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МАТЕРИАЛЫ КОНФЕРЕНЦИИ

ГЕНЕТИКА

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VARIATION IN THE *COI* GENE OF THE FRESHWATER PEARL MUSSEL *Margaritifera margaritifera* FROM RIVER VUOKKIJOKI

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The freshwater pearl mussel *Margaritifera margaritifera* L. is one of the most endangered freshwater mussels in the world. Effective conservation of threatened species requires not only ecological, but also genetic information from the target species and populations. Since low genetic diversity can reduce the ability of a species to adapt to environmental changes, maintaining genetic diversity has been identified as one of the key elements in successful conservation programs. We examined genetic variation of the freshwater pearl mussel from the river Vuokkijoki, Karelia, Russia. We sequenced a fragment of the cytochrome c oxidase subunit I gene (*COI*) from 22 individuals and compared the data to 32 previously published *COI* sequences available in GenBank. We identified 10 different *COI* haplotypes in the sequenced samples, three of which had not been previously reported. Our results show that the river Vuokkijoki has high genetic diversity and suggest that the colonization of this northern freshwater pearl mussel population might have occurred from multiple

and even distant refugia. Therefore, the freshwater pearl mussel population of the river Vuokkijoki is valuable for the conservation of the whole species.

The freshwater pearl mussel *Margaritifera margaritifera* was a common species in European rivers in the middle of the last century, but now it is critically endangered (e.g., Beasley *et al.*, 1998; Cosgrove *et al.*, 2000; Lopes-Lima *et al.*, 2016). In particular, Central and Southern European freshwater pearl mussel populations are estimated to have declined by 95–100% (Bauer, 1986; 1988). In Northwest Russia, pearl mussel habitats have also declined (Ieshko *et al.*, 2009; Makhrov *et al.*, 2011, 2014). At present, viable pearl mussel populations have survived only in 18 rivers of the Karelia, Arkhangelsk and Murmansk Regions. Because of its decline, the freshwater pearl mussel is included in the EU Habitats Directive Annex II as one of the protected species, and it is listed as an endangered species by the IUCN (International..., 2014).

The freshwater pearl mussel spends the first months of its life as a parasitic glochidium larva on the gills of a suitable host fish, either trout *Salmo trutta* or Atlantic salmon *S. salar* (e.g., Ieshko *et al.*, 2016; Salonen *et al.*, 2016). Larvae grow in the gills of the host fish to the next spring, after which they excyst and fall to the bottom of the river. If the condition of the river bottom is suitable for the young mussels, they will develop to adults. Because of its complex life cycle, the freshwater pearl mussel is regarded as a super indicator species for undisturbed, clean and pristine headwaters and small tributaries (Geist, 2010).

Currently, many freshwater pearl mussel populations do not produce new recruits (Geist, 2010). Due to the extremely long life span of the species (up to 150–200 years (Helema, Valovirta, 2008), the species may seem misleadingly widely distributed, but many of the populations are overaged, and it is a rare species throughout its distribution range. The mussel can be found on both sides of the Atlantic. In North America it is found in the northeast coast of the United States and Canada (Jungbluth *et al.*, 1985; Zyuganov *et al.*, 1994). In Europe, it

occurs in North-West European rivers that flow into the North Atlantic, the Arctic Ocean and the White Sea. In southern Europe, the distribution of the freshwater pearl mussel is limited to the Alps and the Iberian Peninsula (Zyuganov *et al.*, 1994; Araujo, Ramos, 2000).

Effective conservation approaches for endangered species such as the freshwater pearl mussel require the integration of ecological and genetic information (Geist, 2010). Maintaining genetic diversity has been identified as one of the key elements in successful conservation programmes (McNeely *et al.*, 1990; Frankham *et al.*, 2002). The populations with high genetic diversity are expected to be important for conservation, because these populations have greater potential to adapt to changes in the environment. Therefore, targeting resources to conserve populations with high genetic diversity might be more effective than the protection of multiple populations each with lower genetic diversities.

