IECD 2022

MDPI

Proceeding Paper

Insights into the Nuclear and Mitochondrial Genetic Diversity of Local Tuva Population of Domestic Reindeer †

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† Presented at the 2nd International Electronic Conference on Diversity (IECD 2022)—New Insights into the Biodiversity of Plants, Animals and Microbes, 1–15 March 2022; Available online: https://iecd2022.sciforum.net/.

Abstract: In our study, we examined genetic diversity of Tuva reindeer based on both a high-density SNP genotypes analysis (n = 12) and a complete cytochrome b (cytb) sequences (1140 bp) (n = 6). To find out a possible genetic contribution toward the Tuva reindeer population structure, SNP genotypes and cytb sequences of all officially recognized breeds in Russia were added to our datasets. All genetic diversity indices calculated based on both nuclear and mitochondrial genomic data were lowest in Tuva population. *Fst*-based Neighbor-Net tree showed that Tuva population was most distant, while other breeds formed well-separated clusters according to their geographic locations. The low level of genetic diversity of the Tuva population observed in our study, based on studies involving a genome-wide approach, as well as a complete cytochrome b sequences, indicated the need to take appropriate measures to avoid negative consequences for this domestic reindeer.

Keywords: *Rangifer tarandus*; Tuva population; SNP; cytochrome b; genetic diversity

Academic Editor: Matthieu Chauvat

Published: 14 March 2022

1. Introduction

The reindeer of the Tuva population belong to one of the southernmost groups of domestic reindeer inhabiting the autonomous republic of Tyva (Tuva) in south-central Russia. Despite the fact that this group of reindeer does not have an official breed status, unlike the other four breeds [1], Tuvan reindeer are of great importance to the socio-cultural activities of the indigenous peoples. The South Siberian and North Mongolian groups of reindeer herders breed small herds of reindeer in the taiga and alpine tundra and use the reindeer mainly as beasts of burden and riding animals for hunting and for obtaining dairy products [2]. Recently, attention has been drawn to the dramatic rate of Tuva reindeer population decline from 15,000 in 1990 to 1400 individuals in 2019. Reduction in population size frequently leads to a decrease in genetic diversity [3]. Populations with low genetic diversity have limited capacities to adapt to fast changing environments [4] display lower fertility [5] and are prone to infectious diseases [6]. To effectively manage reindeer populations and overcome the negative effects of their decline, it is necessary to apply modern approaches for assessing and preserving the biodiversity of this important species [7]. Molecular genetic techniques have revolutionized our ability to characterize genetic variation and rationalize genetic selection [8]. Employment of nuclear markers is

one of the most powerful means for studying the genetic diversity, calculation of genetic distances, detection of bottlenecks and admixture because of high degree of polymorphism, random distribution across the genome, codominance and neutrality with respect to selection [9]. Mitochondrial DNA (mtDNA) is also considered a good tool for genetic diversity and evolutionary studies due to near-neutrality, maternal inheritance and clock-like nature of its substitution rate [10]. Herein, we aimed at using both a high-density SNP genotypes analysis and a complete cytochrome b (cytb) sequences to examine genetic diversity, to characterize population structure and to establish population relationships of Tuva reindeer population with all officially recognized reindeer breeds in Russia.

2. Experiments

2.1. Ethics Statement

The study does not involve any endangered or protected animals and all procedures were conducted according to the ethical guidelines of the L.K. Ernst Federal Science Center for Animal Husbandry. The tissue samples of domestic reindeer were collected by trained personnel under strict veterinary rules. The muscle tissue samples of the wild reindeer were collected during scientific expeditions after obtaining collection permits granted by the Department of Hunting of the Republic of Sakha in compliance with the Russian Federation Law No. 209-FZ of 24 July 2009.

