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STUDY OF THE GENETIC DIVERSITY OF DOMESTIC AND WILD REINDEER (*Rangifer tarandus* L., 1758) POPULATIONS USING NUCLEAR AND MITOCHONDRIAL GENOMIC MARKERS

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Abstract

The reindeer (*Rangifer tarandus* L., 1758) is an important biological species that plays a key role in the supporting livelihood of the peoples of the Far North of Russia. Due to climate change and anthropological impacts, this species may be endangered, therefore, in the modern world, the study and conservation of the genetic diversity of the reindeer is relevant. In this work, for the first time, the genetic variability responsible for the differentiation of domestic and wild forms of the reindeer was studied using an integrated molecular genetic approach, which consisted in the analysis of nuclear and mitochondrial genomes. Our aim was to evaluate the genetic diversity, genetic structure, and phylogenetic relationships of domestic and wild populations of reindeer bred in the Russian Federation based on complete mitochondrial DNA *CytB* gene sequences and microsatellite loci polymorphism. The research was carried out in 2022. Cuts from reindeer antlers served as material. The sample included wild reindeer from the tundra population (WLD), as well as domestic reindeer from Nenets (NEN), Chukchi (CHU), Even (EVN) and Evenk breeds comprising the Krasnoyarsk (EVK_KRA) and Yakut (EVK_YAK) populations. For the study of mtDNA, 123 unrelated individuals were selected. Microsatellite analysis was performed in 213 individuals of domestic breeds and 119 representatives of the wild population. The complete sequences of the *CytB* gene were determined using next generation sequencing (NGS) on a miSeq sequencer (Illumina, Inc., USA). Polymorphism of nine STR loci (NVHRT21, NVHRT24, NVHRT76, RT1, RT6, RT7, RT9, RT27, RT30) was investigated by fragment analysis using an ABI3130xl genetic analyzer (Applied Biosystems, USA). To assess the genetic diversity of each group of reindeer, indicators of mitochondrial variability (number of polymorphic sites S, average number of nucleotide differences K, number of haplotypes H, haplotype diversity H_D, nucleotide diversity π) and microsatellite variability (rarefied allelic richness A_R, observed heterozygosity H_O, unbiased expected heterozygosity uH_E, unbiased inbreeding coefficient F_{IS}) were calculated. The degree of genetic differentiation between groups was assessed based on pairwise F_{ST} and JostD values. Statistical processing of the raw data was performed using the programs MEGA 7.0.26, PopART 1.7, PartitionFinder 2, Arlequin 3.5.2.2, MrBayes 3.2.7, FigTree 1.4.3, DnaSP 6.12.01, SplitsTree 4.14.5, STRUCTURE 2.3.4 and R packages diveRsity, pophelper, adegenet and ggplot2. Analysis of mtDNA *CytB* gene sequences showed that all studied populations were characterized by high haplotype diversity, H_D = 0.519 (CHU)-0.997 (WLD), and nucleotide diversity, π = 0.00238 (CHU)-0.00626 (WLD). Based on the mtDNA analysis no clear genetic structure was revealed in the studied reindeer populations. Analysis of microsatellite variability showed that values of allelic richness ranged from 6.188 in CHU to 8.76 in WLD. In all six populations, observed heterozygosity ranged from 0.566 (CHU) to 0.687 (EVK_YAK) and 0.693 (WLD). All studied reindeer groups were characterized by a deficit of heterozygotes, as indicated by positive values of the fixation index, F_{IS} = 0.11 (EVK_YAK)-

0.262 (EVK_KRA). Network analysis showed the differentiation of the Chukotka breed from the rest groups, as evidenced by the highest F_{ST} and JostD values, which varied from 0.203 and 0.488 for EVK_KRA to 0.212 and 0.564 for EVN, respectively. Based on both nuclear and mitochondrial markers, wild reindeer populations showed higher genetic diversity compared to domestic populations. It may be assumed that selection work with domestic reindeer breeds led to the creation of unique populations that differ from the original wild relatives. However, both domestic and wild reindeer populations, which were studied in this work, were characterized by high genetic variability.

Keywords: reindeer, *Rangifer tarandus*, genetic diversity, phylogenetic assessment, mitochondrial DNA, microsatellite loci

The reindeer (*Rangifer tarandus* L., 1758) is a bioresource that is important for maintaining ecological balance via the influence on vegetation and as a livelihood for many indigenous peoples of the Arctic North. The reindeer was probably essential for human migration and colonization of the Eurasian Arctic and Subarctic after the last ice age. Recently, the reindeer has also been involved to create protected areas [1, 2]. Unlike most other livestock species whose wild forms are extinct (e.g., cattle, horses), endangered (e.g., donkeys, llamas, alpacas), or geographically restricted (e.g., sheep, goats), wild reindeer populations are still widespread in Northern Eurasia and North America (caribou). Almost 50% of approximatelly 3,000,000 deer in the Old World are wild animals, and in many areas wild and domestic herds coexist closely [2, 3]. This provides a unique opportunity to analyze the interaction between domestic and wild populations.

Reindeer are mainly distributed in the Arctic region of the Northern Hemisphere, including Norway, Finland, Sweden, Russia, Greenland, the United States, Mongolia, China and Canada. Fossils found indicate that during the Pleistocene *Rangifer* lived south of the ice sheet in both Eurasia and North America, as well as in Beringia, covering the Bering Land Bridge, Alaska and a large part of Siberia.

