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Article

Resolving the Taxonomic Status of Chukotkan Snow Sheep (*Ovis nivicola*) Using Genome-Wide Single-Nucleotide Polymorphism (SNP) and Mitochondrial Data

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Abstract

The intraspecific taxonomy of snow sheep (*Ovis nivicola*) is one of the most controversial issues in Caprinae systematics. Although eight subspecies have been described using morphological traits, the validity of several taxa, particularly those in the eastern part of their geographical range, remains disputed. We investigated the phylogenetic relationships and genetic diversity of snow sheep in the Chukotka Autonomous Okrug using genome-wide single-nucleotide polymorphism (SNP) data (935 loci after filtering) and complete mitochondrial genomes from 57 individuals collected across the Russian Far East (Chukotka Autonomous Okrug, Kamchatka Krai, Magadan Oblast, and Republic of Sakha (Yakutia)). Post SNP-genotyping nuclear genomic studies using principal component analysis (PCA), ADMIXTURE, pairwise F_{ST} and Neighbor-Net revealed two clearly differentiated groups within Chukotka. One included individuals from the Koryak Mountains that are genetically indistinguishable from the Koryak subspecies (*O. n. koriakorum*) of northern Kamchatka. The other one encompassed individuals from the Anadyr Plateau and the Chukotka Mountains cluster with the Okhotsk subspecies (*O. n. alleni*) of the Kolyma Mountains. Bayesian phylogeny of complete mitochondrial genomes fully corroborated the nuclear results: Koryak Mountains samples formed a monophyletic clade, while Anadyr–Chukotka samples grouped with Kolyma Mountains individuals. Genetic diversity indices ($_uH_E$, A_R, F_{IS}) in both Chukotka groups were comparable to other studied populations and showed no signs of inbreeding depression. Our results provide important insights and can be used to develop science-based strategies for preserving the population-genetic diversity of snow sheep.

1. Introduction

The snow sheep (*Ovis nivicola* Eschscholtz, 1829), an endemic species of the mountain systems of the Russian Far East and Eastern Siberia, was first described by Johann Friedrich Eschscholtz in 1829. Its geographical range encompasses Kamchatka, Chukotka, Yakutia, the Magadan and Amur Oblasts, and Khabarovsk Krai. Isolated populations inhabit the Kodar Mountains (Zabaykalsky Krai) and the Putorana Plateau (Krasnoyarsk Krai). The snow sheep's closest taxonomic relatives are the North American wild sheep—the bighorn (*O. canadensis* Shaw, 1804) and the thinhorn (*O. dalli* Nelson, 1884)—all of which are united in the subgenus *Pachyceros* Gromova, 1936.

Among all populations of *O. nivicola*, the Chukotka population remains one of the most poorly resolved and controversial taxonomic units, primarily because of the region's remoteness and the limited availability of biological material. Although its presence in the region has been known since the late 19th century [1], it was not described as a distinct subspecies until 1990 [2], and this status is not universally accepted by researchers. Previously, the Chukotkan population was typically classified as belonging to the Yakut subspecies [3–5]. The distinctiveness of the Chukotka snow sheep and its potential subspecific status were first proposed by Chernyavsky (1984) [6], who noted that it exceeds the Koryak subspecies in skull, body, and horn dimensions. Furthermore, its coloration lacks the yellowish tint characteristic of the Yakut subspecies and the white patches behind the axillae typical of the Okhotsk subspecies. However, in later work, Chernyavsky (2004) [7] criticized the description of the Chukotka subspecies by Zheleznov, pointing to violations of the rules of zoological nomenclature. He noted that the diagnosis was not supported by specific numerical data indicating the number of skulls measured. He also noted that information on the precise collection locality of the type specimen and the name of the collector was absent.

This taxonomic uncertainty is reflected in its conservation status. The Chukotka snow sheep is currently listed in the Red Data Book of the Russian Federation; however, in different sources, it is classified either as a separate subspecies [8,9] or as a Chukotkan population of the Yakut subspecies [10].

Historically, the primary taxonomic characters used for subspecies delineation were morphological parameters such as the size and shape of horns, characteristics of the bony horn cores, as well as pelage pattern and coloration. However, according to a number of scientists, notably Raul Valdez (1985) [11], these traits are variable. For instance, horn growth is influenced by environmental factors and habitat conditions, and fur color can change with the seasons.

To resolve contentious issues in systematics, molecular genetic methods are now widely used. The analysis of nuclear and mitochondrial DNA allows for a more objective determination of phylogenetic relationships. For example, genetic studies revealed that specimens from Uzbekistan, long classified as urial (*O. vignei*), in fact belong to argali (*O. ammon*) and show evidence of urial admixture [12]. Similarly, in snow sheep, these approaches provided the definitive basis for recognizing the Kharaulakh population as a distinct subspecies [13,14]. The research demonstrated that this group, inhabiting northern Yakutia, has a distinct origin from other studied populations and that snow sheep from the nearby Orulgan Range carry genetic components of both the Kharaulakh sheep and other populations of the Yakut subspecies. Thus, genetic research represents a promising tool

for refining the intraspecific systematics of the snow sheep and resolving long-standing questions that have persisted since the species' initial description.

The aim of the present study was therefore to clarify the intraspecific phylogeny and assess the genetic diversity of the snow sheep inhabiting the Chukotka Peninsula based on the analysis of genome-wide single-nucleotide polymorphism (SNP) data and complete mitochondrial genomes. Specifically, we tested whether the Chukotka population forms a genetically distinct lineage consistent with subspecific status or whether it is genetically indistinguishable from adjacent populations within the species' range.

2. Materials and Methods

2.1. Sample Collection and Molecular Analyses

The study material consisted of muscle tissue samples from snow sheep collected in various regions of the Russian Far East: Kamchatka Krai, Magadan Oblast, and the Republic of Sakha (Yakutia) (Figure 1). Samples from the Chukotka Autonomous Okrug (AO) were obtained from museum skins and bones (modern finds), as the Chukotka subspecies is listed in the Red Data Book and protected from direct sampling.

