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SHORT COMMUNICATIONS

Genetic Diversity of Charrs of the Commander Islands Based on the Analysis of Mitochondrial DNA

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Abstract—Nucleotide sequences of the mitochondrial DNA cytochrome *b* (*CytB*) gene fragment and the control region (*D-loop*) of Dolly Varden (*Salvelinus malma*) from the Commander Islands and the Kol River of the Kamchatka Peninsula were examined. A high level of genetic variability of island populations comparable to that of the mainland population of western Kamchatka was demonstrated. The belonging of the Commander Islands chars to the genetic lineage of northern Dolly Varden *Salvelinus malma malma* was confirmed.

Keywords: Salvelinus malma, mitochondrial DNA, Commander Islands, *CytB*, D-loop
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Charrs of the genus *Salvelinus* are one of the most interesting group of salmonids in terms of microevolution, characterized by complex population structure, high ecological plasticity, geographical variability, and morpho-ecological diversity. The evolutionary status of many forms of charrs is beyond the classical understanding of the biological species; their taxonomic position is a matter of long scientific discussion [1–3]. The extraordinary morphological diversity and complex interpopulation relationships of charrs led to the fact that researchers do not have a consensus on species identification and taxonomic status of the individual forms. Owing to inaccessibility, many populations are still scarcely explored. In the 1980s, the morphology of chars from the Commander Islands was studied [2], but their genetic studies have not been conductedyet.

Six samples from the Commander Islands were investigated; for comparison, a continental sample from the Kol River (western Kamchatka) was used. The characteristics of the samples are demonstrated in Table 1; in total, 183 individuals were analyzed. In all samples, the sequences of two mtDNA fragments were determined. The examined 567-bp fragment of the control region (D-loop) corresponds to the positions 1–567 bp of the complete mtDNA genome of Dolly Varden (GenBank #KJ746618) (positions 1–567 bp of the control region); the 973-bp cytochrome *b* (*CytB*) gene fragment corresponds to the positions 15457–16429 of the complete mtDNA genome of Dolly Varden (GenBank #KJ746618) (positions 85–1057 bp of the *cytB* gene).

DNA was isolated and purified using the silica-based method [4]. Amplification was performed in a total volume of 15 μL (30 mM Tris-HCl (pH 8.6), 16.6 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.6 mM dNTP, 3 pmol of each primer, about 100 ng DNA, and 0.5 units of *Taq* polymerase (Sileks)).

Amplification of the *CytB* gene was carried out under the following reaction conditions: initial DNA denaturation at 95°C for 2 min; 35 cycles of denaturation at 94°C for 15 s, primer annealing at 57°C for 30 s, and DNA extension at 72°C for 1 min 30 s; and final extension at 72°C for 10 min with primers L14795 TAATGGCCAACCTCCGAAAA and H15844 AGCTACTAGGGCAGGCTCATT [5].

The reaction conditions for amplification of the D-loop fragment were different: initial DNA denaturation at 95°C for 2 min; 42 cycles of denaturation at 94°C for 20 s, primer annealing at 52°C for 45 s, and DNA extension at 72°C for 30 s; and final extension at 72°C for 10 min with primers HN20 GTGTTATGCTTTAGTTAAGC and Tpro2 ACCCTTAACTCCCAAAGC [6].

Sequencing was performed on an ABI 3500 automated sequencer using the BigDye v. 1.1 sequencing kit from Tpro2 primer in the case of the control region and from the both ends in the case of cytochrome *b*.

Processing of chromatograms and multiple sequence alignment was carried out in the Geneous 6.0.5

Table 1. Characteristics of the samples used in the study

Locality	Sample size	Sample symbol	Sampling year
Gavanskaya River (Bering Island)	29	BHA	2013
Podutesnaya Bay (Bering Island)	29	BPU	2013
Tovarischeskaya Shaiba River (Bering Island)	24	BTS	2013
Buyan River (Bering Island)	27	BBU	2013
Gladkovskaya Bay (Mednyi Island)	30	NML	2014
Peschanaya River (Mednyi Island)	14	SND	2014
Kol River (Western Kamchatka)	30	KL	2008

Table 2. Nucleotide and haplotype diversity of Dolly Varden of the Commander Islands and Kol River (Kamchatka)