Based on morphological and historical data, populations of modern reindeer are classified into three ecological groups: forest (sedentary deer), tundra (migratory deer), and high arctic island deer [4]. These ecological groups include nine subspecies, of which seven [5] have survived to date. Domestic reindeer in the Russian Federation belong to four approved breeds, the Nenets, Even, Evenk and Chukchi [6].

The reindeer, like other Holarctic species, may become an endangered species due to climate change and human impact. Thereof, evaluation of phylogenetic structure at the species level is important to conserve genetic diversity [5] which allows species to adapt to environment and develop local adaptations [7]. The issue of studying the genetic diversity of reindeer populations is the subject of many works based on the use of nuclear and mitochondrial markers.

J.-C. Zhai et al. [13] characterized the genetic diversity of eight populations of reindeer from the Greater Khingan mountains using 11 microsatellite loci. The authors revealed a deficit of heterozygotes in all populations and a low degree of genetic differentiation. T.E. Deniskova et al. [14] assessed the genetic diversity of 15 populations of the Nenets breed using 14 microsatellite loci. Later, in 2020, V.R. Kharzinova et al. [15] studied the population structure of 528 domesticated reindeer of four breeds from the Russian Federation. In the same year, Yu. Stolpovsky et al. [16] studied 397 individuals of domestic and wild reindeer bred in various climatic zones of Russia. Analysis of microsatellite loci showed that 70% of the allelic diversity occurres in the wild reindeer populations.

In 2018, V.R. Kharzinova et al. [17] performed the first genotyping of reindeer of four Russian breeds using the BovineHD BeadChip and submitted a complete characterization of the genetic diversity of these breeds, as well as their ecotypes from four federal districts of the Far North of Russia. The Chukchi breed

and the Yakut intrabreed ecotype Khargin had low genetic diversity. Thereof, the preservation and increase of genetic variability in these groups is a priority [17].

As markers of the mitochondrial genome, two highly variable mtDNA regions, the *CytB* gene and the control region (D-loop), are used in the reindeer population studies. In 2018, C.D. Wilkerson et al. [19] based on the analysis of mtDNA D-loop and *CytB* gene sequences, identified 4 haplogroups (A, B, C and D) and 32 haplotypes in woodland caribou on the island of Newfoundland. Island caribou were characterized by a fairly high genetic diversity (H_D = 0.894 and π = 0.00216), with the exception of deer from the Avalon Peninsula, in which only three haplotypes were identified with a relatively low degree of haplotype (H_D = 0.569) and nucleotide (π = 0.00052) diversity. Phylogenetic analysis allowed the authors to trace the direction of the post-glacial recolonization of the island by reindeer [19].

Currently, an integrated approach is gaining popularity when several types of molecular genetic markers are used for a more accurate analysis to obtain complete information about the genetic diversity of animals. In 2012, F. Barbanera et al. [11] successfully investigated the poaching of the Cypriot moufflon (*Ovis orientalis ophion*) using 12 microsatellite loci as molecular markers together with the mitochondrial *CytB* gene. Later, in 2021, another poaching crime was uncovered in the Kabardino-Balkarian Republic. A. Rodionov et al. [12], using a complex approach based on 14 microsatellites and SNP genotyping (DNA chip), proved the fact of poaching of the Caucasian tur (*Capra caucasica*).

M.A. Cronin et al. [20] quantified genetic variation in 11 North American caribou herds using 18 microsatellite loci and *CytB* gene sequences. Such a comprehensive analysis confirmed the intraspecific classification of the reindeer into three ecotypes: living in the tundra on barren land, the mountain form and the forest form. Later, the same authors characterized the genetic diversity of domestic reindeer from Alaska, Siberia, and Scandinavia in comparison with wild caribou using 18 microsatellite loci and sequences of the mitochondrial *CytB* gene. The authors revealed differences in the frequencies of haplotypes and microsatellite loci in domestic reindeer and wild caribou. High genetic diversity for both markers was characteristic of wild deer {21].

In 2018, a research team from China [22] examined the genetic variation in a single Aolugui reindeer population using 10 microsatellite loci together with the *CytB* gene and revealed the varying degrees of inbreeding in the population. mtDNA polymorphism indicated a relatively low genetic diversity (H_D = 0.468±0.091, π = 0.0017±0.001), and five unique haplotypes were identified. The authors propose to form strategies for the conservation of the species and restoration of the population based on the data obtained [22].

A combination of several markers is now commonly used to quantify the genetic diversity of reindeer. Howevere, similar attempts to characterize Russian reindeer populations have not yet been made. In the presented work, we for the first time evaluated the genetic diversity of reindeer from the Russian Federation, revealed their phylogenetic relationships and assessed the degree of differentiation of the studied animals using an integrated approach based on the analysis of nuclear and mitochondrial genomes.

Our goal was to evaluate the genetic diversity, genetic structure, and phylogenetic relationships of domestic and wild Russian populations of reindeer based on complete mitochondrial DNA *CytB* gene sequences and microsatellite loci polymorphism.

Materials and methods. The research was carried out in 2022 using sections of antlers. The sampe included biomaterial collected from wild reindeer of the

tundra population (WLD), domestic reindeer of the Nenets (NEN) (Komi Republic), Chukchi (CHU) (Iultinsky district, Chukotka Autonomous Okrug), Even (EVN) (Neryungri district, Sakha Republic) breeds, as well as the Krasnoyarsk (EVK_KRA) (Krasnoyarsk Territory) and Yakut (EVK_YAK) (Aldan district, Republic of Sakha) populations of the Evenk breed. A total of 123 unrelated individuals were selected for mtDNA study. For microsatellite analysis, 213 domestic reindeer and 119 reindeer from wild populations were selected.