Figure 1. Sampling locations of snow sheep (*Ovis nivicola*) populations analyzed in this study. Colored triangles indicate sampling localities of the studied populations, while black points represent populated places.

Within the Chukotka Autonomous Okrug, two geographical groups were identified: individuals from the Koryak Mountains (KORYAK (Chukotka), n = 7) and those from the Anadyr–Chukotka region (CHUKOTKA, n = 8), which included samples from the Anadyr Plateau and the Chukotka Mountains. In Kamchatka Krai, samples from the Koryak Mountains were assigned to the Koryak snow sheep group (KORYAK, n = 8), while individuals from the Sredinny Range were assigned to the Kamchatkan snow sheep (KAMCHATKA, n = 8). Samples from Magadan Oblast were classified as representatives of the Okhotsk subspecies (OKHOTSK, n = 10). In Yakutia, two populations were studied:

from the Suntar-Khayata Range (YAKUTIAN (Suntar-Khayata), n = 8) and from the Moma (Momsky) Range (YAKUTIAN (Momsky), n = 8). The total sample size was 57 individuals, enabling a comparative analysis of the different geographical groups of this species (Table 1).

Table 1. Sample information for the molecular genetic analysis of snow sheep (*Ovis nivicola*) from the Chukotka Autonomous Okrug and other reference populations.

Population (Subspecies)	Region	Sample Size (n)		Reference *
		Genome-Wide SNP Analysis	Mitochondrial Genome Analysis	
Kamchatka *O. n. nivicola*	Sredinny Range (Kamchatka Krai)	8	4	This study
Koryak *O. n. koriakorum*	Koryak Mountains (Kamchatka Krai)	8	4	This study
Koryak (Chukotka) *O. n. koriakorum*	Koryak Mountains (Chukotka Autonomous Okrug)	7	4	This study
Chukotka *O. n. tschuktschorum*	Chukotka Mountains, Anadyr Plateau (Chukotka Autonomous Okrug)	8	4	This study
Okhotsk *O. n. alleni*	Kolyma Mountains (Magadan Oblast)	10	4	This study
Yakutia (Suntar-Khayata) *O. n. lydekkeri*	Suntar-Khayata Range (Republic of Sakha (Yakutia))	8	4	Dotsev et al. (2018) [13]
Yakutia (Momsky) *O. n. lydekkeri*	Moma (Momsky) Range (Republic of Sakha (Yakutia))	8	4	Dotsev et al. (2018) [13]

Notes: * Reference is given for the genome-wide SNP analysis methodology. All complete mitochondrial genome sequences presented here were newly determined for this study.

All samples were initially preserved in 95% ethanol following collection and subsequently stored at −20 °C in the laboratory until processing. Genomic DNA (gDNA) extraction was performed using Nexttec columns (Nexttec Biotechnology GmbH, Leverkusen, Germany) according to the manufacturer's protocol. The extracted gDNA was used for both nuclear genome genotyping and whole-genome sequencing (WGS) to assemble mitochondrial DNA (mtDNA) genomes. The analyses for the nuclear genome and mtDNA differed: the nuclear genome was genotyped using the Illumina OvineSNP50 and OvineHD arrays designed for domestic sheep (*O. aries*), while complete mitochondrial genomes were assembled from the WGS data.

2.2. Sequence Data Processing and Bioinformatic Analyses

Quality control of the merged genotyping data was performed using PLINK 1.9 [15]. All studied samples had at least 90% of SNPs successfully genotyped (--mind 0.1). SNPs with unknown positions, as well as markers genotyped in less than 90% of individuals (--geno 0.1), with a minor allele frequency of <5% (--maf 0.05), and those in linkage disequilibrium (--indep-pairwise 50 5 0.5) were excluded from the analysis.

After the two Illumina arrays were merged and quality control was performed, a set of 935 polymorphic loci was selected for all downstream analyses.

Principal component analysis (PCA) was also performed in PLINK 1.9, and the results were visualized using the R package ggplot2 (version 4.0.0) [16]. Cluster analysis was con-

ducted using ADMIXTURE v1.3 [17] for K = 1–10, with ten independent replicate runs performed for each K using different random seeds to assess stability of inferred ancestry components. Cross-validation (CV) with 10 folds was applied to evaluate model fit. Replicate Q matrices were aligned and visualized using CLUMPAK [18] (https://clumpak.evolseq.net/, accessed on 15 December 2025) to confirm consistency of cluster assignments across runs. Final results were plotted with the R package pophelper [19]. Phylogenetic relationships were inferred using the Neighbor-Net algorithm in SplitsTree4 [20] based on pairwise F_{ST} genetic distances [21] calculated with the R package StAMPP [22]. Genetic diversity indices were calculated using the R package diveRsity [23]. Multilocus heterozygosity (MLH) was calculated using the R package inbreedR [24].

The complete mitochondrial genome was assembled using a snow sheep reference sequence from the NCBI GenBank database (NC_039431) [25]. For this purpose, raw sequencing reads were aligned to the reference using bwa-mem2 [26] and subsequently processed with SAMtools [27].

Alignment of the mitochondrial genomes was performed with MUSCLE 3.8.31 [28]. The best-fit nucleotide substitution models were determined using PartitionFinder 2 [29]. A phylogenetic tree was inferred from a concatenated alignment of two rRNA and 13 protein-coding genes using the Bayesian algorithm in BEAST 2.7.7 [30] and visualized in FigTree 1.4.2. The thinhorn sheep (*O. dalli*) reference sequence NC_039432 from the NCBI database [25] was used as an outgroup.

A map showing sample collection sites was created using the R package maps [31].