Previously, Machordom *et al.* (2003) used allozyme and mitochondrial DNA markers to describe genetic variation and genetic structure among freshwater pearl mussel populations in Europe. From 46 individuals, they found only two16S rRNA haplotypes and 10 *COI* haplotypes, which were resolved into two different mitochondrial lineages: a northern lineage, which stretches from Ireland to the Kola Peninsula, and a southern lineage, which stretches from Ireland to the Iberian Peninsula. Other studies using microsatellite markers (Geist *et al.*, 2003, 2010; Geist, Kuehn, 2005, 2008; Bouza *et al.*, 2007) have shown higher diversity in the northeastern populations than in the southern and central European populations. Microsatellite study by Karlsson *et al.* (2014) revealed host-dependent genetic variation between *M. margaritifera* originating from salmon and trout rivers in Norway. The purpose of this study was to describe the genetic variation of the freshwater pearl mussel (*M. margaritifera*) in river Vuokkijoki, Karelia, Russia, based on *COI* sequences, and compare these sequences to those found from other populations published in the NCBI GenBank nucleotide database (Nucleotide..., 2016).

MATERIAL AND METHODS

Study area/Sampling. Samples were collected in autumn 2015 from the river Vuokkijoki (Vuokinjoki), Karelia, Russia (fig. 1). The river is 9.2 km long, empties into Lake Kuito, and the entire water/river system flows into the White Sea (Ieshko *et al.*, 2016). The fish host of *M. margaritifera* in the river Vuokkijoki is Atlantic salmon (Ieshko *et al.*, 2016). In total, 30 mussels were collected. For a source of DNA, we took small tissue pieces, circa 5 mm in diameter, from the mantle tissue using a non-destructive mantle biopsy method (Berg *et al.*, 2005). After sampling, the mussels were returned alive back to the river. Tissues were preserved in 1.5 ml Eppendorf tubes with absolute ethanol. The pearl mussel analysis was carried out with The Federal Supervisory Natural Resources Management Service, the State Nature Inspectorate Permit № 57 of June 30, 2015.

DNA. Total DNA was extracted from mantle tissue using the NucleoSpin Tissue-Kit (Macherey-Nagel), following the manufacturer's instructions for preparation of tissue material. Isolated total DNA was used in polymerase chain reactions (PCR) to amplify the mitochondrial gene cytochrome c oxidase subunit I (*COI*) using the following primers: 5'-GGTCAACAAATCATAAAGATATTGG-3' (Folmer *et al.*, 1994) and 5'-TCAGGGTGACCAAAAAATCA-3' (Machordom *et al.*, 2003). The reactions had a total volume of 20.0 μL and contained the following components: 50 ng of genomic DNA, 1.0 μM of each primer, 0.2 mM of each dNTP, 2.0 mM $MgCl_2$, 1 × PCR buffer ($(NH_4)_2SO_4$, (Fermentas, USA), and 0.4 U Taq DNA Polymerase (Fermentas, USA). PCR was carried out using a S1000 Thermal Cycler (Bio-Rad, USA) with the following conditions: 94°C (3 min), 34 cycles of denaturation at 94°C (30 s), annealing at 50°C (1 min), extension at 72°C (1 min) and a final extension at 72°C (7 min). Agarose gel electrophoresis was used to verify that the PCR amplifications were successful. Before sequencing, the amplified *COI* fragments were purified using the Exo-SAP method. We used the ABI PRISM BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) following the

manufacturer's instructions in sequencing. Purified sequencing products were separated using the 3730 DNA Analyzer (Applied Biosystems, USA). The raw *COI* sequence data were edited and aligned with Sequencing Analysis Software 6 (Applied Biosystems, USA), MEGA 6.06 software (Tamura *et al.*, 2013) and ClustalW (Thompson *et al.*, 1994) programs.

Statistical analyses. The nucleotide database at NCBI (National Center for Biotechnology Information), GenBank, was utilized to search for previously published *COI* sequences obtained from *M. margaritifera*. We obtained 9 sequences originating from samples from Russia, 8 sequences originating from samples from Ireland and 15 sequences originating from samples from Spain (Table 1). *COI* sequences from *Margaritifera auricularia* and *Potomida littoralis* (GenBank accession numbers: AF303315 and AF303348) were also obtained and used as outgroups in our phylogenetic reconstruction (see below). The sequences downloaded from GenBank were aligned with the sequences we obtained from the river Vuokkijoki samples using MEGA 6.06 and trimmed to 657 nucleotides.