DNA was isolated using the DNA-Extran-2 kit (OOO Sintol, Russia) according to the manufacturer's standard protocol. The DNA concentration was measured (a Qubit 4.0 fluorometer, Invitrogen/Life Technologies, USA), and the absorption ratio $OD_{260/280}$ was assessed (a NanoDrop™ 8000 spectrophotometer, Thermo Fisher Scientific, Inc., USA). The DNA concentration ranged from 15 to 50 ng/μl with an $OD_{260/280}$ ratio of 1.8 or higher.

Next generation sequencing (NGS) was carried out in several staps. At the first stage (sample preparation), complete reindeer mitochondrial genomes were generated by amplification of six fragments of 2000 to 4500 bp in length with 120-780 bp overlapping region. The following primer pairs were used: F1 5'-TCCTCCCTTCTAGACTTAATCTGACT-3', R1 5'-CTCCTCCCACGACTAGTTGCAC-3'; F2 5′-ACTCCAACCTATTGCAGATGCCAT-3′, R2 5′-AAGGTTATTTCGACTGCATGTGCGGTTAC-3′; F3 5'-CTAACACTCAGATTAATTAGAGGACA-3', R3 5'-GTACTCCGCGGTTCATATTAATGAGAGG-3'; F4 5′-TGCTTGAGCAGGCATAGAAGGGAC-3′, R4 5′-TGGTGTGTCATTATGACTTGTTGTGCA-3′; F5 5′-GGAGGAATTACACTGGGATTAATAAG-3′, R5 5′-AATACCCTCTACTGCTATTGGCTTGA-3′; F6 5'-GGAACCGTAAAATTGATACAACTCCAA-3', R6 5'-GGGATTGCAAGCTTATATAGTTATGG-3'. Amplification was carried out in the following mode: 2 min at 96° °C (initial denaturation); 30 s at 96 °C, 30 s at 60 °C, 3 min at 72 °C (40 cycles), 10 min at 72 °C (final elongation) (an Applied Biosystems SimpliAmp thermal cycler, Thermo Fisher Scientific, Inc., USA). At the second stage, the libraries were prepared for sequencing using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, USA) according to the manufacturer's standard protocol. The samples were sequenced using paired end reads of 300 bp (a MiSeq instrument, Illumina, Inc., USA). The final stage was the processing of the obtained data.

From the complete deer mtDNA sequences, after alignment using the MUSCLE algorithm [23] in the MEGA 7.0.26 program [24], the *CytB* gene sequences were reassabmled. The sequence of the reindeer *CytB* gene from the NCBI database (https://www.ncbi.nlm.nih.gov/genbank/, GenBank accession number NC_007703.1) served as an outgroup. The median network was constructed using the PopART 1.7 program [25]. The best evolution models were determined in the PartitionFinder 2 program [26] using the adjusted Akaike information test (AICc) [27]. The evolutionary models HKY+I, HKY+I, and GTR+I for the first, second, and third nucleotides in the codon turned out to be optimal. F_{ST} analysis was performed using the Arlequin 3.5.2.2 program [28]. The Bayesian phylogenetic tree was built using MrBayes 3.2.7 software [29] followed by visualization in a graphical viewer FigTree 1.4.3 [30]. Monte Carlo search with Markov chains was performed using four chains with 10,000,000 steps, trees were selected every 500 generations (the first 25% of the selected trees were rejected using the burn-in algorithm).

The parameters of genetic diversity, i.e., the number of polymorphic sites (S), the average number of nucleotide differences (K), the number of haplotypes (H), haplotype diversity (H_D), nucleotide diversity (π), arithmetic mean errors (±SEM) were calculated using the DnaSP 6.12.01 program. The population expansion hypothesis was tested by calculating the Fu's Fu neutrality statistic and

the Tajima's D test in DnaSP 6.12.01 [31].

Polymorphism analysis of 9 microsatellites (NVHRT21, NVHRT24, NVHRT76, RT1, RT6, RT7, RT9, RT27, RT30) was performed as previously described [32]. The resulting DNA fragments were visualized by fragment analysis using Gene Mapper v. 4 (Applied Biosystems, USA). Analysis of population genetic parameters, including rarefied allelic diversity (A_R), observed (H_O) and unbiased expected (uH_E) heterozygosity, as well as unbiased inbreeding coefficient (F_{IS}) with a 95% confidence interval (CI), was performed using the diveRsity R package with subsequent visualization in the pophelper package [33]. The degree of genetic differentiation was assessed based on the matrices of pairwise values F_{ST} [34] and JostD [35]. To build phylogenetic trees using the Neighbor-Net algorithm, we used SplitsTree 4.14.5 software [36] and the diveRsity R package, followed by visualization in the pophelper package.

The genetic structure of the studied groups of reindeer was assessed using Principal Component Analysis (PCA) in the adegenet R package [37] and with visualization in the ggplot2 R package [38], as well as by clustering in STRUCTURE 2.3.4 program [39] using a mixed model (the number of assumed clusters K is from 1 to 10, the length of the burn-in period is 100,000, the Monte Carlo Markov chain model is 100,000). For each value of K, 10 iterations were performed. The STRUCTURE HARVESTER application [40] was used to determine the optimal number of clusters (ΔK) according to the method of G. Evanno et al. [41]. The source files were formed in the R 3.5.0 software environment (R Core Team) [42].

Results. The use of next generation sequencing (NGS) technology made it possible to obtain complete sequences of the mitochondrial *CytB* gene in the studied reindeer populations.

