GENETIC STUDIES PROBLEMS OF EUROPEAN GRAYLING (*THYMALLUS THYMALLUS*) OF RUSSIAN EUROPEAN NORTH

EKATERINA V. PONOMAREVA[1], ALEKSANDR A. VOLKOV[2],
MARIIA V. PONOMAREVA[1], ELENA A. SHUBINA[3]
[1]*Lomonosov Moscow State University, Faculty of Biology, Department of Ichthyology, 119234, Moscow, Russia*
[2]*Laboratory of Molecular Genetics, Russian Federal Research Institute of Fisheries and Oceanography, 107140, Moscow, Russia*
[3]*Belozersky Institute of Physico-Chemical Biology (MSU), 119234, Moscow, Russia*

GENETSKA STUDIJA LIPLJANA (*THYMALLUS THYMALLUS*) NA SEVERU EVROPSKOG DELA RUSIJE

Apstrakt

U ovom radu je izložen komercijalni i akvakulturni značaj porodice lipljana (*Thymallidae*) na teritoriji Rusije. Posebna pažnja je poklonjena genetičkim studijama ove familije, uzimajući u obzir da populacije evropskog lipljana u Rusiji još uvek nisu u dovoljnoj meri istražene. Rad zapravo predstavlja polaznu genetičku studiju evropskog lipljana u svrhu konzervacije ove vrste. Studija uključuje prikupljene uzorke od 5 jedinki evropskog lipljana (*T. thymallus*) iz slivova reka Severna Dvina i Kuloj, 2 sibirska lipljana (*T. arcticus*) iz sliva reke Ob i jednog uzorka lipljana iz gornjeg toka reke Jenisej. Analiza MtDNA je urađena koristeći 3 fragmenta gena ATP6, CR i COI. Po prvi put su dobijeni genetički podaci riba iz reke Kuloj. Od uzoraka svih riba, jedino su identifikovani haplotipovi evropskog lipljana.

Ključne reči: Thymallus thymallus, CR, ATP6, COI, mtDNA haplotipovi
Keywords: Thymallus thymallus, CR, ATP6, COI, mtDNA haplotypes

INTRODUCTION

On the territory of Russia *Thymallidae* are widespread almost in all water basins of Siberia, Far East and European North. But in Central and Southern Russia, European grayling (*T. thymallus*) inhabits the Volga and the Ural Rivers' basins and some populations are in-

cluded in the regional Red Books. Scientific concerns are connected both with commercial use and protection of the grayling. Detailed genetics aspects of European grayling conservations are formulated in Gum *et al.* (2009). Morphologic and systematics analyses of graylings were conducted in Russia for a long time (Svetovidov, 1936; Tugarina & Khramtzova, 1980; Zinoviev, 1980; Knizhin, *et al*. 2004, etc.). At the same time, systematics of genus *Thymallus* was constantly being reconsidered and revised. Grayling populations from Asian part of Russia (east of the Ural Mountains) are regarded as the most important ones for theoretical studies due to diversity of their forms.

The commercial use of grayling in Russia has many aspects. Grayling is highly appreciated for its taste qualities, and conserved by salting, smoking or drying. Grayling is one of favorite objects of recreational angling, its presence being important factor of regional tourism strategies. Grayling is perspective fish for water bodies stocking; the hatcheries exist in Sayan, Baikal, Siberia and Ladoga. Also, grayling is frequently reared on trout farms. At the moment, principles and techniques of grayling farm rearing is sufficiently developed. But the real practice of grayling used as an aquaculture object and fishery object still needs more attention in Russia.

The ichthyological studies (as well as fisheries) of European North of Russia have traditionally concentrated on the most valuable commercial species, namely Atlantic salmon (*Salmo salar* L.) and Brown trout (*S. trutta* L.), while European grayling populations of large territories stay underexplored, though in the Yemtsa, a tributary of the Northern Dvina, were caught up to 2 tons of grayling. While grayling populations of Asian part of Russia were extensively studied, both morphologically and genetically (Knizhin, *et al*., 2004; Froufe *et al*., 2005; Weiss *et al*., 2006), genetic studies of European grayling of Russia are still based on small quantity of samples (Koskinen *et al*., 2000; 2002). Only one sample from the territory of Archangelsk region (the Sjamzhenga River, eastern tributary of the Northern Dvina) was studied, mtDNA haplotypes characteristic for Siberian grayling (*T. arcticus*) were found at two specimens (Koskinen *et al*., 2000). It was suggested that hybridization zone of European and Siberian grayling is situated more to the west than was being supposed earlier. But the question of hybridization zone border is still not resolved. The objective of our study involves Koskinen *et al.* (2000) statement: „Molecular investigation, including assessment of nuclear loci, for a dense sample from the Archangelsk region and east of the Urals needs to be conducted to conclusively resolve this issue".