The best suitable evolutionary model indicated by analysis in MEGA 6.06 was the Hasegawa–Kishino–Yano model (HKY85 model; Hasegawa *et al.*, 1985). This model was used to calculate genetic distances between haplotypes. DnaSP v5 software package (Librado, Rozas, 2009) was used to calculate the number of haplotypes, haplotype frequencies and haplotype diversities. The genetic distance matrix based on the HKY85 model was used to construct a Maximum Likelihood dendrogram, and the Bootstrap method was used to test the phylogeny with 1000 replicates in MEGA 6.06 software.

RESULTS

Although we collected 30 mantle tissue samples, only 22 high quality *COI* sequences were obtained. Lengths of the edited *COI* sequences were 658 base pairs, and there were 11 variable nucleotide positions and 10 haplotypes (table

2). The reading frame started from the second nucleotide position and there were no stop codons in the sequence. When the previously published *COI* sequences were added to the alignment, a non-synonymous nucleotide substitution (transition) at position 482 was noted in haplotype HT11, which changed the amino acid alanine to isoleucine.

Haplotype frequencies, richness and diversity indexes are presented in Table 3. For the whole dataset, haplotype frequencies ranged from one to 35. The most frequent haplotypes were HT8 with 35 individuals and HT5 with 14 individuals. HT8 was the most common haplotype in Spain and Ireland, while haplotype HT5 was common in the river Vuokkijoki, other Russian populations and in Ireland. The number of haplotypes, i.e. haplotype richness, varied in Russia from 10 in the river Vuokkijoki to two in the rivers Thurma and Pana. From Ireland there were two, and from Spain there were three different haplotypes recorded in GenBank. Three of the haplotypes we sequenced had not been published previously (haplotypes 1, 9 and 10). The haplotype diversity index was 0.83 in the river Vuokkijoki. When we combined the sequences obtained in this study and the data from GenBank, country specific haplotype diversity indices are 0.85 for Russia, 0.45 for Spain and 0.43 for Ireland (Table 3).

The Maximum Likelihood dendrogram is presented in fig. 2. Freshwater pearl mussel haplotypes formed their own group with 100% bootstrap support. Russian haplotypes were clearly separated from Spanish haplotypes, except for HT8, the most common Spanish haplotype, which was found also in the river Vuokkijoki and the river Nore (Ireland). Another haplotype from Ireland, HT5, was the most common haplotype in the river Vuokkijoki, and it was clearly grouped with other Russian haplotypes.

DISCUSSION

In contrast to a previous study of genetic diversity in the *COI* gene of *M margaritifera* (Machordom *et al.*, 2003), we found considerable genetic variation in haplotype richness (number of haplotypes) in a sample from the river Vuokkijoki. We analyzed only 22 specimens from a single Russian river in contrast to Machordom *et al.* (2003) who surveyed 46 specimens from multiple locations. Moreover, even though the sample was small, we found 10 *COI* haplotypes, three of which were unpublished and possibly unique to this population. Our analysis of a sample from the river Vuokkijoki is in concordance with studies of microsatellite markers that have indicated higher genetic diversity of *M. margaritifera* populations in the Northeast, than in populations from southern and central Europe (Geist *et al.*, 2003, 2010; Geist, Kuehn, 2005, 2008; Bouza *et al.*, 2007). For example, allelic richness of microsatellites, which is comparable to the measure of haplotype richness, was less than 2.0 in all Central European populations (Geist, Kuehn, 2005) and had an average of 2.1 in Iberian populations, (Bouza *et al.*, 2007). Almost all Swedish populations had allelic richness of 2.7 or higher (2.7 to 3.6), and in populations from Finnish Lapland, allelic richness ranged between 4.0 and 4.7 (Geist, Kuehn, 2008; Geist *et al.*, 2010). The genetic diversity of the freshwater pearl mussel, indicated both by microsatellites and mtDNA sequences, seems to increase towards the northernmost populations in its range of distribution. This could be explained by multiple postglacial colonization routes of the freshwater pearl mussel to their current northeastern populations, but also could be explained by the rapid destruction and decline of the southern and central European populations.

In their study, Machordom *et al.* (2003) identified two different mitochondrial lineages, a northern (river Dereen) and a southern (river Nore) lineage. According to their data, the northern lineage extends northwards from Ireland to the Kola Peninsula and the southern lineage extends from Ireland to the south. Our results do not support a strict geographical division between the

lineages, since the southern haplotype (from the river Nore) was also found in the river Vuokkijoki.