2.2. Sample Collection and DNA Extraction

The biomaterial of the Tuva reindeer, selected for our study, was picked during the corral work on the herd and was stored at–20 °C until DNA extraction. Genomic DNA was extracted with Nexttec columns (Nexttec Biotechnology GmbH, Germany) following the manufacturer's instructions. The DNA samples were quantified using a method of visualization in bands by 1% agarose gel electrophoresis. The concentration of the dsDNA was measured on a Qubit 3.0 fluorimeter (Thermo Fisher Scientific (formerly Life Technologies), Wilmington, DE, USA). DNA purity was determined by evaluating the absorption ratio of A260/A280 on a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

2.3. SNP Genotyping and Data Analysis

DNA samples of 12 Tuvan reindeer (TUV) were genotyped using the Illumina BovineHD Genotyping BeadChip. After conducting all quality control steps, the final data set comprised 6783 SNPs. To find out a possible genetic contribution toward the Tuva reindeer population structure, SNP genotypes of all officially recognized breeds in Russia obtained in previous studies [1] were employed in the current dataset, namely, the Nenets (NEN, n = 21), the Even (EVN, n = 13), the Evenk (EVK, n = 12) and the Chukotka (CHU, n = 12). Additionally, SNP genotypes of 10 wild reindeer were included in the analyses as an outgroup (WLD). PLINK 1.07 [11], SplitsTree 4.14.5 [12] software and R packages "diveRsity" (StAMMP) [13] and 'ggplot2' [14] were used for statistical analyses.

2.4. Cytochrome b Gene Sequences and Data Analysis

DNA samples of the Tuva reindeer (n = 6) as well as the Nenets (NEN, n = 12), the Even (EVN, n = 8), the Evenk (EVK, n = 6), the Chukotka (CHU, n = 5) breeds and the wild population (WLD, n = 10) were analyzed by nucleotide sequence analysis of the cytochrome b (cytb) gene. The whole sequences of cytb gene (1140 bp) was PCR-amplifed and sequenced by the Sanger method. The alignment was performed using the MUSCLE algorithm [15] in MEGA 7.0.26 software [16]. Arlequin 3.5.2.2 [17], DnaSP 6.12.01 [18] programs and SplitsTree 4.14.5 [12] software were used for statistical analyses.

3. Results

The genetic diversity parameters estimated based on the SNP genotypes and cytb gene sequences of all studied groups of reindeer are listed in Table 1 and Table 2.

Table 1. Parameters of genetic diversity of the studied groups of reindeer calculated from SNP genotypes.

Breed/Population [1]	n [2]	A_R [3]	H_O [4]	uH_E [5]	uF_{IS} [6]
CHU	12	1.597 ± 0.006	0.181 ± 0.002	0.184 ± 0.002	0.011[0.004;0.018]
EVN	13	1.676 ± 0.005	0.195 ± 0.002	0.201 ± 0.002	0.028[0.021;0.035]
EVK	12	1.69 ± 0.005	0.2 ± 0.002	0.203 ± 0.002	0.013[0.006;0.02]
NEN	21	1.63 ± 0.005	0.189 ± 0.002	0.193 ± 0.002	0.017[0.011;0.023]
TUVA	12	1.547 ± 0.006	0.169 ± 0.002	0.173 ± 0.002	0.019[0.011;0.027]
WLD	10	1.678 ± 0.006	0.193 ± 0.002	0.199 ± 0.002	0.022[0.014;0.03]

[1] CHU, Chukotka; EVN, Even; EVK, Evenk; NEN, Nenets; TUVA, Tuva population; WLD, wild population; [2] n, sample size; [3] A_R, allelic richness; [4] H_O, observed heterozygosity; [5] uH_E, unbiased expected heterozygosity; [6] F_{IS}, inbreeding coefficient based on the difference between uH_E and H_O with a 95% confidence interval (CI; in square brackets).

Table 2. Parameters of genetic diversity of the studied groups of reindeer calculated from sequence of mitochondrial cytb gene.

Breed/Population [1]	n [2]	S [3]	H [4]	K [5]	HD [6]	π [7]
CHU	5	15	4	6.8	0.900 ± 0.161	0.00596 ± 0.00129
EVN	8	11	6	4.286	0.893 ± 0.111	0.00476 ± 0.00059
EVK	6	13	5	5.4	0.933 ± 0.122	0.00474 ± 0.00091
NEN	12	11	5	5.758	0.530 ± 0.136	0.00542 ± 0.00057
TUVA	6	10	5	4.533	0.933 ± 0.122	0.00398 ± 0.00110
WLD	10	26	9	7.511	0.978 ± 0.054	0.00659 ± 0.00079

[1] CHU, Chukotka; EVN, Even; EVK, Evenk; NEN, Nenets; TUVA, Tuva population; WLD, wild population; [2] n, sample number; [3] S, number of variable sites; [4] H, number of haplotypes; [5] K, average number of nucleotide differences; [6] HD, haplotype diversity; [7] π, nucleotide diversity.