3. Results

3.1. Population Structure and Genetic Differentiation

Principal component analysis (PCA; Figure 2A,B) showed that the first three principal components accounted for 20.94% (PC1), 11.22% (PC2), and 7.66% (PC3) of the genetic variance. All studied specimens were assigned to clusters corresponding to their populations. The group most closely related to the snow sheep from the Anadyr–Chukotka region was the Okhotsk subspecies, with the clusters of these two populations partially overlapping. An overlap was also observed between the cluster of the Okhotsk sheep and the Yakutian population from the Suntar-Khayata Range. Samples collected in the Chukotka Autonomous Okrug from the Koryak Mountains were genetically aligned with the Koryak subspecies of snow sheep.
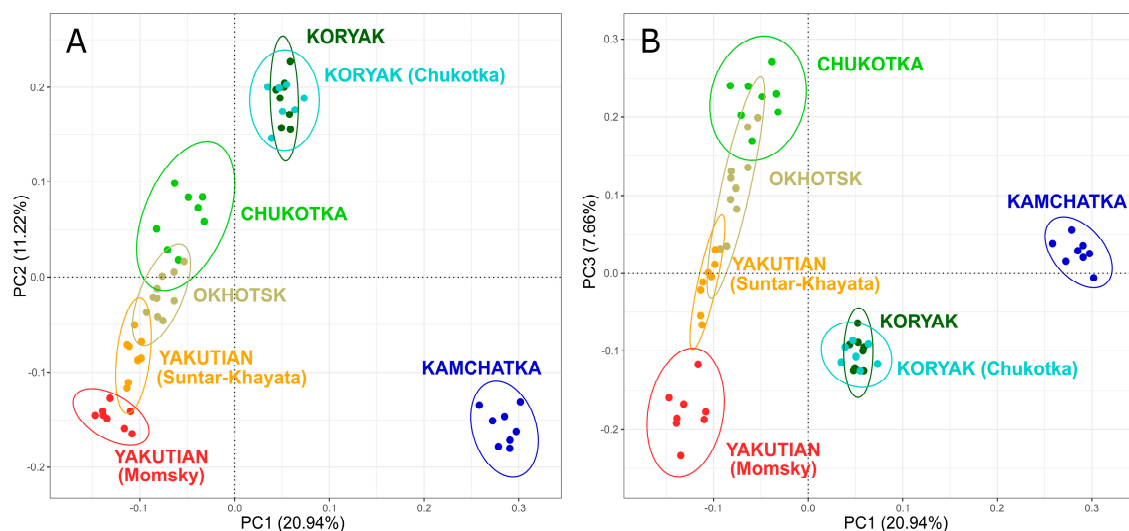

Figure 2. Principal Component Analysis (PCA) of snow sheep populations based on genome-wide SNP genotyping. (**A**) Projection on PC1 and PC2. (**B**) Projection on PC1 and PC3.

To investigate the composition of ancestral genetic components in the studied snow sheep populations, we performed a cluster analysis using the ADMIXTURE algorithm for K = 1–10, assuming multiple ancestral populations. CV error values were calculated for each K, revealing a minimum at K = 3 (CV error = 0.48429), which indicates that three ancestral components best explain the population structure (Figure 3). Ten independent replicate runs for each K converged to a single mode (10/10), and barplots generated by CLUMPAK confirmed the stability of cluster assignments, demonstrating that the observed patterns of ancestry are robust and reproducible.
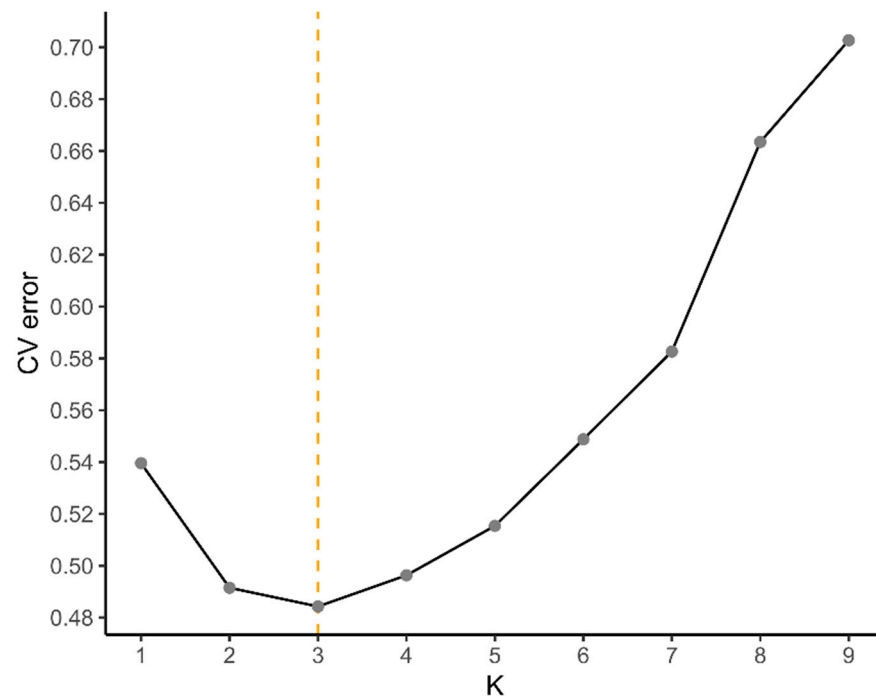

Figure 3. Cross-validation (CV) error plot for determining the most likely number of ancestral populations (K) in the ADMIXTURE analysis. The orange vertical line represents the optimal K as inferred from the CV error.

At K = 2 (CV error = 0.49150), distinct clusters corresponded to the Kamchatka and Yakutian populations, with no additional components detected (Figure 4). The Koryak population, including samples from the Koryak Mountains within the Chukotka Autonomous Okrug, displayed nearly equal contributions from both clusters. Individuals from the Chukotka and Okhotsk populations were primarily associated with the Yakutian cluster while exhibiting minor contributions from the Kamchatka cluster.