Sample	*N*	*H*	*S*	π	*h*	P_i
BHA	26	13	16	0.002171 ± 0.001280	0.8954 ± 0.0414	3.369231 ± 1.783824
BPU	28	7	12	0.002008 ± 0.001195	0.6772 ± 0.0816	3.116402 ± 1.666463
BTS	24	10	13	0.001961 ± 0.001180	0.9167 ± 0.0257	3.043478 ± 1.642150
BBU	26	7	11	0.001711 ± 0.001050	0.8185 ± 0.0430	2.655385 ± 1.462772
NML	29	11	14	0.001987 ± 0.001183	0.8818 ± 0.0364	3.083744 ± 1.650087
SND	14	4	4	0.001204 ± 0.000822	0.7363 ± 0.0748	1.868132 ± 1.136009
KL	29	13	13	0.001524 ± 0.000951	0.8645 ± 0.0458	2.364532 ± 1.326579

N, sample size; *H*, number of haplotypes; *S*, number of variable sites; π, nucleotide diversity; *h*, haplotype diversity; P_i, mean number of pairwise differences between the haplotypes.

software package [7]. To represent the phylogenetic relationships between haplotypes, the method of maximum parsimony [8] was used as implemented in the TCS software program [9]. Genetic polymorphism and the level of pairwise differentiation of the samples were evaluated in the Arlequin v. 3.5.1.3 software program [10].

All sequences were deposited in the GenBank database under the accession numbers from KT962126 to KT962138 (D-loop) and from KT962139 to KT962167 (*CytB*). For each individual, the D-loop and *CytB* sequences were concatenated, and the obtained composite haplotypes were used for further analysis. Comparison of the concatenated 1540-bp sequences (1–567 bp D-loop and 568–1540 bp *CytB*) revealed the presence of 40 haplotypes (I-1–I-40). The number of haplotypes found in most island samples is comparable to that in the population of the Kol River (Table 2). Thus, the level of mtDNA genetic diversity in island populations of the Commander chars is comparable to that in the mainland population. In spite of the existing opinion on lower genetic polymorphism of island populations, we didn't observe this phenomenon. The occurrence of haplotypes in the populations is represented in Table 3.

All tested samples are characterized by a relatively high level of haplotype and nucleotide diversity (Table 2). The lower, compared to other samples, values of the nucleotide diversity and the mean number of pairwise differences between haplotypes in the Peschanaya River (SND) can be explained by the small sample size. The haplotype structure of the sample (Table 3) suggests that, if its volume were greater, it would not be statistically significantly different from the sample of Gladkovskaya Bay, located on the same coast of Mednyi Island.

