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Genetic Characteristics of Wild and Domestic Reindeer Based on the Analysis of mtDNA Cytb Gene †

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† Presented at the 1st International Electronic Conference on Biological Diversity, Ecology and Evolution, 15–31 March 2021; Available online: https://bdee2021.sciforum.net/.

Abstract: Reindeer *(Rangifer tarandus)* in Russia is presented by a number of wild and domestic populations. Both wild and domestic reindeer play an important role in lives of indigenous people. Investigation of biodiversity of this species is very important for developing conservation and breeding programs. Our research was aimed at determining haplotype variability and genetic diversity of the wild and domestic reindeer. MtDNA cytb gene (1140 bp) of the wild reindeer from the Taimyr region (WLD, n = 16) and domestic reindeer from the Nenets-Autonomous district (NEN, n = 15) and Tuva Republic (TUVA, n = 5) were sequenced. It was shown that the number of variable sites was higher in WLD—35, than in NEN and TUVA—17 and 5, respectively. Haplotype diversity was 0.958 ± 0.036 in WLD, 0.762 ± 0.096 in NEN and 0.900 ± 0.161 in TUVA. Average number of nucleotide differences was 7.942 in WLD, 4.324 in NEN and 2.800 in TUVA. The median-joining network revealed that WLD and NEN had shared haplotypes with each other, while TUVA had private haplotypes. Thus, the obtained results of the current study demonstrated that the wild reindeer were characterized by higher genetic diversity than both domestic groups. Tuva reindeer clustered separately from the other populations and were characterized by higher haplotype diversity than the Nenets conspecifics that had a higher average number of nucleotide differences.

Keywords: *Rangifer tarandus*; cytochrome b; mitochondrial DNA; genetic diversity

Citation: Kharzinova, V.; Dotsev, A.; Bardukov, N.; Upadhyay, M.; Krebs, S.; Kunz, E.; Medugorac, I., I; Zinovieva, N. Genetic Characteristics of Wild and Domestic Reindeer Based on the Analysis of mtDNA Cytb Gene. **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

Reindeer *(Rangifer tarandus L. 1758)* is an ungulate that inhabits the whole circumpolar area of the northern hemisphere, and it is the last animal to be domesticated by humans [1,2]. According to the Arctic Council's project "Sustainable Reindeer Breeding" [3], Russia has approximately 2/3 of the world's domestic reindeer stock browsing in tundra, forest tundra, boreal forest (taiga), and mountainous regions covering over 3 million km2. The domestic population of the reindeer is represented by four officially recognized breeds, as well as breed groups that differ from each other in terms of their exterior constitutional characteristics. Along with domestic reindeer, the small peoples of the North are able to be provided with food and necessary materials for housing and clothing by the wild reindeer.The largest wild population inhabits the central Arctic—Taimyr, Evenkia, Yakutia—about 480 thousand animals. Nowdays, reindeer population number has drastically decreased, likely because of changes in economic priorities, global climate change, and industrial development. 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. The genetic diversity and population structure of the reindeer in Russia have been described in detail based on the analysis of nuclear genome markers [4,5], while the current information on the genetic variation of the reindeer based on sequences of the mtDNA cytochrome b gene is still lacking. Herein, the current work was aimed at determining haplotype variability and genetic diversity of the wild and domestic reindeer based on the analysis of the of the mitochondrial cytochrome B (*cytb*) gene polymorphism

2. Experiments

2.1. Ethics Statement

The principles of laboratory animal care were followed, and all procedures were conducted according to the ethical guidelines of the L.K. Ernst Federal Science Center for Animal Husbandry. The wild reindeer muscle tissue samples were collected during scientific expeditions between 2019–2020 after obtaining collection permits granted by the Department of Hunting of the Taymyr Dolgano-Nenetsky District, in compliance with the Russian Federation Law No. 209-FZ of 24 July 2009. The tissue samples of domestic reindeer were collected by trained personnel under strict veterinary rules during the corral work on the herd throughout 2019–2020.