Increasing the number of clusters to three led to a homogeneous cluster for the Koryak population. The Anadyr–Chukotka and Okhotsk populations were mainly associated with the Yakutian component, but they also carried additional contributions from the Koryak cluster.

When four ancestral components were considered (K = 4, CV error = 0.49633), the Anadyr–Chukotka and Okhotsk populations together formed a distinct cluster. However, several individuals from the Okhotsk population retained genomic components characteristic of the Yakutian cluster.

Pairwise F_{ST} genetic distances between the studied snow sheep populations (Table 2) revealed genetic identity between the populations of Koryak sheep inhabiting the northern part of Kamchatka Krai and the Chukotka Autonomous Okrug. For the Anadyr–Chukotka snow sheep, F_{ST} values ranged from low (F_{ST} = 0.053 with the Okhotsk subspecies) to high

(F_{ST} = 0.326 with the Kamchatka subspecies). The genetic distances from the Koryak and Yakutian subspecies were moderate (F_{ST} = 0.113 and 0.095, respectively).
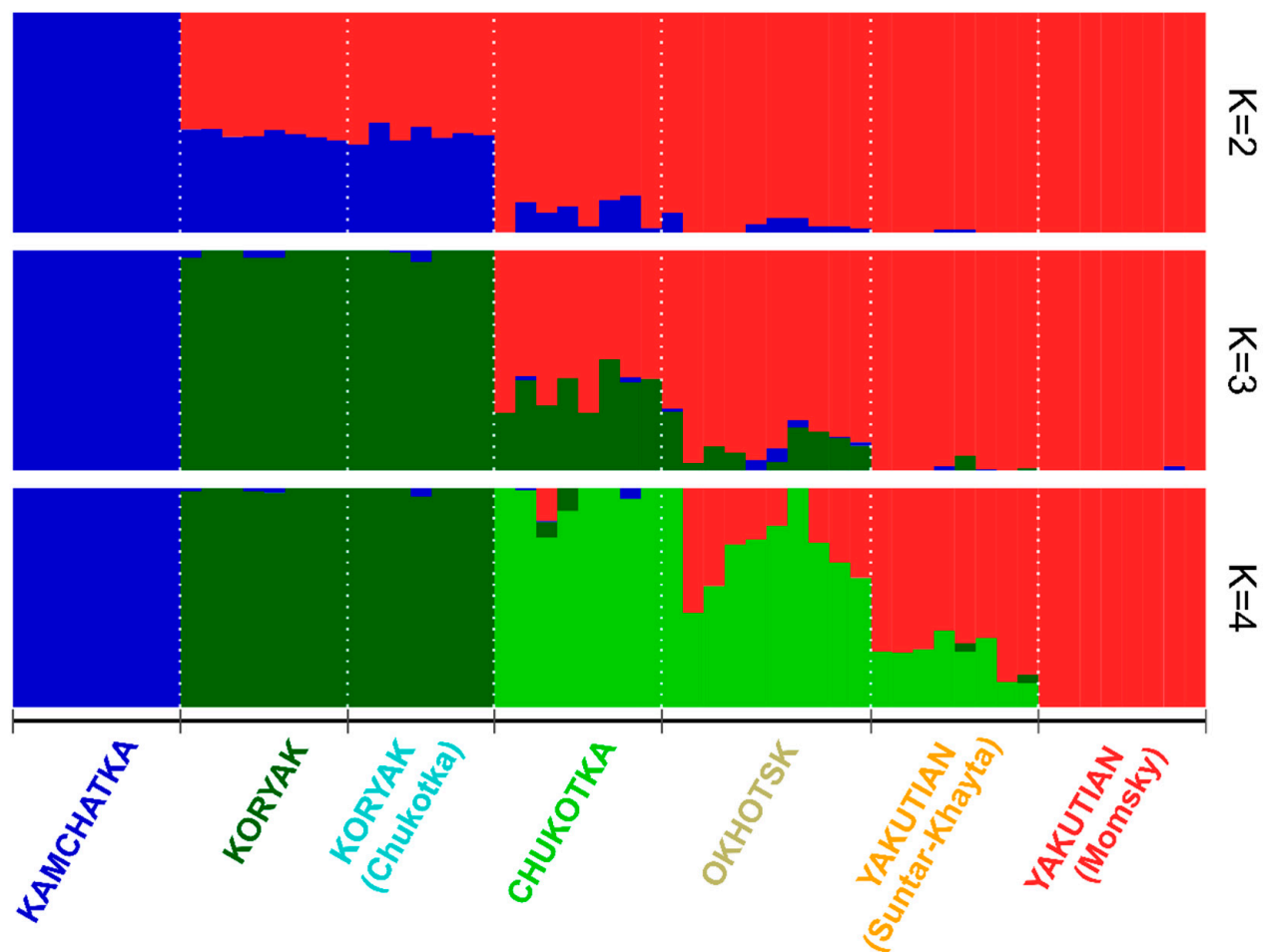
Figure 4. Population structure analysis of snow sheep using the ADMIXTURE algorithm. Each vertical bar represents an individual partitioned into colored segments that represent the proportion of its ancestry from K inferred ancestral populations.

Table 2. Pairwise F_{ST} genetic distances between studied populations (subspecies) of snow sheep.