MATERIALS AND METHODS

Samples were collected according to our study objectives (Table 1). ATP6 gene and control region (CR, D-loop) of mtDNA were chosen because of using in phylogenetic *Thymallidae* studies (Froufe *et al*., 2005; Weiss *et al*., 2006; Marić *et al*., 2011) and mtDNA COI, as more conservative fragments which used for DNA barcode project (Ivanova *et al*., 2007).

Sample from the Toimushka River (the Northern Dvina basin) was taken from archived scales, containing degraded DNA less than 700 bp. As a result of short DNA fragments, PCR products were either weak or undetected. Hence internal primers were designed for amplification. PCR conditions are being optimized to gain stable amplification products.

Table 1.

Samples	Basin	N	Year	Tissue
Toimushka river	Northern Dvina	24	1999	archived scales
Ustya river	Northern Dvina	1	2014	fin (ethanol)
Oboksha river	Northern Dvina	2	2015	fin (ethanol)
Kelda river	Kuloy river	10	2104	fin (ethanol)
Laka river	Kuloy river	14	2015	fin (ethanol)
Bolshoe Shchuchye Lake	lower Ob` river	10	2007	liver (ethanol)
Bolshoe Hadata Lake	lower Ob` river	16	2007	liver (ethanol)
Maimamysh river	upper Enisey river	3	2013	fin (ethanol)

RESULTS AND DISCUSSION

The preliminary results of CR and ATP6 sequences for tributaries of the Northern Dvina and the Kuloy rivers were obtained. Only one haplotype was found within CR sequences and three haplotypes were found within ATP6 gene. A cladogram is constructed, based on obtained and GeneBank sequences. Our samples were displayed in red color (Fig. 1 and 2).

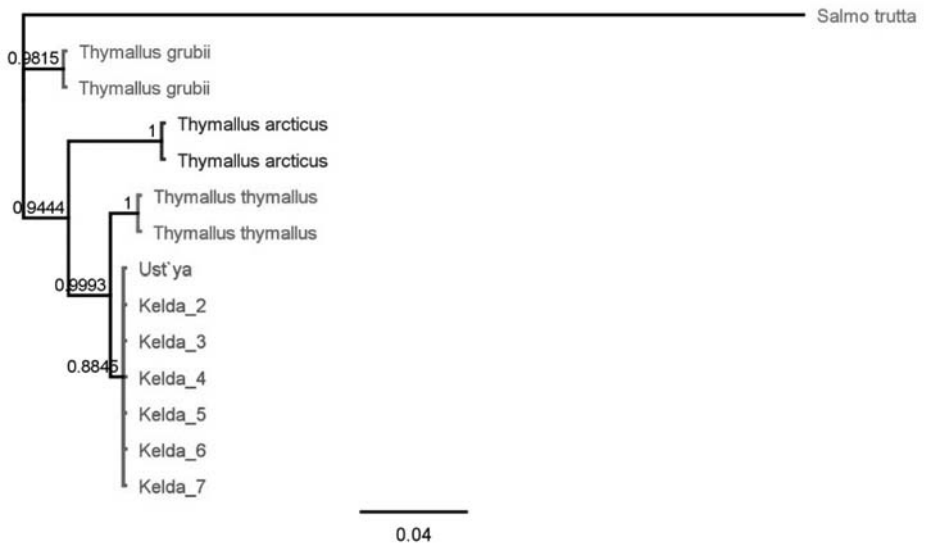

Figure 1. Consensus tree of mtDNA control region (CR) haplotypes for *Thymallus sp.* based on algorithm MrBayes 3.2.1., HKY model. The tree is rooted with haplotype from European brown trout (*S. trutta*). Haplotypes of our specimens are in red color.