CONCLUSIONS

Compared to other freshwater pearl mussel populations in Europe, the population in the river Vuokkijoki has high genetic diversity based on *COI* gene sequences. The freshwater pearl mussel population of the river Vuokkijoki is, therefore, very valuable for the conservation of the whole species. For example, the river Vuokkijoki population could serve as a source population for future conservation actions, which might include reintroductions of mussels into rivers where they have gone extinct, or for strengthening current populations of low diversity prone to inbreeding depression. Both mitochondrial lineages, which were previously thought to extend only from Ireland to the north and from Ireland to south, were also present, indicating that the river Vuokkijoki may have been re-colonized from multiple refugia.

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REFERENCES

Araujo R., Ramos M.A. Action plan for *Margaritifera margaritifera* in Europe // Council of Europe. Strasbourg: Council of Europe Pub, 2000. T-PVS 10. 38 pp.

Bauer G. Reproductive strategy of the freshwater pearl mussel *Margaritifera margaritifera*// J. Animal Ecol. 1987. V. 56. P. 691–704.

Bauer G. Threats to the freshwater pearl mussel in Central Europe // Biol.Conserv. 1988. V. 45.P. 239–253.

Beasley C.R., Roberts D., Mackie T.G. Does the freshwater pearl mussel, *Margaritifera margaritifera* L., face extinction in Northern Ireland? // Aquat. Conserv.: Mar. Freshwater Ecosyst. 1998. V. 8. P. 265–272.

*Berg D.J., Haag W.R., Guttman S.I., Sickel J.B.*Mantle biopsy: a technique for non-destructive tissue-sampling of freshwater mussels //J. North Amer.Benthologic. Soc. 1995.V. 14.P. 577–581.

Bouza C., Castro J., Martines P., Amaro R., Fernandes C., Ondina P., Outeiro A., San Miguel E. Threatened freshwater pearl mussel *Margaritifera margaritifera* L. in NW Spain: low and very structured genetic variation in southern peripheral populations assessed using microsatellite markers // Conserv. Genet. 2007. V. 8. P. 937–948.

Cosgrove P.J., Young M.R., Hastie L.C., Gaywood M., BoonP.J. The status of the freshwater pearl mussel *Margaritifera margaritifera* Linn. in Scotland // AquaticConservation: Mar. Freshwater Ecosyst. 2000. V. 10. P. 197–208.

FolmerO., Black M., Hoeh W., Lutz R., Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates // Mol. Mar. Biol. Biotechn. 1994. V. 3. P. 294–299.

Frankham R., Ballou J.D., Briscoe D.A. Introduction to conservation genetics. Cambridge: Cambr. Univ. Press, 2002. 617 p.

Geist J. Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera* L.): a synthesis of conservation genetics and ecology // Hydrobiologia. 2010. V. 644. P. 69–88.

*Geist J., Kuehn R.*Genetic diversity and differentiation of central European freshwater pearl mussel (*Margaritifera margaritifera* L.) Populations: implications for conservation and management // Mol. Ecol. 2005. V. 14. P. 425–439.

Geist J., Kuehn R. Host-parasite interactions in oligotrophic stream ecosystem: the roles of life-history strategy and ecological niche // *Mol. Ecol.*, 2008, V. 17. P. 997–1008.

Geist J., Rottmann O., Schröder W., Kühn R. Development of microsatellite markers for the endangered freshwater pearl mussel *Margaritifera margaritifera* L. (Bivalvia: Unionoidea) // Mol. Ecol. Notes. 2003. V. 3. P. 444–446.

*Geist J., Söderberg H., Karlberg A.,Kuehn R.*Drainage-independent genetic structure and high genetic diversity of endangered freshwater pearl mussels (*Margaritifera margaritifera*) in northern Europe // Conserv.Genet. 2010. V. 11. P. 1339–1350.

Hasegawa M., Kishino K., Yano T. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA // J. Mol. Evol. 1985. V. 22. P. 160–174.

Helama S., Valovirta I. The oldest recorded animal in Finland: ontogenetic age and growth in *Margaritifera margaritifera* (L. 1758) based on internal shell increments // Mem. Soc. Fauna Flora Fenn. 2008. V. 84. P. 20–30.