Populations (Subspecies)	1	2	3	4	5	6	7
1. Kamchatka *O. n. nivicola*	0						
2. Koryak *O. n. koriakorum*	0.265	0					
3. Koryak (Chukotka) *O. n. koriakorum*	0.262	0	0				
4. Chukotka *O. n. tschuktschorum*	0.326	0.113	0.109	0			
5. Okhotsk *O. n. alleni*	0.309	0.111	0.110	0.053	0		
6. Yakutian (Suntar-Khayata) *O. n. lydekkeri*	0.331	0.133	0.129	0.095	0.051	0	
7. Yakutian (Momsky) *O. n. lydekkeri*	0.382	0.188	0.182	0.147	0.089	0.079	0

A phylogenetic tree was constructed based on pairwise F_{ST} genetic distances using the Neighbor-Net algorithm (Figure 5). The placement of the studied populations on the dendrogram was consistent with the results obtained in the previous analyses.
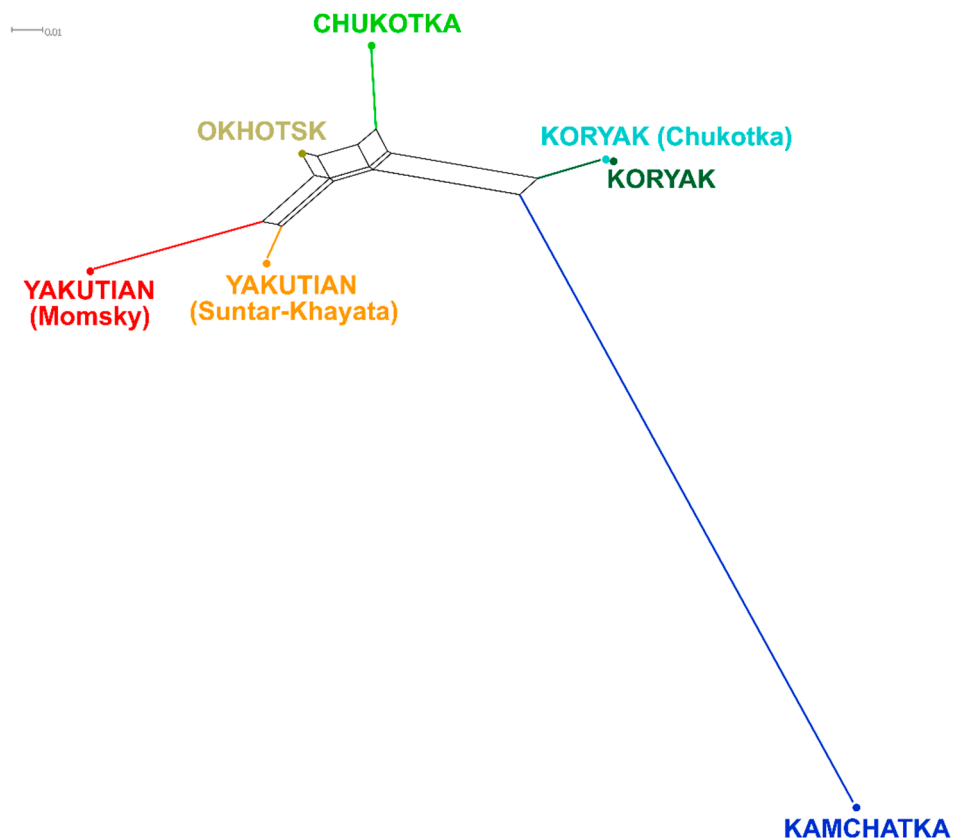

Figure 5. Neighbor-Net network constructed from pairwise F_{ST} genetic distances illustrating the phylogenetic relationships among snow sheep populations.

The resultant network clearly showed strong genetic differentiation of KAMCHATKA from all other major subspecies groups. Populations from the Koryak Mountains (both KORYAK and KORYAK (Chukotka)) formed a single cohesive cluster. The closest group to CHUKOTKA was OKHOTSK. Yakutian populations, although sharing a recent common ancestor, were nevertheless differentiated from each other.

3.2. Genetic Diversity

The genetic diversity indices (Table 3) for the Anadyr–Chukotka sheep were relatively high. Higher values of unbiased expected heterozygosity ($_uH_E$) and allelic richness (A_R) were recorded only in the Okhotsk sheep and the Yakutian sheep from the Suntar-Khayata Range. In the group of Koryak sheep from the Chukotka Autonomous Okrug, these indices were only slightly lower. The lowest level of genetic diversity was identified in the population of the Kamchatka subspecies ($p < 0.001$).

The inbreeding coefficient (F_{IS}) for the Anadyr–Chukotka snow sheep was 0.003 and did not significantly differ from zero, indicating that this population was in equilibrium. In contrast, a slight heterozygote deficiency ($F_{IS} = 0.031$), significantly different from zero, was detected in the Koryak sheep inhabiting the Chukotka Autonomous Okrug, suggesting a somewhat higher level of inbreeding. An elevated inbreeding coefficient was also identified in the Okhotsk snow sheep.

Table 3. Genetic diversity indices in the studied populations (subspecies) of snow sheep.

Population (Subspecies)	n	H_O	uH_E	F_{IS} [CI 95%]	A_R
Kamchatka *O. n. nivicola*	8	0.140 ± 0.007	0.143 ± 0.006	0.016 [−0.013; 0.045]	1.407 ± 0.016
Koryak *O. n. koriakorum*	8	0.223 ± 0.008	0.228 ± 0.007	0.018 [−0.007; 0.043]	1.622 ± 0.015
Koryak (Chukotka) *O. n. koriakorum*	7	0.225 ± 0.008	0.232 ± 0.007	0.031 [0.005; 0.057]	1.644 ± 0.016
Chukotka *O. n. tschuktschorum*	8	0.234 ± 0.008	0.234 ± 0.007	0.003 [−0.021; 0.027]	1.657 ± 0.015
Okhotsk *O. n. alleni*	10	0.217 ± 0.006	0.237 ± 0.006	0.065 [0.044; 0.086]	1.688 ± 0.014
Yakutian (Suntar-Khayata) *O. n. lydekkeri*	8	0.252 ± 0.007	0.251 ± 0.007	−0.004 [−0.027; 0.019]	1.688 ± 0.015
Yakutian (Momsky) *O. n. lydekkeri*	8	0.219 ± 0.007	0.217 ± 0.007	−0.004 [−0.027; 0.019]	1.627 ± 0.015

The allelic richness (A_R) values for both groups from the Chukotka territory were approximately at the same level as the other studied populations, with the exception of the Kamchatka subspecies, for which these values were significantly lower ($p < 0.001$).