2.2. Sample Collection and DNA Extraction

A total of 36 individuals, including wild (n = 16) and domestic (n = 20) reindeer, were analyzed. The wild reindeer muscle tissue samples were represented by the Taymyr population from western Taymyr (WLD). The coordinate range of the covered area in Taymyr varied from 70° to 74° N and from 91 to 107° E. The domestic reindeer tissue samples were taken from the reindeer of the Nenets-Autonomous district (NEN, n = 15) and Tuva Republic (TUVA, n = 5). 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. Data Processing

The whole sequences of cytb gene (1140 bp) of of wild and domestic reindeer were analyzed by Sanger sequencing. To construct a median joining network [6], PopART 1.7 software [7] was applied. Determination of the best models of evolution was carried out separately for each nucleotide in the program PartitionFinder 2 [8] using the Akaike information corrected criterion (AICc) [9]. DnaSP 6.12.01 program [10] was used to calculate genetic diversity parameters: number of polymorphic sites (S), average number of nucleotide differences (K), number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π).

3. Results

Genetic diversity indices of reindeer populations calculated from nucleotide sequence of mitochondrial cytb gene are presented in Table 1.

Table 1. Genetic diversity indices of reindeer populations calculated from nucleotide sequence of mitochondrial cytb gene.

Breed/Population	Code	n	S	H	HD	K	π
Nenets domestic	NEN	15	17	6	0.762 ± 0.096	4.324	0.00379 ± 0.0006
Taimyr wild	WLD	16	35	12	0.958 ± 0.036	7.942	0.00697 ± 0.00052
Tuva domestic	TUVA	5	5	4	0.900 ± 0.161	2.800	0.00246 ± 0.00054

n—sample number; S—number of variable sites; H—number of haplotypes; HD—haplotype diversity; k—average number of nucleotide differences; π—nucleotide diversity.

It was shown that in total, 22 haplotypes were detected in 36 reindeer with the prevalence of this indicator in the wild individuals. The number of variable sites was higher in WLD – 35, than in NEN and TUVA—17 and 5, respectively. The distribution of the haplotype diversity (Hd) had an interesting pattern – the wild and Tuva reindeer showed the practically equal maximum values of the indicator: 0.958 ± 0.036 in WLD and 0.900 ± 0.161 in TUVA. However, TUVA reindeer group characterized by the the lowest values of the average number of nucleotide differences (K) as well as nucleotide diversity (π).

The median-joining network of the studied reindeer populations based on the analysis of mtDNA cytb gene polymorphism is depicted in Figure 1.