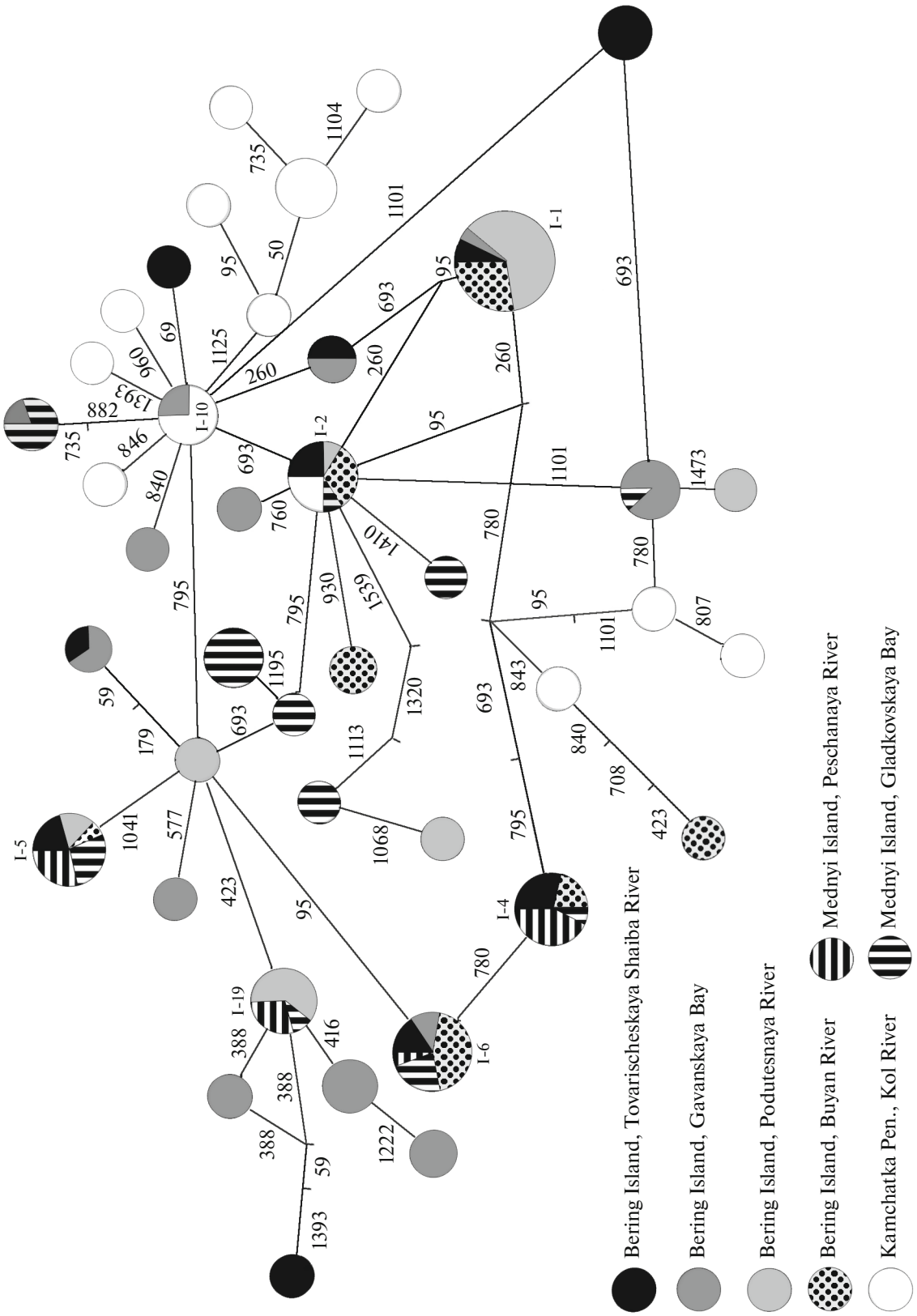
Table 4 shows the results of applying the methods of *F* statistics for evaluating the level of differentiation of the studied populations and statistical significance of the differences between all population pairs. As can be seen from Table 4, all samples were statistically significantly different from each other, except for a pair of rivers, Buyan and Tovarischevskaya Shaiba, which is quite explainable, because the mouths of these rivers are located on one bank of Bering Island at a distance of about 7 km.

The figure shows the net of mitochondrial haplotypes constructed by comparing the concatenated sequences of D-loop and *CytB* gene. The examined *S. malma* individuals carried five mass haplotypes (I-1, I-2, I-4, I-5, I-6), and four of them were found out in the samples collected on the Commander Islands only. Haplotypes found in four or more populations were considered by us as widespread. Chars of the Commander Islands were characterized by a large number of unique (found in a single sample) and rare (in two to three samples) haplotypes. In the sample of chars from western Kamchatka, 11 unique haplotypes, one of which (I-27) was present in nine individuals, were identified. On the Commander Islands and in the

Table 3. The number of Dolly Varden individuals with different haplotypes in the samples from the Commander Islands and Kol River

