and 100 ng of DNA template in the final volume of 25 μl under mineral oil. The PCR thermal profiles included the following steps: initial denaturing of DNA: +95 °C for 5 min – 1 cycle; 30 cycles of synthesis: +95 °C for 1 min, +58 °C for 1 min, +72 °C for 1 min 30 s; and final elongation: +72 °C for 5 min.

The obtained 1141 bp long PCR products were sequenced in both directions using the above-mentioned primers on Evrogen Ru facilities. Sequences have been aligned in BioEdit v. 5.0.9.1 [Hall, 2001]. Calculation of variability parameters and genetic distances as well as Neighbor-Joining tree construction with 1000

bootstrap replications was made using MEGA 4.0 [Tamura et al., 2007]. NJ-tree was constructed using fragments of *cyt b*, 847 bp long, from *Coregonus peled* JX960789 (Ob River, Russia), least cisco (*C. Sardinella*) JX960792 (Shingle Point, Canada), Arctic cisco (*C. autumnalis*) JX960774 (Pechora River, Russia) and Baikal omul (*C. migratorius*) JX960784 (Lake Baikal, Russia) as reference. Mongolian grayling (*Thymallus brevirostris*) JX960865 from GenBank (NCBI) was treated as an outgroup. A novel haplotype of peled was deposited in GenBank as KJ652458.

RESULTS AND DISCUSSION

Allozyme data. In total, products of 21 loci (see Table) were resolved electrophoretically. Fish genotypes were scored according to variation in relative mobility of corresponding allozymes that reflects mutations in enzyme-coding loci.

CK. At *CK-1,2** isoloci of all analyzed fish carried allele **A*(100) that is characteristic of most *Coregonus* species except for Arctic cisco and Baikal omul (allele **C* or **85*). There was zero activity in the minor zone *Ck-3* (*Ck'*) except for one fish which had phenotype 44 typical for Siberian whitefish and some populations of *C. albula – C. sardinella* complex.

G3PDH. At *G3PDH-1** all studied fish had genotype 33, while genotypes 13 and *35* that are occasionally observed in Baikal omul were not detected.

IDH, LDH and *MDH*. Alleles common for all *Coregonus* species were observed at pairs of duplicated loci *IDHP-1,2**, *LDH-1,2** and *sMDH-A1,2**, so these loci had no diagnostic power in this case. At isoloci *sMDH-B1,2** allele **4* was predominant as in both peled and Baikal omul, however rarity of allele **5* and complete absence of allele **2* also indicated absence of influence of Baikal omul gene pool.

MEP. The malic-enzyme *mMEP** (*MEP-1,2**), predominantly expressed in skeletal muscle, had similar polymorphisms at these isoloci in peled and Baikal omulб so it could not be used for identification. At predominantly liver-expressed loci *sMEP** (*MEP-3,4**) we found polymorphism for three alleles – **2, *6* and **8* – characteristic for peled, while genotype 5588, common for Baikal omul, was not found.

PGM. Allele *PGM-1*7* was predominant in the studied sample with rare (0.025) occurrence of **3* allele which is typical profile for peled, while in Baikal omul *PGM-1** is fixed for **3* allele. All the studied fish were homozygous for *PGM-2*5* which is fixed in peled and common in many *Coregonus* species, while Baikal omul lacks this allele having *PGM-2*1* and *PGM-2*3* at polymorphic frequencies.