Ieshko E.P., Geist J., Murzina S.A., Veselov A.E., Lebedeva D.I., Ziuganov V.V. The characteristics of the infection of juvenile Atlantic salmon with glochidia of the freshwater pearl mussel in rivers of Northwest Russia // Knowledge Management Aquat. Ecosyst. 2016. V. 6. P. 417.

Ieshko E.P., Larson B.M., Pavlov Y.L.,Barskaya Y.Y., Lebedeva D.I., Novohatskaya O.V. Population dynamics of glochidia of the freshwater pearl

mussel *Margaritifera margaritifera* L., parasitizing on juvenile Salmonidae fishes in northern water reservoirs // Biol. Bull. 2009. V. 36. № 6. P. 624–629.

International Union for Conservation of Nature. The IUCN Red List of Threatened Species. 2014. Version 2014.3. <www.iucnredlist.org>. Downloaded on 06 May 2015.

Jungbluth J.R., Coomans H.E.,Groh H. Bibliographie der Flussperlmuschel *Margaritifera margaritifera* (Linn. 1785) // Verslagenen Technische Gegevens Institut voor Taxonomische Zoologie: Univ. Amsderdam, 1985. P. 1–220.

Karlsson S., Larsen B.M., Hindar K. Host-dependent genetic variation in freshwater pearl mussel *(Margaritifera margaritifera* L.) // Hydrobiologia. 2014. V. 735. P. 179–190.

Librado P., Rozas J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data // Bioinformatics. 2009. V. 25. P. 1451–1452.

Lopes-Lima M., Sousa R., Geist J., Aldridge D.C., Araujo R., Bergengren J., Bespalaya Y., Bódis E., Burlakova L., Van Damme D., Douda K., Froufe E., Georgiev D., Gumpinger C., Karatayev A., Kebapçi Ü., Killeen I., Lajtner J., Larsen B.M., Lauceri R., Legakis A., Lois S., Lundberg S., Moorkens E., Motte G., Nagel K.-O., Ondina P., Outeiro A., Paunovic M., Prié V., von Proschwitz T., Riccardi N., Rudzīte M., Rudzītis M., Scheder C., Seddon M., Şereflişan H., Simić V., Sokolova S., Stoeckl K., Taskinen J., Teixeira A., Thielen F., Trichkova T., Varandas S., Vicentini H., Zajac K., Zajac T., Zogaris S. Conservation status of freshwater mussels in Europe: state of the art and future challenges // Biol. Rev. 2016. DOI: 10.1111/brv.12244.

Machordom A., Araujo R., Erpenbeck D.,Ramos M.-A. Phylogeography and conservation genetics of endangered European Margaritiferidae (Bivalvia: Unionoidea) // Biol. J. Linn. Soc. 2003. V. 78. P. 235–252.

Makhrov A.A., Ieshko E.P., Shchurov I.L., Shirokov V.A. Distribution of the freshwater pearl mussel (*Margaritifera margaritifera*) in the Republic of

Karelia (North-West Russia) // Toxicol. Environ. Chem. 2011. V. 93. № 9. P. 1731–1747.

Makhrov A., Bespalaya Ju., Bolotov I., Vikhrev I., Gofarov M., Alekseeva Y., Zotin A. Historical geography of pearl harvesting and current status of populations of freshwater pearl mussel *Margaritifera margaritifera* (L.) in the western part of Northern European Russia // Hydrobiologia. 2014. DOI 10.1007/s10750-013-1546-1.

McNeely J.A., Miller K.R., Reid W.V., Mittermeier R.A., Werner T.B. Conserving the World`s Biological Diversity, IUCN, Conserv. Int. WWF-US and the World Bank, Washington DC: World Res. Inst., 1990.

Nucleotide. The Nucleotide database is a collection of sequences from several sources, including GenBank, RefSeq, TPA and PDB. Genome, gene and transcript sequence data provide the foundation for biomedical research and discovery. 2016. <http://www.ncbi.nlm.nih.gov/nucleotide/>.

Salonen J.K., Marjomäki T.J., Taskinen J. An alien fish threatens an endangered parasitic bivalve: the relationship between brook trout (*Salvelinus fontinalis*) and freshwater pearl mussel (*Margaritifera margaritifera*) in northern Europe // Aquat. Conserv.: Mar. Freshwater Ecosyst. 2016. DOI: 10.1002/aqc.2614.