As it follows from the data of Table 1, the lowest values of allelic diversity, as measured by allelic richness, were detected for reindeer of the Chukotka breed and the Tuva population: 1.597 and 1.547, respectively. A similar pattern was revealed regarding heterozygosity indices: the same groups of reindeer were inferior to others, in terms both of the observed (H_O) and of the unbiased expected uH_E heterozygosity: $H_{O\,CHU}$ = 0.181 and $H_{O\,TUVA}$ =0.169; $uH_{E\,CHU}$ = 0.184 and $uH_{E\,TUVA}$ =0.173. Values of uF_{IS} ranged between 0.011 for CHU and 0.028 for EVN and were positive for all groups.

As it follows from the data of Table 2, for all studied reindeer groups, 34 haplotypes and 86 variable sites were detected with a minimum number in the Tuvan population (S = 10). The lowest average number of nucleotide difference between haplotypes was found in EVN (K = 4.286) and TUVA (K = 4.533) while the highest was seen in domestic reindeer of the Nenets breed (K = 5.758) and in the wild population (K = 7.511). However, the Nenets breed exhibited a decline in haplotype diversity: HD = 0.530 while the reindeer of Tuva population showed a decline in nucleotide diversity: π = 0.00398.

Figure 1 presented the results of the neighbor-net analysis conducted based on pairwise F_{ST} values calculated from SNP genotypes (Figure 1A) and cytb gene sequences (Figure 1B).