Analysis of the nucleotide sequences of the mitochondrial *CytB* gene. In the 1140 bp sequences of the mitochondrial *CytB* gene we obtained from 123 individuals, 40 haplotypes were identified. All animals were characterized by high genetic diversity (H_D = 0.918±0.014; π = 0.00448±0.00023) (Table 1). In wild reindeer populations, the highest haplotype (H_D = 0.997±0.013) and nucleotide (π = 0.00626±0.00041) diversity was observed compared to domestic populations (H_D = 0.865±0.021; π = 0.00364±0.00022).

1. Genetic diversity in populations of domestic and wild reindeer (*Rangifer tarandus* L., 1758) based on nucleotide sequences of the mitochondrial gene *CytB* (2022)

Population	*n*	S	K	H	H_D±SEM	π±SEM	Tajima's D	Fu's Fu
CHU	22	17	2.710	5	0.519±0.114	0.00238±0.00066	–1.53176 ns	1.780 ns
EVK_KRA	12	8	3.333	5	0.788±0.090	0.00292±0.00029	1.03140 ns	0.159 ns
EVK_YAK	14	15	4.396	7	0.846±0.074	0.00386±0.00055	–0.27767 ns	0.159 ns
EVN	21	12	3.200	10	0.848±0.059	0.00281±0.00029	–0.14393 ns	–2.304 ns
NEN	21	17	4.038	6	0.663±0.105	0.00354±0.00067	–0.53756 ns	2.014 ns
All domestic populations	90	35	4.153	21	0.865±0.021	0.00364±0.00022	–1.24481 ns	–4.365 ns
WLD	33	48	7.129	24	0.997±0.013	0.00626±0.00041	–1.45464 ns	–10.970
Total	123	61	5.098	40	0.918±0.014	0.00448±0.00023	–1.73284 ns	–19.784

N o t e. *n* — the number of samples, S — number of polymorphic sites, K — average number of nucleotide differences, H — number of haplotypes, H_D — haplotype diversity, π — nucleotide diversity, ns — 0.10 > P > 0.05. CHU — Chukchi breed, EVK_KRA — Evenk Krasnoyarsk breed, EVK_YAK — Evenk Yakut breed, EVN — Evenk breed, NEN — Nenets breed, WLD — wild reindeer.

In the populations of domestic reindeer, the Even breed had the greatest haplotype diversity (H_D = 0.848±0.059). The highest nucleotide diversity and the highest average number of nucleotide differences were found in the Evenki Yakut population (π = 0.00386±0.00055, K = 4.396). The Chukchi breed was characterized by the least genetic diversity in all indicators (H_D = 0.519±0.114, π = 0.00238±0.00066, K = 2.710).

The obtained values of the Tajima's D (–1.24481 ns) and Fu's Fu (–4.365 ns) tests indicated a trend towards complete identity between home populations. According to these values, there is a limited difference in the number of polymorphic sites and the average number of pairwise nucleotide differences between the studied populations. That is, the studied domestic reindeer breeds are in genetic balance, which indicates the state of alleles and genotypes in the gene pool of their populations. This ensures adaptation to environmental changes caused primarily by anthropogenic factors. On the contrary, in wild deer, we found a high negative Fu's Fu (–10.970), indicating the flow of foreign genes due to spatial expansion, while a low D value (–1.45464 ns) indicated a stable state of the population.

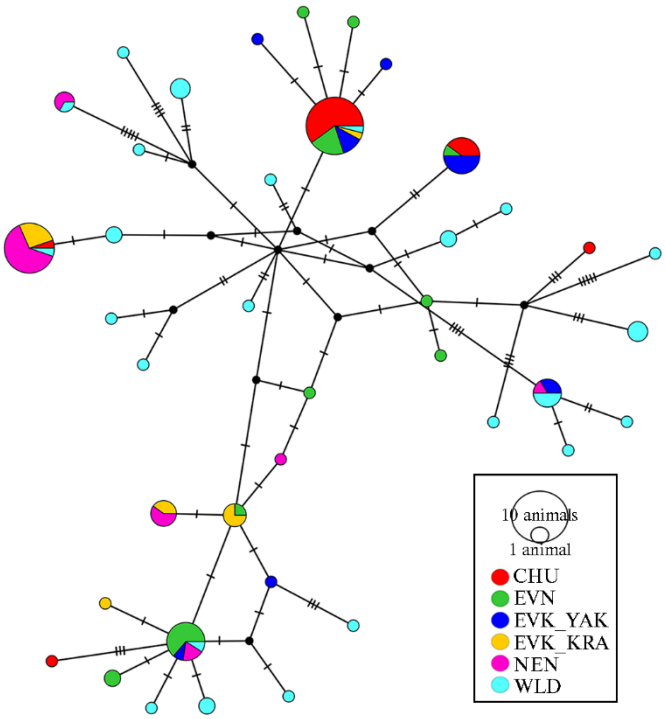

Fig. 1. The median network characterizing the relationships of haplotypes identified in domestic and wild reindeer (*Rangifer tarandus* L., 1758) based on the nucleotide sequences of the mitochondrial *CytB* gene: CHU — Chukchi breed (n = 22), EVK_KRA — Evenk Krasnoyarsk breed (n = 12), EVK_YAK — Evenk Yakut breed (n = 14), EVN — Even breed (n = 21), NEN — Nenets breed (n = 21), WLD — wild reindeer (n = 33). The diameter of the circle corresponds to the number of individuals of the corresponding haplotype. The number of transverse lines indicates the number of nucleotide substitutions. Black circles at network branching points represent hypothetical haplotypes (2022).