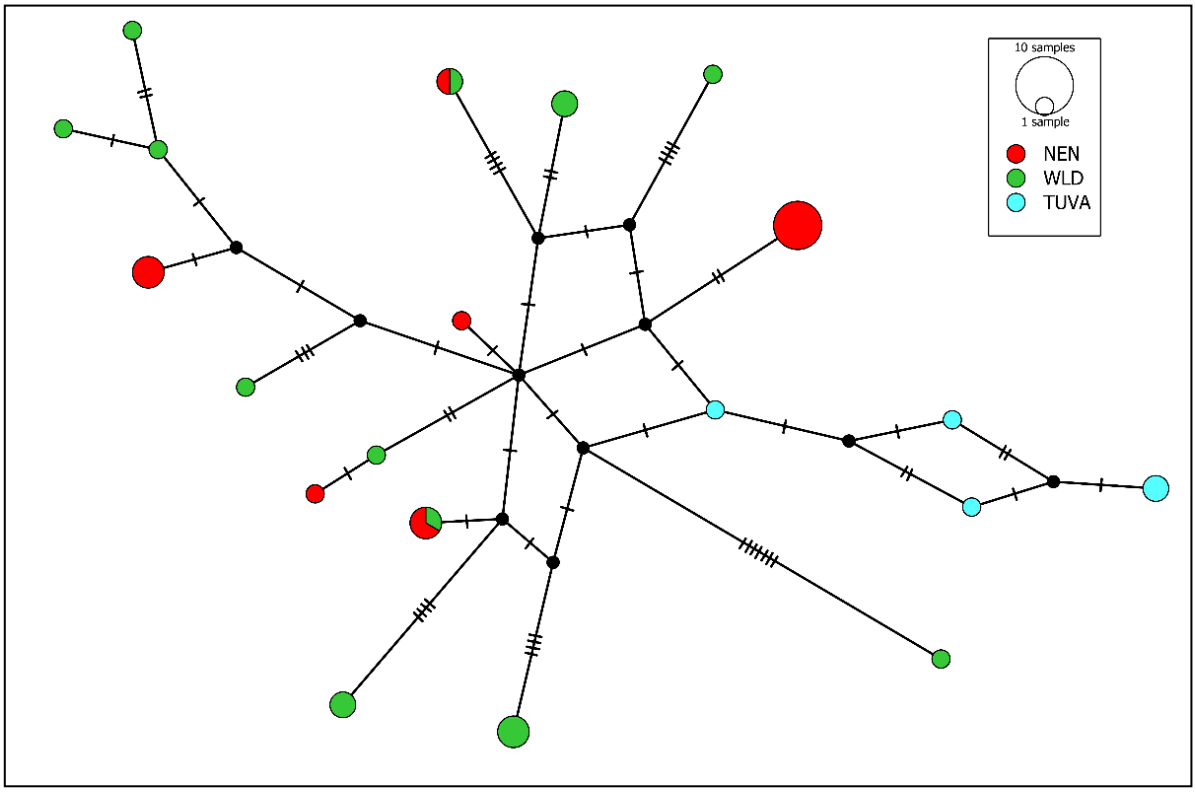

Figure 1. Median joining network of reindeer populations based on the analysis of mtDNA cytb gene polymorphism.

It was revealed that the wild population and the Nenets reindeer breed shared haplotypes with each other and while TUVA had the private haplotypes. Moreover, TUVA reindeer group was the most separated from the others.

4. Discussion

Reindeer is an essential element of Russia's Far North ecosystem that plays an important role in the economy of the North, providing employment and well-being of the small peoples of this vast region, being an object of both fishing and breeding. In addition, it is one of the few species in which the wild form coexists with the domestic one. Reindeer genetic diversity across populations has long fascinated scientists and has been described

using different types of genetic markers. The first results were obtained in the 1960s and wide application of the gel electrophoresis method followed [11]. Further, an application of microsatellite and SNP markers allowed to determine generally high level of differences among the domestic and wild reindeer groups. Examining mitochondrial DNA to determine levels of genetic diversity in populations and phylogenetic relationships between groups of reindeer at the present stage was also investigated using mtDNA analysis. Likewise, analyzing the sequences of the mtDNA control region, Kvie K.S. et al. [12] found that reindeer from the Arctic archipelagos of Svalbard, Franz Josef Land and Novaya Zemlya are phylogenetically closely related, which indicates their common origin. Cronin M.A. et al. [13] studied the variability of D-loop sequences in populations of caribou and domestic reindeer inhabiting the United States and Canada. As a result, it was revealed that caribou and domesticated reindeer differentiate from each other in terms of the frequencies of mitochondrial haplotypes. The results of the current study based on the analysis of mtDNA cytb gene also showed differentiation of domestic and wild reindeer populations in accordance with the frequencies of mitochondrial haplotypes.

5. Conclusions

The obtained results of the current study, based on the analysis of mitochondrial cytochrome b gene sequences, demonstrated that the wild reindeer were characterized by higher genetic diversity than both domestic groups. Tuva reindeer clustered separately from the other populations and were characterized by higher haplotype diversity than the Nenets conspecifics that had a higher average number of nucleotide differences. Our findings will assist in the programs of biodiversity conservation of this essential element of Russia's Far North.

Author Contributions: N.Z., A.D. and V.K. conceived and designed the experiments; V.K., A.D. and N.B. performed the experiments; A.D., M.U., S.K. and E.K. analyzed the data; I.M.I contributed reagents/materials/analysis tools; V.K. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: The study was supported by Ministry of Science and Higher Education of the Russian Federation within theme No. 0445-2019-0024.

Conflicts of Interest: The authors declare no conflict of interest. Ministry of Science and Higher Education of the Russian Federation had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:
mtDNA cytb gene: mitochondrial cytochrome b gene

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