The multilocus heterozygosity (MLH) values (Figure 6), calculated as the proportion of polymorphic loci to the total number of loci examined, clearly illustrated the level of genetic diversity within the studied populations.
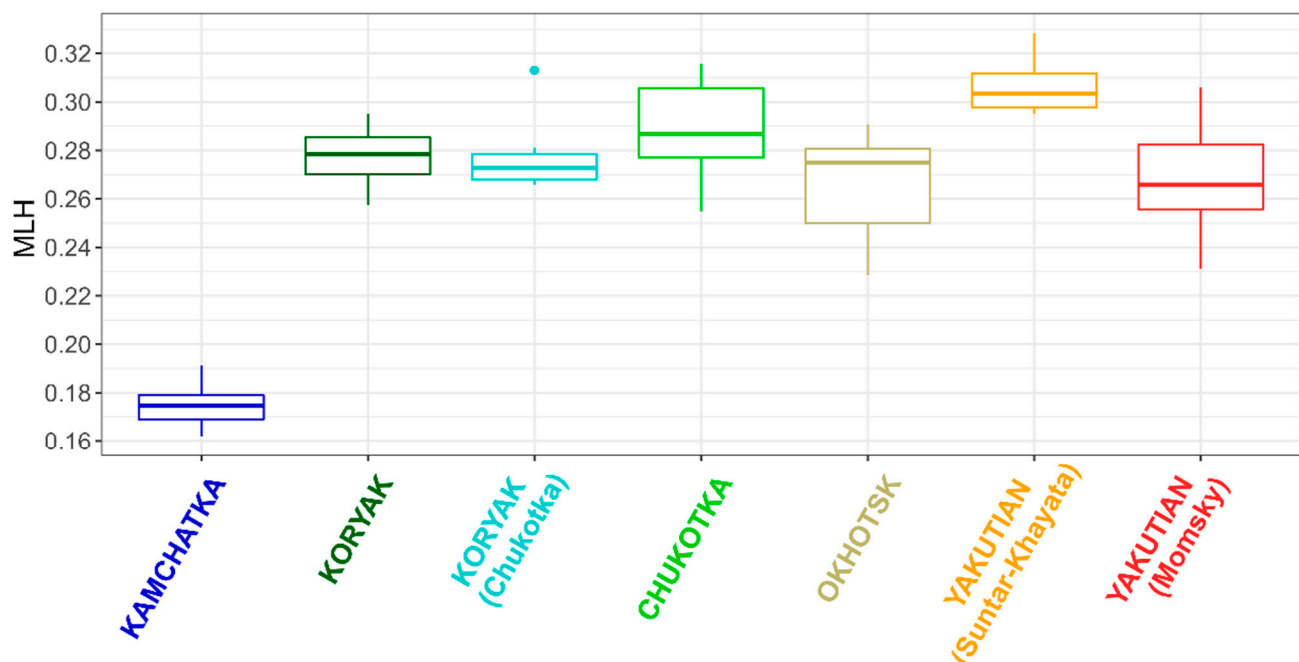
Figure 6. Multilocus heterozygosity (MLH) across studied snow sheep populations. The blue point indicates an outlier outside the range of the boxplot whiskers.

3.3. Phylogenetic Analysis of Mitochondrial Genomes

To provide a more comprehensive characterization of the genetic structure of snow sheep populations inhabiting the Chukotka Autonomous Okrug, we conducted an analysis

of mitochondrial genomes, which have a maternal mode of inheritance, i.e., different when compared to nuclear DNA.

A Bayesian phylogenetic tree constructed based on the complete mitochondrial genomes of the studied snow sheep populations is shown in Figure 7.
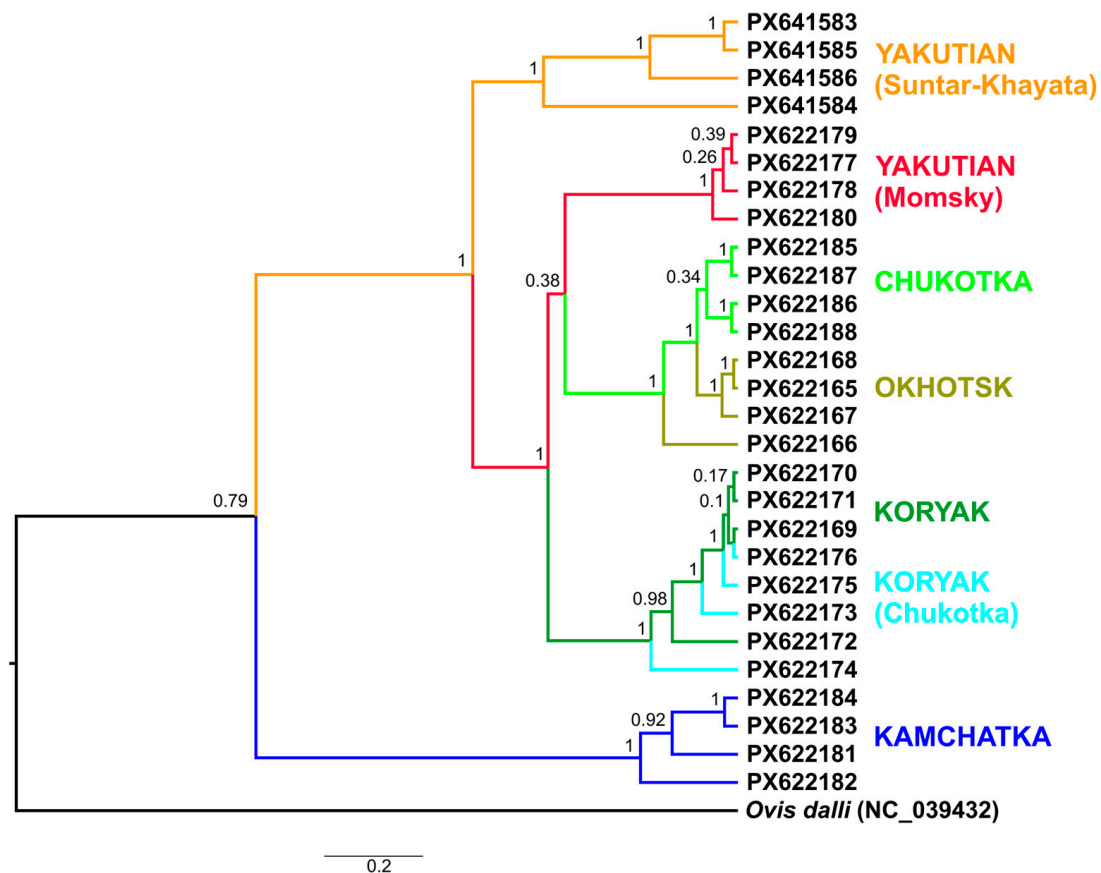