Among the reindeer of both wild and domesticated populations inhabiting the territory of the Russian Federation, we did not reveal a clear differentiation according to the maternal mtDNA marker.

Of all 40 haplotypes, only 8 were common, the remaining 32 haplotypes were found only in one representative from the studied deer sample (Fig. 1). Basically, the diversity of haplotypes was achieved due to populations of wild animals. Of 24 haplotypes identified in wild deer, only 5 were common with populations of domestic animals. Representatives of all 5 populations of domestic deer had one common haplotype with populations of wild reindeer.

In addition to the common haplotypes with wild deer, the domestic deer populations formed 3 common haplotypes. The Krasnoyarsk population of the Evenk breed had one common haplotype each with representatives of the Nenets and Even breeds, and one common haplotype was found in representatives of the

Chukchi, Even and Yakut populations of the Evenk breed.

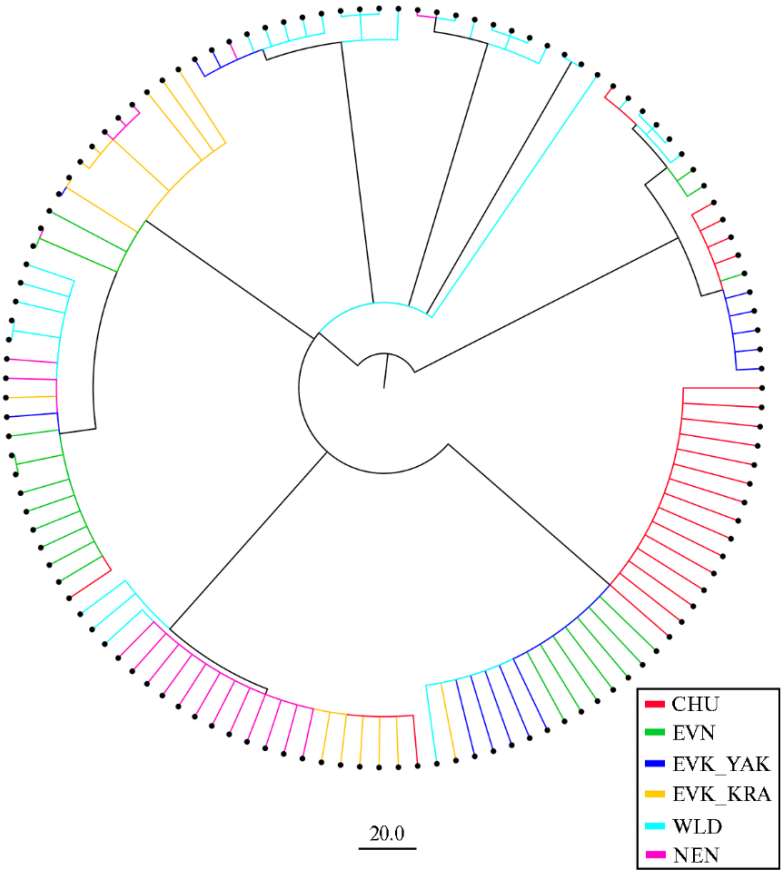

Fig. 2. Bayesian phylogenetic tree for the genetic relationships between domestic and wild reindeer (*Rangifer tarandus* L., 1758) based on the nucleotide sequences of the mitochondrial gene *CytB*: CHU — Chukchi breed (n = 22), EVK_KRA — Evenk Krasnoyarsk breed (n = 12), EVK_YAK — Evenk Yakut breed (n = 14), EVN — Even breed (n = 21), NEN — Nenets breed (n = 21), WLD — wild reindeer (n = 33) (2022).

Analysis of the Bayesian phylogenetic tree (Fig. 2) revealed a clear divergence of the studied groups of deer into two main clusters. One cluster included 17 haplotypes that occurred in CHU, EVN, and EVK_YAK. Individual representatives of these breeds also had haplotypes included in the second cluster. Only domestic reindeer EVK_KRA had haplotypes characteristic only for the second cluster. Wild deer carried haplotypes of both clusters. The Nenets breed was the most distant from CHU individuals, which was confirmed by the highest values of the criterion index F_{ST} = 0.32645 (Table 2, Fig. 3).

2. Pairwise F_{ST} genetic distances based on the nucleotide sequences of the mitochondrial *CytB* gene in populations of domestic and wild reindeer (*Rangifer tarandus* L., 1758) (2022)

Population	CHU	EVN	EVK_YAK	EVK_KRA	NEN	WLD
CHU	0					
EVN	0.16899	0				
EVK_YAK	0.03967	0.13174	0			
EVK_KRA	0.29011	0.08439	0.21856	0		
NEN	0.32645	0.21418	0.27135	0.00824	0	
WLD	0.12380	0.08769	0.05715	0.09139	0.12807	0

N o t e. CHU — Chukchi breed, EVK_KRA — Evenk Krasnoyarsk breed, EVK_YAK — Evenk Yakut breed, EVN — Even breed, NEN — Nenets breed, WLD — wild reindeer.