U*	Acc # GenBank		Bering Island				Mednyi Island		Kamchatka, Kol River KL	Total
	CytB	D-loop	BHA	BPU	BTS	BBU	NML	SND		
I-1	KT962140	KT962128	1	15	2	7				25
I-2	KT962140	KT962126		1	3	4	1		3	12
I-3	KT962145	KT962130				1				1
I-4	KT962143	KT962127			4	3	1	6		14
I-5	KT962142	KT962126		2	3	1	4	4		14
I-6	KT962139	KT962127	2		3	8	4	1		18
I-7	KT962144	KT962126				2				2
I-8	KT962139	KT962129	1							1
I-9	KT962139	KT962131	5							5
I-10	KT962141	KT962126	2						6	8
I-11	KT962146	KT962126	7				1			8
I-12	KT962147	KT962126	1							1
I-13	KT962148	KT962126	1							1
I-14	KT962139	KT962132	2		1					3
I-15	KT962149	KT962126	1				4			5
I-16	KT962141	KT962133	1		1					2
I-17	KT962150	KT962131	1							1
I-18	KT962139	KT962134	1							1
I-19	KT962139	KT962135		6			1	3		10
I-20	KT962151	KT962126		1						1
I-21	KT962143	KT962126		1						1
I-22	KT962152	KT962127		2						2
I-23	KT962153	KT962126			4					4
I-24	KT962141	KT962136			2					2
I-25	KT962154	KT962137			1					1
I-26	KT962155	KT962138							1	1
I-27	KT962156	KT962138							9	9
I-28	KT962156	KT962127							1	1
I-29	KT962157	KT962138							2	2
I-30	KT962158	KT962127							1	1
I-31	KT962159	KT962126							1	1
I-32	KT962160	KT962126							1	1
I-33	KT962156	KT962126							1	1
I-34	KT962161	KT962126							1	1
I-35	KT962162	KT962126							1	1
I-36	KT962163	KT962126							1	1
I-37	KT962164	KT962126					1			1
I-38	KT962165	KT962126					8			8
I-39	KT962166	KT962126					2			2
I-40	KT962167	KT962127					2			2

U*, composite haplotype.



Phylogenetic network of 1540-bp composite D-loop–*CytB* haplotypes (1–567 bp D-loop and 568–1540 bp *CytB*) in the examined samples of *S. malma*. The circle size reflects the relative proportion of individuals carrying this haplotype. Figures at the lines connecting the haplotypes mark the positions of nucleotide substitutions in the concatenated sequence of D-loop–*CytB* haplotypes.

Table 4. Pairwise F_{st} values in the populations of Dolly Varden (below the diagonal) and the levels of their statistical significance (above the diagonal)

	BHA	BPU	BTS	BBU	NML	SND	KL
BHA	–	0.00001	0.00001	0.00002	0.00003	0.00000	0.00000
BPU	0.19824	–	0.00006	0.00109	0.00000	0.00004	0.00000
BTS	0.07778	0.15618	–	**0.07732**	0.00173	0.03762	0.00000
BBU	0.11289	0.11819	**0.03180**	–	0.00016	0.00060	0.00000
NML	0.08851	0.20533	0.05909	0.09816	–	0.00060	0.00000
SND	0.17306	0.24664	0.06036	0.14941	0.12263	–	0.00003
KL	0.10596	0.22577	0.09816	0.14462	0.12372	0.19311	–

Not statistically significant differences are in bold type.

Kol River, two common haplotypes (I-2, I-10), with one of them (I-2) belonging to mass haplotypes, were identified.

The uniqueness of this study lies in the fact that, for the first time, the mitochondrial polymorphism of chars from the Commander Islands was examined in comparison with Dolly Varden from the Kamchatka Peninsula. Despite the prevailing view of the low level of genetic diversity of island populations, Commander charrs showed the opposite, and their level of genetic polymorphism can be considered comparable to that in the mainland population. The topology of the phylogenetic net, the existence of common haplotypes in chars of the Commander Islands and western Kamchatka, and the differences between the haplotypes of the Commander and Kamchatka individuals in one to three nucleotide substitutions point to their genetic closeness. According to Gritsenko [11], Dolly Varden from the Kamchatka Peninsula belongs to the genetic lineage of northern Dolly Varden *Salvelinus malma malma*. Because of this, it can be affirmed that the charr populations of the Commander Islands also belong to the genetic lineage of northern Dolly Varden.

Thus, the level of genetic diversity of mtDNA in island populations of the Commander chars is comparable to that of the Kamchatka representatives of the species (Kol River), as well as to the overall level of genetic diversity at similar sites in the continental populations of *S. malma malma* [12–14]. Despite the existing opinion about the lower genetic polymorphism of island populations, we do not see it in this case. Considering that classical features of formation of island population isolates is the effect of bottleneck and subsequent inbreeding, this fact is a good incentive for further studies of the Commander chars.

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