Figure 7. Bayesian phylogenetic tree of snow sheep based on complete mitochondrial genome sequences. The tree was reconstructed using BEAST 2.7.7 with the thinhorn sheep (*O. dalli*) as an outgroup. Node values represent posterior probabilities. Accession numbers of mitochondrial genome sequences deposited in NCBI GenBank are indicated for each sample.

Analysis revealed that the samples of Anadyr–Chukotka snow sheep were located within a cluster formed by the Okhotsk subspecies, with no significant differentiation observed between these two groups. Snow sheep inhabiting the Koryak Mountains, both within Kamchatka Krai and the Chukotka Autonomous Okrug, were grouped into a single common cluster. Among all the studied groups, the Yakutian population from the Momsky Range showed the greatest phylogenetic similarity to the last common ancestor of the snow sheep populations from the Chukotka Autonomous Okrug.

Thus, the mitochondrial DNA analysis, consistent with the nuclear DNA data, supports the classification of the Anadyr–Chukotka snow sheep as belonging to the Okhotsk subspecies. Furthermore, this analysis confirmed that all populations inhabiting the Koryak Mountains across the two adjacent administrative regions should be assigned to the Koryak subspecies.

4. Discussion

The study of the fauna of the remote territories of the Far East and Siberia has long attracted interest, particularly in relation to adaptation and biogeography in cold environments. Research has faced major difficulties due to the exceptional inaccessibility of

these regions, yet since the 17th century expeditions documented animal species, their distribution, and biological characteristics.

The first mention of sheep in Chukotka dates back to the second half of the 19th century. Karl K. Neumann (cited in [1]) reported in 1871 that "wild sheep are distributed throughout the Chukotka land and reach as far as the Arctic Ocean," and Innokenty P. Tolmachoff noted their presence in the Chukotka Mountains in 1911 (cited in [1]). However, limited material prevented definitive conclusions about their taxonomic status. Tsalkin [3], with a single specimen, noted similarities to Northern Yakut specimens while leaving the systematic position of Anadyr sheep open. Later, a connection with Okhotsk sheep was suggested [32].

The Chukotka snow sheep (*O. n. tschuktschorum*) was first described as a separate subspecies in 1990, with notable morphological differences in this group of animals being highlighted [2]. A taxonomy distinguishing six subspecies of snow sheep was proposed: Kamchatkan, Koryak, Chukotka, Okhotsk, Yakutian, and Putorana [33], which subsequently gained support from other researchers [34]. In later works, the existence of the Chukotka subspecies of snow sheep was not denied [9]. However, other authors did not support the recognition of the Chukotka population as a separate subspecies. Chernyavsky (2004) [7] emphasized that the description of the Chukotka subspecies violated the Rules of Zoological Nomenclature [35].

Currently, the application of DNA technologies allows for the refinement of the phylogenetic status of species, determination of the degree of relatedness between taxa, identification of genetic diversity and reconstruction of evolutionary relationships with high accuracy. Here, we used, for the first time, genome-wide SNP analysis and complete mitochondrial genome analysis for the phylogenetic assessment of the Chukotka population of snow sheep. We compared groups of snow sheep inhabiting the territory of Chukotka with populations of other subspecies (Kamchatkan, Koryak, Okhotsk, and Yakutian).

The application of two types of genetic markers, characterized by different modes of inheritance (genomic biparental and mitochondrial maternal), allowed us to conduct a comprehensive analysis of the studied populations. The analysis of nuclear DNA provides the ability to investigate modern evolutionary processes, including the assessment of the genetic contribution of individuals of both sexes. Mitochondrial DNA is inherited exclusively along the maternal line and, without undergoing recombination, allows for the reconstruction of more distant evolutionary events. Thus, for example, it becomes possible to study the history of a population while excluding the influence of genetic components acquired through the migration of males from neighboring populations.

PCA-assisted analysis showed that all studied samples formed clusters according to their population affiliation. ADMIXTURE-based population structure analysis revealed three ancestral components in the studied sample set associated with the Kamchatkan, Koryak, and Yakutian populations. No differences were found between samples from the Koryak Mountains collected in both the Kamchatka Krai and the Chukotka Autonomous Okrug. Individuals from the Anadyr–Chukotka region exhibited an admixture of genetic components from the Yakutian and Koryak clusters, bringing them closer to the Okhotsk snow sheep. The calculation of pairwise F_{ST} genetic distances and the respective Neighbor-Net dendrogram confirmed the results of the previous analyses.

