AGRICULTURAL BIOLOGY,
2021, V. 56, Iss. 6, pp. 1134-1147
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.32/.38:575.162:577.2

doi: 10.15389/agrobiology.2021.6.1134eng
doi: 10.15389/agrobiology.2021.6.1134rus

A STUDY OF MATERNAL VARIABILITY OF RUSSIAN LOCAL SHEEP BREEDS BASED ON ANALYSIS OF CYTOCHROME b GENE POLYMORPHISM

O.A. KOSHKINA[1], T.E. DENISKOVA[1] ✉, A.V. DOTSEV[1], E. KUNZ[2], M. UPADHYAY[2], S. KREBS[3], A.D. SOLOVIEVA[1], I. MEDUGORAC[2], N.A. ZINOVIEVA[1]

[1]*Ernst Federal Research Center for Animal Husbandry*, 60, pos. Dubrovitsy, Podolsk District, Moscow Province, 142132 Russia, e-mail olechka1808@list.ru, horarka@yandex.ru (✉ corresponding author), asnd@mail.ru, anastastasiya93@mail.ru, n_zinovieva@mail.ru;
[2]*Population Genomics Group, Department of Veterinary Sciences*, *Ludwig Maximilian University (LMU) Munich*, Germany, 80539 Munich, Veterinaerstr. 13, e-mail elisabeth.kunz@gen.vetmed.uni-muenchen.de, u.maulik@gen.vetmed.uni-muenchen.de, ivica.medjugorac@gen.vetmed.uni-muenchen.de;
[3]*Gene Center, Ludwig-Maximilians-University, (LMU) Munich*, Germany, 80539 Munich, Veterinaerstr. 13, e-mail krebs@genzentrum.lmu.de
ORCID:
Koshkina O.A. orcid.org/0000-0003-4830-6626
Deniskova T.E. orcid.org/0000-0002-5809-1262
Dotsev A.V. orcid.org/0000-0003-3418-2511
Kunz E. orcid.org/0000-0002-6949-4120
Upadhyay M. orcid.org/0000-0002-4296-0021
Krebs S. orcid.org/0000-0001-5112-9507
Solovieva A.D. orcid.org/0000-0003-2628-9554
Medugorac I. orcid.org/0000-0002-6891-3039
Zinovieva N.A. orcid.org/0000-0003-4017-6863

The authors declare no conflict of interests
Acknowledgements:
The equipment of the Center for Biological Resources and Bioengineering of Agricultural Animals (Ernst Federal Research Center for Animal Husbandry) was used.
Performed with the financial support of the Russian Science Foundation (RSF No. 21-66-00007). Sheep samples were obtained as part of the assignment of the Ministry of Science and Higher Education of the Russian Federation (No. 0445-2019-0024).
Received October 1, 2021

Abstract

Analysis of mitochondrial DNA (mtDNA) polymorphism is one of the most effective modern approaches to assess the genetic diversity of livestock species. The mtDNA sequencing is the most efficient approach for identifying mtDNA haplogroups in sheep (*Ovis aries*). Although this approach is widely used abroad, a systematic and comprehensive study of Russian sheep breeds with its aid has not yet been conducted. In this work, we analyzed the polymorphism of the complete sequence of the cytochrome b (*CytB*) gene in Russian sheep breeds of various origins. For the first time, we established the belonging of sheep from 25 Russian breeds to haplogroups and showed haplotype relationships between coarse wool, fine wool and semi-fine wool sheep breeds based on the analysis of polymorphism of the mitochondrial cytochrome b gene. The maternal variability of a wide range of local sheep breeds in comparison with transboundary breeds was assessed. In this research, we aimed to evaluate genetic diversity and to determine the haplotype variability and haplogroup belonging of Russian local sheep breeds based on the *CytB* gene sequences. The study was performed on 106 samples from 25 Russian sheep breeds in 2020-2021. Tissue samples (ear notches) were retrieved from the biological collection "Bank of genetic material of domestic and wild animal species and poultry" (registered by the Ministry of Education and Science of the Russian Federation No. 498808), which is established and maintained at the Ernst Federal Research Center for Animal Husbandry. The final study sample included nine fine-wool breeds, including Baikal (n = 3), Dagestan Mountain (n = 4), Groznensk (n = 5), Kulundin (n = 5), Manych Merino (n = 5), Salsky (n = 5), Soviet Merino (n = 3), Stavropol (n = 5) and Volgograd (n = 5); five semi-fine wool breeds, including Altai mountain(n = 5), Kuibyshev (n = 1), North Caucasian meat-wool (n = 5), Russian longhaired (n = 3) and Tsigai (n = 2); eleven coarse-wool breeds, including Romanov (n = 3), Andean black (n = 5), Buubei (n = 5), Karakul (n = 3), Karachaev (n = 5), Kuchugur (n = 3), Lezgin (n = 5), Tushin (n = 5), Tuva short-fat-tailed (n = 4), Edilbai (n = 5) and Kalmyk (n = 5). The complete sequences of the *CytB* gene of the studied sheep breeds were determined using the next generation sequencing (NGS) technology. To achieve this goal, three overlapping mtDNA fragments (overlapping region of more than 290 bp) with lengths of 6500, 5700, and 6700 bp were amplified. The ob-