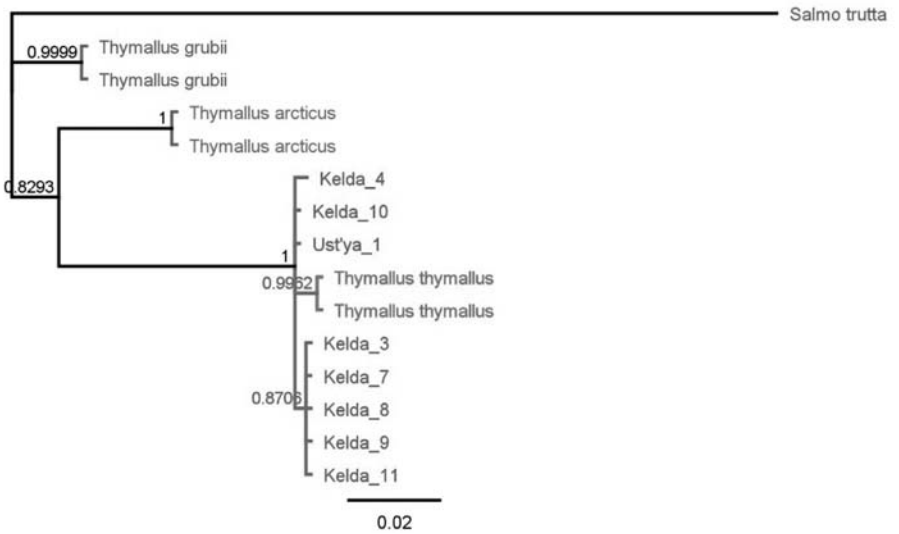

Figure 2. Consensus tree of mtDNA ATP6 gene haplotypes for *Thymallus sp.* based on algorithm MrBayes 3.2.1., HKY+G model. The tree is rooted with haplotype from European brown trout (*S. trutta*). Haplotypes of our specimens are in red color.

Genetic data from the grayling population living in the Kuloy River were obtained for the first time. Though it could seem more probable to find Siberian grayling haplotype in rivers which, like Kuloy, are situated closer to the Urals Mountains, haplotypes close to Siberian grayling were not found in studied specimens. To obtain further genetic information, microsatellite analysis is planned.

ACKNOWLEDGMENTS

We express our thanks to Yu.G. Vedernikov for help in collecting samples and to N.V. Gordeeva for granted samples.

REFERENCES

Froufe, E., Knizhin, I., Weiss, S. (2005): Phylogenetic analysis of the genus *Thymallus* (grayling) based on mtDNA control region and ATPase 6 genes, with inferences on control region constraints and broad-scale Eurasian phylogeography. Molecular Phylogenetics and Evolution, 34: 106–117.

Gum, B., Gross, R., Geist, J. (2009): Conservation genetics and management implications for European grayling, *Thymallus thymallus*: synthesis of phylogeography and population genetics. Fisheries Management and Ecology, 16: 37–51.

Ivanova N., Zemlyak T., Hanner R., Hebert P. (2007): Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes, 7: 544–548.

Knizhin, I., Weiss, S., Antonov, A.L., Froufe, E., (2004): Morphological and genetic diversity of Amur graylings (*Thymallus; Thymallidae*). Journal of Ichthyology, 44: 59–76.

Koskinen, M.T., Nilsson, J., Veselov, A.J., Potutkin, A.G., Ranta, E., Primmer C.R. (2002): Microsatellite data resolve phylogeographic patterns in European grayling, *Thymallus thymallus*, Salmonidae. Heredity, 88: 391–401.

Koskinen, M.T., Ranta, E., Piironen, J., Veselov, A., Titov, S., Haugen, T.O., Nilsson, J., Carlstein, M., Primmer, C.R. (2000): Genetic lineages and postglacial colonization of grayling (*Thymallus thymallus, Salmonidae*) in Europe, as revealed by mitochondrial DNA analyses. Molecular Ecology, 9: 1609–1624.

Svetovidov, A.N. (1936): Graylings (genus *Thymallus*, Cuvier) of Europe and Asia. Proceedings of Institute of Zoology Academy of Sciences USSR, III, 183–301 (in Russian).

Tugarina, P.Y., Khramtzova, V.S. (1980): Morphizioloogicheskaya kharakteristka amurskogo khariusa *Thymalllus grubei* Dyb. Jorunal of Ichthyology, 20: 590–605 (in Russian).

Weiss, S., Knizhin, I., Kirillov, A., Froufe, E. (2006): Phenotypic and genetic differentiation of two major phylogeographical lineages of arctic grayling *Thymallus arcticus* in the Lena River, and surrounding Arctic drainages. Biological Journal of the Linnean Society, 88: 511–525.

Zinoviev, E.A. (1980): Parallelizm izmenchivosti u evropeiskogo i sibirskogo khariusov. Lososevidniye rybi. ZIN AN SSSR, Leningrad, 69–80 (in Russian).