Based on the complete mitochondrial genomes, a Bayesian phylogenetic tree was reconstructed, which allowed for the identification of five clusters. As with the nuclear DNA analysis, all samples from the Koryak Mountains formed a monophyletic clade, and samples from the Anadyr–Chukotka region formed a common cluster with representatives from the Kolyma Mountains.

At the same time, several methodological limitations should be considered when interpreting our results. After filtering, the nuclear dataset comprised 935 SNPs derived from a genotyping array originally developed for domestic sheep (*O. aries*). The use of such SNP arrays in non-model and wild ungulates is nevertheless widespread and has proven informative for population genetic analyses in related taxa, including the North American bighorn and thinhorn sheep, even when the number of retained loci is relatively limited. For example, Miller et al. [36] applied the OvineSNP50 BeadChip to the bighorn (*O. canadensis*) and thinhorn (*O. dalli*) sheep, retaining hundreds of polymorphic SNPs for structure analyses, and later studies have used the Ovine Infinium HD SNP array to genotype thousands of loci in bighorn sheep for trait and structure analyses [37,38]. The retained number of loci is sufficient for multivariate and clustering analyses such as PCA, ADMIXTURE, and F_{ST}-based approaches, which are known to be robust to moderate SNP densities, although it does not allow for fine-scale demographic inference (e.g., runs of homozygosity or precise estimates of effective population size). It should be noted that all analytical approaches applied in this study yielded highly concordant results, consistently recovering the same major genetic groupings, indicating that the primary population structure inferred here is robust and unlikely to be an artifact of marker number.

Sample sizes were limited for some populations in our study, reflecting the logistical difficulties of sampling in remote regions of Chukotka. We explicitly acknowledge this constraint as a limitation of the study. To reduce potential bias, we used unbiased expected heterozygosity ($_uH_E$), which is appropriate for small sample sizes, and additionally calculated multilocus heterozygosity (MLH), a metric less sensitive to variation in sample size. The consistency of clustering patterns across independent ADMIXTURE replicates and the concordance between nuclear and mitochondrial markers further support the robustness of the major genetic patterns identified in this study.

Ascertainment bias associated with domestic sheep SNP arrays is expected to bias absolute estimates of genetic diversity downward, particularly measures such as heterozygosity. While this limits the interpretation of absolute diversity values, relative comparisons among populations and inference of population structure are expected to remain robust because all analyzed individuals belong to a single species (*Ovis nivicola*) and are similarly diverged from the reference species (*O. aries*).

The genetic differentiation observed in snow sheep (*O. nivicola*) likely stems from biogeographic barriers and historical processes limiting gene flow across the species' range. Snow sheep primarily inhabit isolated mountain ranges, avoiding intervening rivers and lowlands where they are vulnerable to predators and harsh conditions, leading to habitat fragmentation and reduced dispersal [39,40]. For example, major river basins in northeastern Siberia may act as barriers, separating southern and northern mountain systems. Pleistocene glaciations further contributed, with the Beringian refugia enabling survival but post-glacial fragmentation promoting divergence, as seen in our distinct clades and admixture patterns. Similar barriers drive structuring in the North American thinhorn sheep (*O. dalli*), where rivers and valleys restrict connectivity [41], and in the bighorn sheep (*O. canadensis*), where glacial history shapes phylogeographic patterns [42,43]. We hypothesize that the Anadyr River basin, combined with discontinuities in the intervening mountain systems, represents the primary contemporary barrier limiting gene flow between the Koryak and Anadyr–Chukotka snow sheep populations. This is consistent with the species' strong preference for continuous high-elevation rugged terrain and aligns well with the observed nuclear and mitochondrial differentiation. In contrast, populations in the Anadyr Plateau and Chukotka Mountains exhibit minimal genetic distinction from the Okhotsk population, likely due to relatively uninterrupted habitat connectivity as the

Kolyma, Anadyr, and Chukotka mountain systems transition smoothly without major riverine or lowland interruptions.

Our molecular data suggest that snow sheep in the Chukotka Autonomous Okrug form two genetically distinct groupings, potentially corresponding to the Koryak (*O. n. koriakorum*; Koryak Mountains) and Okhotsk (*O. n. alleni*; Anadyr Plateau and Chukotka Mountains) subspecies. These results are of significant importance and can inform conservation programs for snow sheep. Individuals from the Anadyr Plateau and Chukotka Mountains are genetically close to the Okhotsk population, forming one management unit, while Koryak sheep across different administrative regions form another. Conservation efforts should aim to maintain the integrity of these units through monitoring, habitat protection, and avoiding translocations between them. Moreover, it is imperative to continue research on the Chukotka populations of snow sheep, expanding the geographical coverage of sample collection within the autonomous okrug to ensure their long-term conservation.

5. Conclusions

Our molecular data revealed that two distinct groupings of snow sheep inhabiting the territory of the Chukotka Autonomous Okrug should be assigned to different subspecies. We demonstrated that animals from the Koryak Mountains, located within two adjacent administrative regions, Chukotka AO and Kamchatka Krai, are genetically indistinguishable and should be considered a single taxonomic unit, *O. n. koriakorum*. The snow sheep inhabiting the Kolyma Mountains (Magadan Region), the Anadyr Plateau, and the Chukotka Mountains are genetically very close to one another and appear to represent a single genetically coherent group, potentially corresponding to the Okhotsk subspecies (*O. n. alleni*).

Calculations of expected and observed heterozygosity, as well as allelic richness for both snow sheep populations in the Chukotka AO, indicate that their level of genetic diversity is comparable to that of other studied groups and does not currently raise concerns.

Our work represents the first comprehensive molecular genetic study of snow sheep populations in the Chukotka Autonomous Okrug, providing data on their phylogenetic status and genetic diversity. However, a deeper assessment of their evolution and the development of rational conservation programs for the Chukotka snow sheep require continued molecular genetic studies utilizing a larger number of samples from across the species' various habitats.

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