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. // Mol. Biol. Evol. 2013. V. 30. P. 2725–2729.

Thompson J.D., Higgins G.D., Gibson T.J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, composition-specific gap penalties and weight matrix choice // Nucl. Acids Res. 1994. V. 22. P. 4673–4680.

*Zjuganov V.A., Zotin A., Nezlin L., Tretiakov V.*The freshwater pearl mussels and their relationships with salmonid fish. M.: VNIRO, 1994. 104 p.

Table 1. Sequences used in this study with their haplotypes and NCBI GenBank accession numbers.

Haplotype	Population	Sample size	Accession number	Publication
HT1	Russia (Vuokkijoki)	1	KX056488	This study
HT2	Russia (Thurma)	1	AF303333	Machordom et al. 2003
	Russia (Vuokkijoki)	2	*	This study
HT3	Russia (Pana)	2	AF303335, AF303337	Machordom et al. 2003
	Russia (Vuokkijoki)	1	*	This study
HT4	Russia (Varzuga)	1	AF303339	Machordom et al. 2003
	Russia (Vuokkijoki)	5	*	This study
HT5	Ireland (Dereen)	2	AF303331, AF303332	Machordom et al. 2003
	Russia (Varzuga)	2	AF303338, AF303340	Machordom et al. 2003
	Russia (Vuokkijoki)	8	*	This study
HT6	Russia (Thurma)	1	AF303334	Machordom et al. 2003
	Russia (Vuokkijoki)	1	*	This study
HT7	Russia (Pana)	1	AF303336	Machordom et al. 2003
	Russia (Varzuga)	1	AF303341	Machordom et al. 2003

	Russia (Vuokkijoki)	1	*	This study
HT8	Ireland (Nore)	6	AF303342–AF303347	Machordom et al. 2003
	Spain (Ulla)	1	AF303316	Machordom et al. 2003
	Spain (Tambre)	2	AF303317, AF303318	Machordom et al. 2003
	Spain (Mandeo)	2	AF303319, AF303320	Machordom et al. 2003
	Spain (Tera)	3	AF303323–AF303325	Machordom et al. 2003
	Spain (Narcea)	3	AF303327–AF303329	Machordom et al. 2003
	Russia (Vuokkijoki)	1	*	This study
HT9	Russia (Vuokkijoki)	1	KX056489	This study
HT10	Russia (Vuokkijoki)	1	KX056490	This study
HT11	Spain (Landro)	2	AF303321, AF303322	Machordom et al. 2003
	Spain (Narcea)	1	AF303326	Machordom et al. 2003
HT12	Spain (Narcea)	1	AF303330	Machordom et al. 2003
Total 12		54		

* indicates samples used in this study, that are identical with previously submitted sequences in the GenBank. ** Machordom *et al.*, 2003.

Table 2. Variable nucleotide positions and their nucleotides between different COI haplotype sequences.

Haplotypes	Variable nucleotide positions (13/658)												
	7	14	34	73	82	110	244	347	370	397	473	511	571
HT1	G	T	C	G	A	T	T	T	T	T	C	A	T
HT2	G	T	C	G	A	C*	T	T	T	T	C	A	T
HT3	G	T	C	G	A	T	T	T	T	T	C	G*	T
HT4	A*	T	C	G	A	T	T	T	T	T	C	A	T
HT5	G	T	C	G	A	T	T	C*	T	T	C	A	C*
HT6	G	T	C	G	G*	T	T	T	T	T	C	A	T
HT7	G	T	T*	G	G*	T	T	T	T	T	C	A	T
HT8	G	T	T*	G	A	T	A*	T	C*	T	C	A	T
HT9	G	T	C	A*	A	T	T	C*	T	T	C	A	C*
HT10	G	T	C	G	A	T	T	C*	T	C*	C	A	C*
HT11	G	T	T*	G	A	T	A*	T	C*	T	T*	A	T
HT12	G	C*	T*	G	A	T	A*	T	C*	T	C	A	T

* Non-synonymous nucleotide substitution, which changed Alanine to Isoleucine.

Table 3. Haplotype diversity (h) and richness (H) in different *Margaritifera margaritifera* populations.