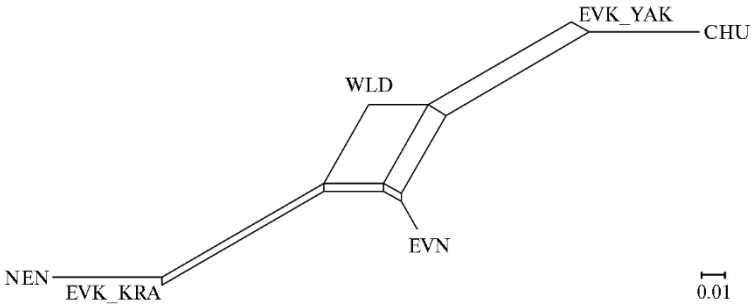

Fig. 3. Genetic relationships between populations of domestic and wild reindeer (*Rangifer tarandus* L., 1758) visualized as a Neighbor Net graph of the F_{ST} genetic distance matrix based on the nucleotide sequences of the mitochondrial *CytB* gene: CHU — Chukchi breed, EVK_KRA — Evenk Krasnoyarsk breed, EVK_YAK — Evenk Yakut breed, EVN — Even breed, NEN — Nenets breed, WLD — wild reindeer (2022).

We determined the closest genetic distances between EVK_KRA and NEN representatives whose fixation index was 0.00824.

We did not reveal a clear breed clustering of the studied groups of domestic deer. Most breed representatives carried similar mtDNA haplotypes, but some individuals had completely distant mitochondrial genotypes. This can be explained by the accidental mating of domestic and wild deer.

Microsatellite analysis. In this work, we used nine microsatellite loci to analyze 332 individuals of domestic and wild reindeer from the Russian Federation.

3. Characterization of genetic variability in populations of domestic and wild reindeer (*Rangifer tarandus* L., 1758) based on polymorphism of 9 microsatellite loci (2022)

Population	*n*	H_O	uH_E	uF_{IS} (95 % CI > 0)	A_R
EVN	33	0,655±0,041	0,746±0,022	0,129 [0,055; 0,203]	6,286±0,402
EVK_YAK	31	0,687±0,027	0,775±0,018	0,11 [0,042; 0,178]	7,014±0,389
EVK_KRA	15	0,576±0,072	0,767±0,031	0,262 [0,085; 0,439]	6,571±0,477
CHU	43	0,566±0,071	0,681±0,051	0,148 [–0,049; 0,345]	6,188±0,719
NEN	91	0,657±0,032	0,766±0,026	0,141 [0,08; 0,202]	7,036±0,441
WLD	119	0,693±0,036	0,841±0,018	0,177 [0,105; 0,249]	8,760±0,565

N o t e. *n* — number of samples, H_O — observed heterozygosity, uH_E — unbiased expected heterozygosity, uF_{IS} — unbiased inbreeding coefficient with 95% confidence interval, A_R — rarified allelic diversity. In parentheses, there are the range of uF_{IS} variability at a 95% confidence interval. CHU — Chukchi breed, EVK_KRA — Evenk Krasnoyarsk breed, EVK_YAK — Evenk Yakut breed, EVN — Even breed, NEN — Nenets breed, WLD — wild reindeer.

Analysis of genetic diversity (Table 3) showed that the population of wild reindeer was characterized by relatively high values of allelic diversity (A_R = 8.76) compared to the populations of domesticated reindeer. This parameter ranged from 6.188 in CHU to 7.036 in NEN. Similarly, the highest rates of observed and unbiased expected heterozygosity (H_O = 0.693; uH_E = 0.841) were in wild populations vs. domestic populations. The Chukchi breed was characterized by the lowest values of these indicators (H_O = 0.566; uH_E = 0.681). EVK_YAK among domestic reindeer was distinguished by the highest genetic diversity (H_O = 0.687; uH_E = 0.775). In all populations, there was heterozygote deficiences, as evidenced by the positive values of the coefficient of inbreeding uH_E, which ranged from 0.11 in EVK_YAK to 0.262 in EVK_KRA. The values of the confidence interval of the inbreeding coefficient in CHU were close to zero (–0.049, 0.345), which indicates the state of genetic balance in this deer population.

The genetic distances between the studied deer populations were estimated in pairs based on the values of the F_{ST} and JostD test (Table 4, Fig. 4). An analysis of the structure of the genetic network made it possible to identify two conditional

clusters (see Fig. 4). The first was represented by the EVN and EVK_YAK populations, which indicates their genetic relationship. This is confirmed by the lowest values of FST and JostD between them (0.045 and 0.089, respectively) (see Table 4). The second cluster was formed by the populations EVK_KRA, NEN, CHU, and WLD. In turn, the CHU population was the most distant from the others, which is explained by the geographical remoteness of its range.

4. Pairwise genetic distances FST and JostD based on polymorphism of 9 microsatellite loci for populations of domestic and wild reindeer (*Rangifer tarandus* L., 1758) (2022)

Population	EVN	EVK_YAK	EVK_KRA	CHU	NEN	WLD
EVN	0	0.089	0.219	0.538	0.198	0.194
EVK_YAK	0.045	0	0.171	0.564	0.130	0.180
EVK_KRA	0.085	0.068	0	0.488	0.141	0.150
CHU	0.212	0.207	0.203	0	0.364	0.408
NEN	0.099	0.068	0.066	0.175	0	0.178
WLD	0.055	0.051	0.039	0.147	0.053	0

N o t e. CHU — Chukchi breed, EVK_KRA — Evenk Krasnoyarsk breed, EVK_YAK — Evenk Yakut breed, EVN — Even breed, NEN — Nenets breed, WLD — wild reindeer.