tained polymerase chain reaction (PCR) products were used to prepare libraries, which were then sequenced by the method of paired terminal reads of 300 bp each with a MiSeq System Sequencer (Illumina, Inc., USA). The *CytB* gene sequence was recovered from the complete mtDNA sequence after alignment, which was performed using the MUSCLE algorithm in the MEGA 7.0.26 software. All studied breeds had high haplotype (HD = 0.400-1,000) and nucleotide diversity (π = 0.00058-0.00760). In total, we identified 82 haplotypes. Tuva short-fat-tailed sheep breed was represented by only one haplotype. The AMOVA results showed that genetic diversity was mainly determined by intrabreed differences (90.55 %). Four haplogroups including A, B, C and D were identified in the study sample. Such a haplogroup diversity might be explained by a wide geographical range of habitats of the studied animals. The most frequent haplogroups in Russian local sheep breeds were B (n = 64) and A (n = 34), which are typical for sheep of European and Asian origin respectively. Seven animals were assigned to haplogroup C, and haplogroup D was represented by one animal. The results contribute to a deeper understanding of the processes of migration and settlement of domestic sheep in Eurasia.

Keywords: domestic sheep, mitochondrial DNA, cytochrome b gene, haplogroups, haplotypes

Domestic sheep (*Ovis aries*) are one of the most economically significant livestock species, providing humans with food (meat and milk) and raw materials for light industry (wool, sheepskin, and astrakhan) [1]. Since domestication (between 11,000 and 10,500 BC), sheep have spread across all continents, except for Antarctica [2]. This has led to various local breeds with a unique composition of traits due to adaptation and artificial selection with the aim of producing livestock products [3].

Genetic diversity (variation in alleles and genotypes present in a population) reflects the size, history, ecology, and fitness of a population [4]. It plays an important role in ensuring the formation of traits that are responsible for the improvement, survival, and adaptation of a species [5]. Climate change, emerging diseases, scarcity of land and water resources, and changing market demands make the conservation and sustainable use of livestock genetic resources even more important [6]. The study of the genetic variability of the world gene pool of modern native sheep breeds makes it possible to comprehensively assess genetic diversity and indicators of selection, deepen knowledge about the breeds' origin and distribution, and determine the impact of human activity on these animals since domestication [7-9].

Single nucleotide polymorphisms (SNPs), although widely used in the study of the genomes of farm animals [10, 11], represent only one type of common genomic variation. Another effective approach for assessing genetic diversity that has not lost its relevance is the study of mitochondrial DNA (mtDNA) polymorphism [12, 13]. MtDNA demonstrates a high degree of polymorphism and is characterized by the absence of recombination. This makes it possible to study the genetic relationships between breeds and to track both ancient and relatively recent evolutionary events.

Phylogenetic studies have often focused on mitochondrial genes encoding ribosomal DNA (12S and 16S), but their use in broad taxonomic analysis is constrained by the predominance of insertions and deletions (indels); this greatly complicates the alignment of sequenced nucleotide sequences [14]. In this regard, 13 protein-coding genes, in which indels are rarely found due to a shift in the reading frame, are considered more suitable targets in the mitochondrial genome.

The cytochrome B (*Cytb*) gene has several advantages over other mtDNA genes. First, it has a wider range of phylogenetic signals than other mitochondrial genes. Second, nucleotides in the third codon position of *Cytb* show a high base substitution frequency, which is approximately three times higher than the rate of 12S or 16S rDNA, leading to accelerated molecular evolution [15]. Third, this gene evolves quite quickly, making it possible to distinguish

closely related species as well as phylogenetic groups within the same species [16, 17]. Therefore, mtDNA sequencing is the approach for identifying haplogroups. Although this approach is widely used [18-20], it has not been used to conduct a systematic and comprehensive study of Russian sheep breeds.