SOD. Almost all the fish possessed allele *sSOD-A** coding for slow electrophoretic variant otherwise observed as most common vari-

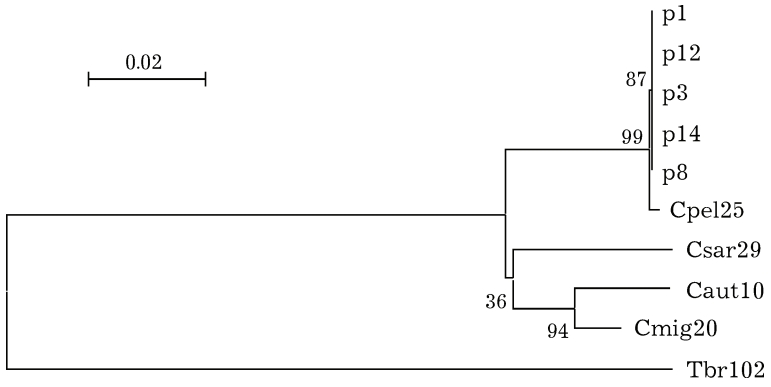

Fig. 2. Neighbor Joining tree reflecting relationships of *cyt b* haplotype found in peled from Ulaagchny Khar Lake with other *Coregonus species*: Cpel25 – peled (*C. peled*, JX960789), Csar29 – least cisco (*C. sardinella*, JX960792), Caut10 – Arctic cisco (*C. autumnalis*, JX960774), Cmig20 – Baikal omul (*C. migratorius*, JX960784), Tbr102 – Mongolian grayling (*Th. Brevirostris*, JX960865). Numbers representing bootstrap values (out of 100 replications) for support of clades are placed at the nodes. Scale at the bottom represents a unit for tree length measurement

ant in peled. The same allele is present as most frequent in Ussuri cisco, chadary whitefish and some populations of vendace but is relatively rare in most populations of least cisco, Broad whitefish, muksun and is not found in Baikal omul, Arctic cisco and Siberian whitefish. Two fish were heterozygous for *AB* at *sSOD** and this frequency of *B* allele is also characteristic for most peled populations.

Differences in allele composition among major phylogenetic lineages within genus *Coregonus* were strongly pronounced [Bodaly et al., 1991, 1994; Politov et al., 2000, 2002, 2007; Sendek, 2002] that allows reliable identification of hybrids among representatives of these clades [Reist et al., 1992] including peled hybrids [Luczynski et al., 1992]. Thus, according to analysis of allozyme variability in Ulaagchny Khar Lake, presence of alleles typical for Baikal omul was not detected.

MtDNA data. Among the aligned sequences of mtDNA *cyt b* fragments, 847 bp long, a single variant was found in all five analyzed specimens from Ulaagchny Khar (haplotype KJ652458). Comparison with other species introduced into the lake showed close similarity with peled with high bootstrap support (99 %) but not with Baikal omul and least cisco (Fig. 2). The only found haplotype differed from the haplotype deposited in GenBank (NCBI) – *Coregonus peled* from Ob' River basin (JX960789) – by absence of two transitions.

MtDNA variation was successfully employed earlier for identification of *Coregonus* species and hybrids, including peled [Politov et al., 2002, 2007].

CONCLUSIONS

Having analyzed two independent genetic markers, nuclear allozyme loci and mitochondrial DNA *cyt b* gene sequences we demonstrated that all the individuals in the population belonged to the same species – the peled, *Coregonus peled* (Gmelin). This does not disprove the existence of other species or interspecific hybrids in Ulaagchny Khar Lakethat can be found by more extensive screening. However, we can conclude that representatives of coregonid species other than peled are absent among the studied samples that were identified as pure *C. peled*. Taking into account mass release of both species in 1980s it is evident that naturalization of peled was much more successful than that of Baikal omul. Shift in values of such characters as numbers of gill rakers, scales in lateral line and vertebrae described earlier in *Coregonus* sp. Inhabiting Ulaagchny Khar Lakeand interpreted as a result of introgression with Baikal omul [Dulmaa et al., 1998] can be caused by adaptation of peled to a new environment rather than by hybridization.

This study was in part supported by Programs of Fundamental research of the Russian Academy of Sciences: "Biodiversity of Living Nature" (subprogram "Gene Pools of Living Nature and Their Conservation") and "Evolution of Organic World and Planetary Processes" (subprogram II).

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