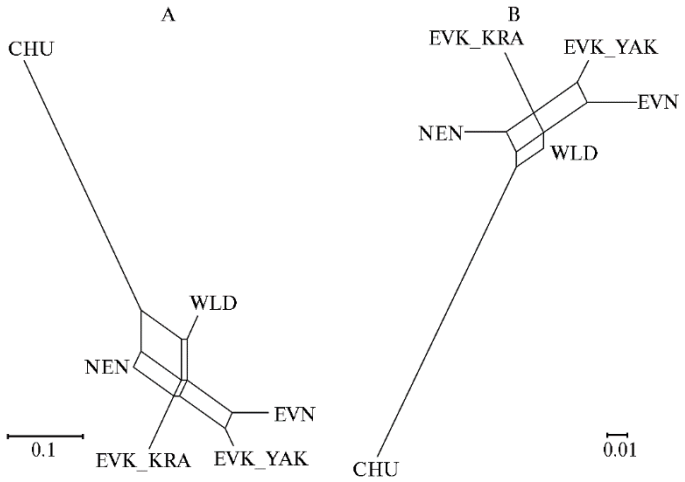

Fig. 4. Genetic relationships between populations of domestic and wild reindeer (*Rangifer tarandus* L., 1758) visualized as a Neighbor Net graph based on the matrix of values of genetic distances JostD (A) **and FST** (B) **for polymorphism for 9 microsatellite loci:** CHU — Chukchi breed, EVK_KRA — Evenk Krasnoyarsk breed, EVK_YAK — Evenk Yakut breed, EVN — Even breed, NEN — Nenets breed, WLD — wild reindeer (2022).

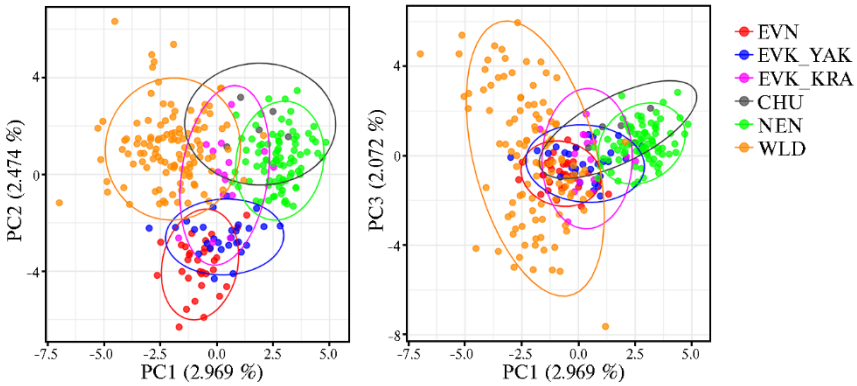

Fig. 5. Principal component analysis of relationships of domestic and wild reindeer (*Rangifer tarandus* L., 1758) based on polymorphism *of* 9 microsatellite loci*:* CHU — Chukchi breed (n = 22), EVK_KRA — Evenk Krasnoyarsk breed (n = 12), EVK_YAK — Evenk Yakut breed (n = 14), EVN — Even breed (n = 21), NEN — Nenets breed (n = 21), WLD — wild reindeer (n = 33) (2022).

To determine the population structure of the studied groups of deer, we used PCA analysis (Fig. 5) and cluster analysis (Fig. 6).