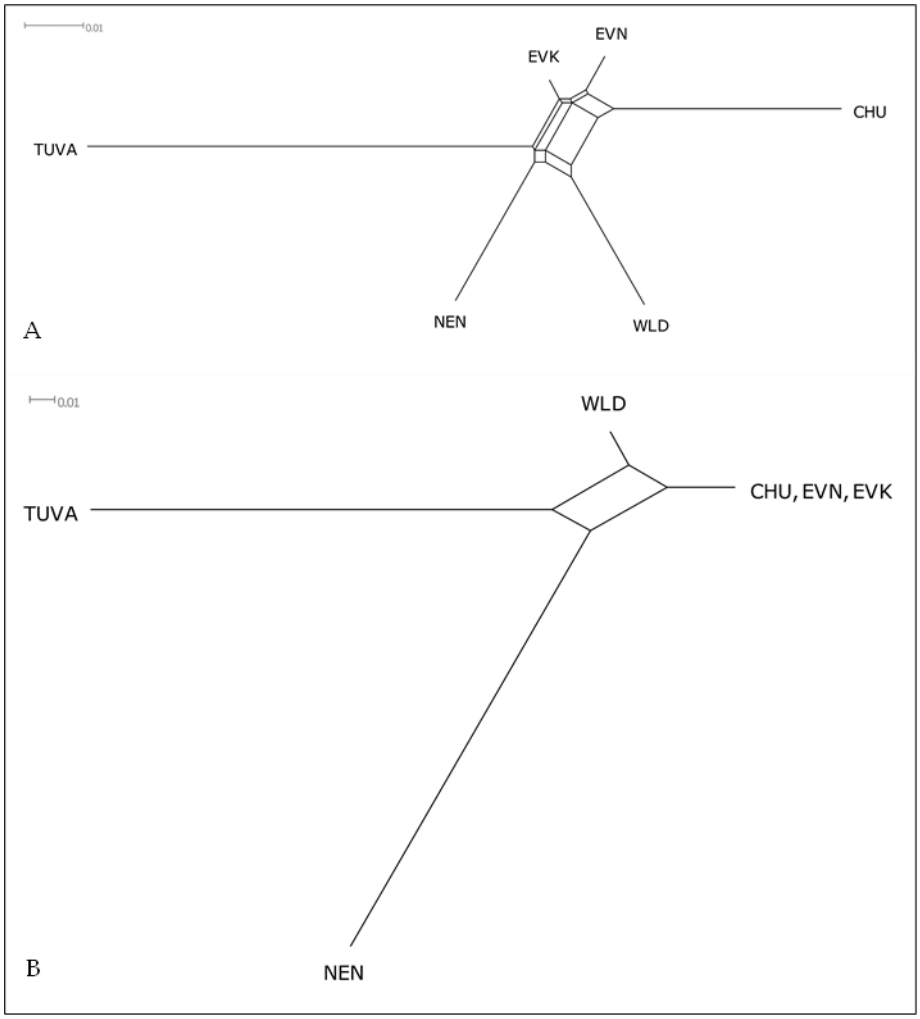

Figure 1. Neighbor-Net tree of the s reindeer based on F_{ST} genetic distances based on the SNP genotypes and cytb gene sequences. CHU, Chukotka breed; EVN, Even breed; EVK, Evenk breed; NEN, Nenets breed; TUVA, Tuva population; WLD, wild population.

A revealed consistency of the Neighbor-Net trees constructed based on genotypes of the nuclear and the mtDNA genomes, reflected a clear distancing of the Tuva population from the domestic reindeer, which are belong to officially recognized breeds and from the wild reindeer. The Nenets breed and the wild population were found to be separated by own branches while other three breeds were placed close to the each other.

4. Discussion

Despite the fact that the reindeer of Tuva population is less genetically studied compared to the others groups, the issues regarding its genetic diversity have been covered in some papers. Stolpovsky et al. [19], evaluating the genetic differentiation and phylogeny of Tuva reindeer in comparison with groups of domestic and wild animals revealed that this population was characterized by lower values for both allelic and genetic diversity parameters, which was consistent with the results obtained in our work. Furthermore, the authors stated that Tuva population had low migratory activity, which indicated its relatively isolated habitation and the process of domestication. A rather high level of genetic diversity in Tuvan reindeer was showed by the results of examination of the mitochondrial DNA D loop region sequences, based in which, in a sample of 29 individuals seven haplotypes were distinguished [2]. An incongruence between our findings and the mentioned above is suggestive of the decrease in the genetic diversity of this reindeer population over the course of time.

5. Conclusions

In our study, we succeed in a better understanding of the current genetic diversity and population structure of domestic Tuva reindeer inhabiting the south of Eastern Siberia using modern molecular genetic approaches such as a high-density SNP genotypes analysis and a complete cytochrome b (cytb) sequences. We believe that our findings will be useful in solving problems of conservation and increase in the number of this important species for the normal functioning of ecosystems and for the life and culture of indigenous peoples.

Author Contributions: Conceptualization, K.V.R., D.A.V. and Z.N.A.; methodology, D.A.V., U.M., K.E., S.A.D., K.S., B.N.V., M.I.; software, D.A.V., B.N.V., U.M., K.E., K.S., M.I.; validation, D.A.V., B.N.V., U.M., K.E., K.S., M.I.; formal analysis, K.V.R., Z.N.A.; investigation, K.V.R., D.A.V., B.N.V., S.A.D.; resources, K.V.R.; data curation, K.V.R.; writing—original draft preparation, K.V.R.; writing—review and editing, Z.N.A.; visualization, K.V.R., D.A.V.; supervision, K.V.R., Z.N.A.; project administration, K.V.R., Z.N.A.; funding acquisition, K.V.R., D.A.V., Z.N.A. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Russian Science Foundation (RSF) within Projects no. 21-16-00071 (the 777k SNP genotyping, a complete cytochrome b (cytb) sequences of all reindeer groups. The studies of the Chukotka, the Even, the Evenk, the Tuva population and wild reindeer population) and the framework of the Ministry of Science and Higher Education of the Russian Federation (the studies of the Nenets breed).

Institutional Review Board Statement: The study was approved by the Commission on the Ethics of Animal Experiments of the L.K. Ernst Federal Science Center for Animal Husbandry (the protocol № 4 of 25 December 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to V.I. Fedorov, K.A. Laishev, T.M. Romanenko, A.V. Senchik, L.D. Schimit, I.M. Okhlopkov and N.V. Mamaev for their help in gathering reindeer samples. The authors thank the reindeer herders for their permission to collect samples. The authors would like to express gratitude to the reviewers for their attention to this paper and for their constructive suggestions.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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