River	HT1	HT2	HT3	HT4	HT5	HT6	HT7	HT8	HT9	HT10	HT11	HT12	N	H	h (S.D)
Russia	1	3	3	6	10	2	3	1	1	1			31	10	0,85 (0,04)
Vuokkijoki	1*	2	1	5	8	1	1	1	1*	1*			22	10	0,83 (0,06)
Thurma		1				1							2	2	
Pana			2				1						3	2	
Varzuga				1	2		1						4	3	
Ireland					2			6					8	2	0,43 (0,17)
Dereen					2								2	1	
Nore								6					6	1	
Spain								11			3	1	15	3	0,45 (0,13)
Ulla								1					1	1	
Landro											2		2	1	
Mandeo								2					2	1	
Narcea								3			1	1	5	3	
Tambre								2					2	1	
Tera								3					3	1	

Total	1	3	3	6	14	2	3	35	1	1	6	2	54	10

Unique haplotypes. ** Calculation is impossible. "–" – not found; N – number of haplotypes.

Подписи к рисункам ст. Välilä *et al.*

Fig. 1. Location of the studied river Vuokkijoki.

Fig. 2. Maximum Likelihood dendrogram based on the Hasegawa–Kishino–Yano substitution model (Hasegawa, *et al.*, 1985), numbers indicate nodes with bootstrap support for 1000 replications.

Fig. 1 к ст. Välilä *et al.*

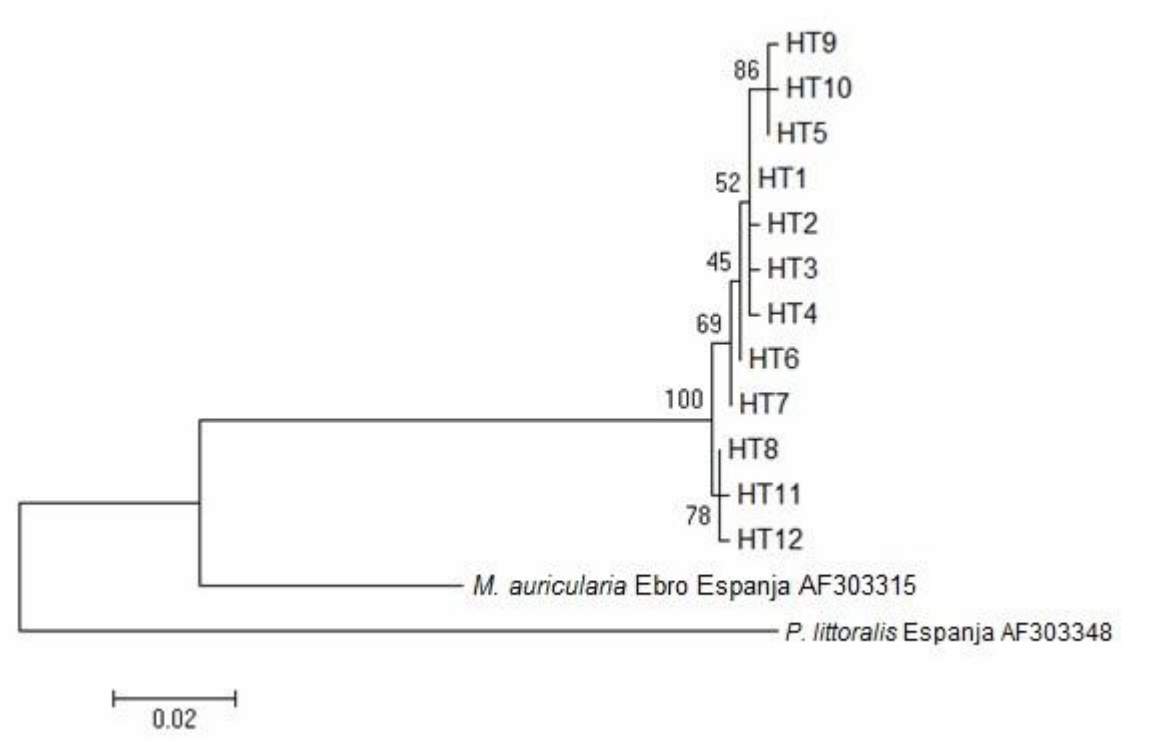

Fig. 2 к ст. Välilä *et al.*

Сер. биол. № -17

Для переписки.

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