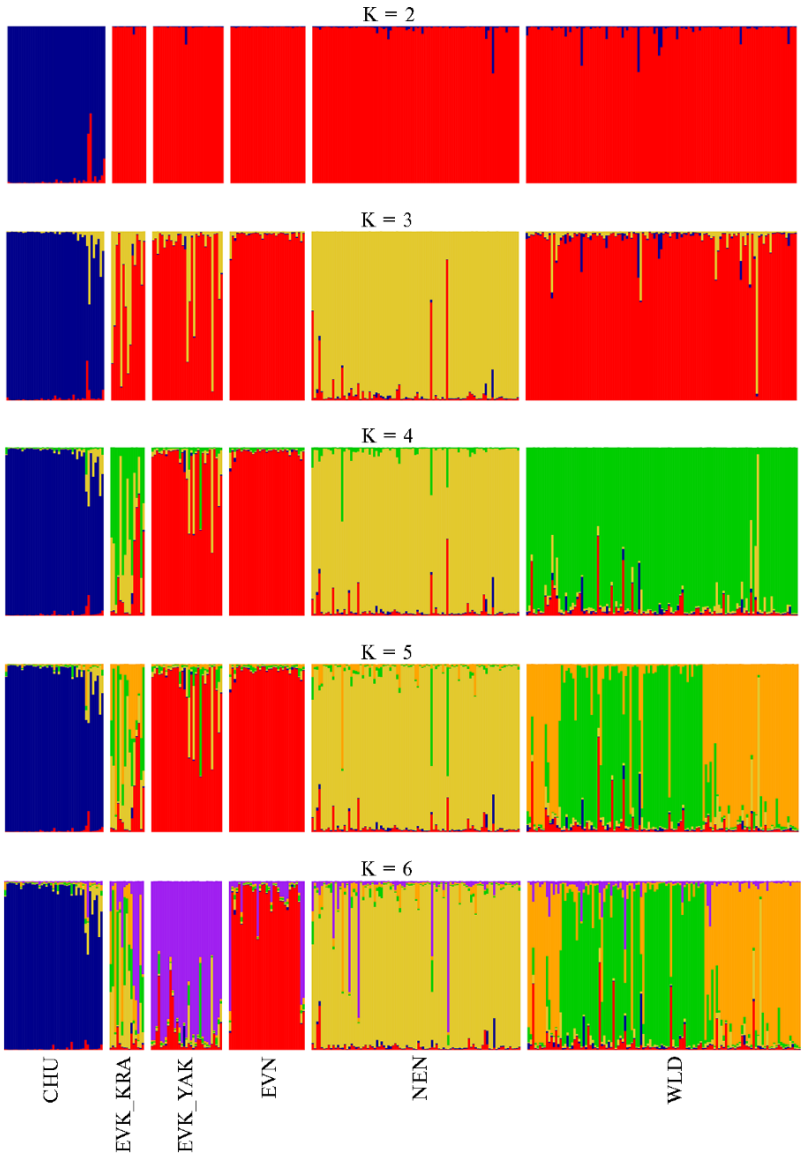

Fig. 6. Cluster analysis of populations of domestic and wild reindeer (*Rangifer tarandus* L., 1758) based on the polymorphism of 9 microsatellite loci (the STRUCTURE 2.3.4 program for a different number of clusters, K = 2, K = 3, K = 4, K = 5, K = 6): CHU — Chukchi breed (n = 22), EVK_KRA — Evenk Krasnoyarsk breed (n = 12), EVK_YAK — Evenk Yakut breed (n = 14), EVN — Even breed (n = 21), NEN — Nenets breed (n = 21), WLD — wild reindeer (n = 33) (2022).

The results of X-ray diffraction analysis demonstrated genetic differentiation between breeds and combined deer into clusters corresponding to similar ones on the phylogenetic tree (see Fig. 4). All studied reindeer populations showed a convergent nature of the genetic composition, forming intersecting clusters. The contribution to the total genetic variability attributable to the first, second, and third principal components was 2.969, 2.474 and 2.072%, respectively. Genetic differentiations for the first two main components and for the first and third main components were similar to each other. PC1 separated CHU and NEN from wild populations. Individuals of EVK_YAK, together with EVN, separated from other

animals by PC2. All studied animals were assigned to the PC3 axis.

Despite the fact that the algorithm based on the value of ΔK revealed the optimal number of clusters for the entire analyzed sample of reindeer, equal to 2 (K = 2, ΔK = 136.79), K = 4, K = 5 and K = 6 also proved to be effective for cluster analysis.

At K = 2, two main genetic pools were identified: the first pool consisted of three breeds, the Even, Evenk, and Nenets, as well as the mebers of the wild population, the second pool was formed only by individuals of the Chukchi breed. At K = 3, we found a clear separation of NEN from other populations. At K = 4, separation of EVK_KRA and wild individuals occurred. At K = 5, there was a separation of wild deer into two main groups with elements of genetic impurities of domestic breeds observed. Also, at K = 5, the WLD population was divided, which can presumably be associated with the large areas of habitat of the selected wild individuals. EVN was separated from EVK_YAK only at K = 6. It should be noted that EVK_KRA demonstrated complete mixing with representatives of all populations except CHU.

For the indigenous peoples of the Arctic North of Russia, the reindeer plays an important biological role, as it is a source of food, clothing and shelter, as well as a means of transportation [1]. In the world, the study of the genetic diversity of reindeer is carried out using a combination of several markers [20-22]. However, to date, the Russian populations of reindeer have been characterized by only one type of marker. In our work, to study domestic and wild reindeer, we used for the first time an integrated approach based on the analysis of the mtDNA *CytB* gene polymorphism and microsatellite loci. Haplotype and nucleotide diversity in the Russian reindeer populations (H_D = 0.519-0.997; π = 0.002-0.006) was comparable to values obtained in previous studies of Russian and Norwegian deer (H_D = 0.570-0.978; π = 0.002-0.019) [1], Aolugui populations from China (H_D = 0.468; π = 0.0017) (22), as well as Canadian reindeer (H_D = 0.890; $\underline{\pi}$ = 0.0022) [1], which indicates the adequacy of our alculating methodology.

The obtained values of observed heterozygosity (uH_E = 0.681-0.841) were close to the data obtained in other Russian populations of reindeer, e.g., H_E = 0.670 [14], H_E = 0.62-0.73 [16], H_E = 0.699 [15], H_E = 0.6491-0.7608 [13] and in deer populations from China, e.g., H_E = 0.650 [22]. Allelic diversity A^R = 6.188-8.760 was also coparable with the results of other researchers, e.g., A_R = 5.730-7.070 [14], A_R = 3.700-7.400 [21]. An analysis of the structure of the genetic network showed the differentiation of the Chukchi deer from the rest populations, which was demonstrated by V.R. Kharzinova et al. [17] who studied the genetic diversity of reindeer using single nucleotide sequence analysis. In the studies of other researchers, as well as in the present work, wild deer populations were characterized by a higher genetic diversity compared to domestic ones. The revealed pattern is most likely due to two factors, the breeding of domestic reindeer and migrations of the wild population which ensure a more intensive exchange of genetic material.

Thus, a combination of several markers revealed high genetic diversity in four breeds of domestic reindeer and the wild tundra reindeer population. In terms of genetic diversity, the sample of tundra wild reindeer exceeded the domestic population represented by individuals of the Nenets, Chukchi, Even breeds, as well as the Krasnoyarsk and Yakut populations of the Evenki breed. The phylogenetic analysis of the mitochondrial *CytB* gene nucleotide sequences did not reveal an isolated genetic structure among the reindeer populations. However, there is a clear divergence of the studied deer groups into two main clusters, which indicates the common origin of animals of the maternal line within one cluster. All statistical approaches that we used in the analysis of the genetic structure of the studied

reindeer by microsatellites (principal component analysis, phylogenetic and cluster analysis) revealed a clear genetic differentiation of domestic and wild reindeer. The results obtained are important both for improving reindeer selection and breeding, and as the basis for recommendations on nature management and protection of wild reindeer as the most important commercial resource traditional for the indigenous peoples of the Far North.

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