In 1996, Wood et al. [19] identified two haplogroups in domestic sheep from New Zealand. Then in 1998, after comparing the distribution of haplotypes in several breeds in Germany, Russia, and Kazakhstan, Hiendleder et al. [20] identified these haplogroups as Asian (haplogroup A) and European (haplogroup B). In 2005, based on the results of studies on local breeds from China and Turkey, Guo et al. [18] and Pedrosa et al. [21] expanded the composition of haplogroups to three generally recognized phylogenetic branches (with the inclusion of haplogroup C). Haplogroup C sequences were found at low frequency in sheep living in Portugal [22], suggesting gene flow from the Fertile Crescent to the Iberian Peninsula. Haplogroup C has also been shown to contain more genetic diversity than haplogroup A or B [21]; however, unlike haplogroup B, it does not correspond to any of the wild animals of the *Ovis* genus. Subsequently, in 2006, Tapio et al. [23] found a control region sequence in one Karachaev sheep that clustered separately from the three distinct clusters of domestic sheep mtDNA. This study provided evidence of the presence of a fourth maternal lineage, named haplogroup D. Lastly, in 2007, based on the analysis of polymorphism of the fragment of the control region and *Cytb* mtDNA in sheep, Meadows et al. [24] identified the fifth haplogroup, E.

Genetic analysis showed that haplogroups A and B are found in domestic sheep from all geographic regions (average combined frequency, 89%). Haplogroup A is mainly found in Asian populations [19, 25], whereas haplogroup B has a high frequency of occurrence in European and Asian populations. In contrast, haplogroup C is less common (mean frequency: 18%); only a small number of individuals have been identified in Asia (within the Fertile Crescent) and Europe (within the Caucasus and Iberian Peninsula) [23, 25, 26]. Haplogroups D and E have been identified more recently and are the least represented of the five lineages; sheep with these haplogroups have so far only been found in the Caucasus and Turkey [23, 24].

Through the use of mtDNA to determine the genetic diversity of sheep, insights into the history of sheep domestication and human-influenced global migration have been obtained [27]. In 2007, Pardeshi et al. [28] characterized the mtDNA diversity of three breeds of Indian sheep which all belonged to maternal line A. The Indian sheep network did not have a well-defined central haplotype, no haplotype exchange between populations was observed, and there was a strong breed structure. This haplotype structure of Indian sheep indicates that the history of these breeds was characterized by complete reproductive isolation and a very low frequency of crossing between populations. This is likely because Indian sheep farming is indeed based on maintaining cultural and traditional barriers that prevent genetic exchange between breeds [28].

In 2013, Zhao et al. [29] examined mtDNA variability in local sheep raised in seven regions of China. Phylogenetic analysis of mtDNA D-loop sequences from 16 indigenous Chinese sheep breeds confirmed the presence of three maternal haplogroups (A, B, and C) with high genetic diversity. Additionally, Lv et al. [27] identified two stages of migration in the history of the East Eurasian sheep. The authors concluded that the Mongolian Plateau region was a secondary center of settlement, acting as a "transport hub" in Eastern Eurasia. Sheep from the Middle Eastern center of domestication migrated through the Caucasus and Central Asia and arrived in northern and southwestern China

(haplogroups A, B, and C) and the Indian subcontinent (haplogroups B and C) [27].

The estimated time of divergence between the five main haplogroups occurred before domestication, as demonstrated by archaeological evidence [30]. For example, the time of divergence between the two most common lineages, A and B, was estimated to be 1.6-1.7 million years ago based on the *Cytb* sequences [20]. In addition, Pedrosa et al. [21] suggested that the divergence times of line C and lines A and B are approximately 0.42-0.76 and 0.45-0.75 Ma, respectively, based on analysis of *Cytb* sequences. However, a recent study [25] used 12 protein-coding genes to provide a different estimate of the divergence between lineages: 0.590±0.17 Ma between A and B, and 0.26±0.09 Ma between C and E. In 2020, Liu et al. [31] conducted a complete genome mtDNA sequencing study on Tibetan sheep and obtained similar results. This supports the existence of two maternal lines (haplogroups A and B) with high genetic diversity in 15 populations of Tibetan sheep in China. The ancestors of the maternal lines may have been mouflons (*O. gmelina*) and argali (*O. ammon*) [31]. Despite the wide coverage of mtDNA studies abroad, the Russian sheep breeds remain poorly understood. Sheep breeding has always been an important branch of animal husbandry in the Russian Federation because it provides the population with wool, which is in huge demand due to harsh climatic conditions. In the 1990s, sheep breeding in the Russian Federation fully met the domestic needs of the country [32], but by 2007 there was a sharp decrease in the number of sheep (by 65%) and the textile industry (by 85–90%) [33]. Many factors contributed to this, including a lack of demand for fine and crossbred wool, change of ownership, price disparity for industrial and agricultural products, an inundation of the domestic market with cheap imported goods made of wool, cotton, and leather, unpreparedness, and vulnerability of prices of the Russian commodities in the market [34]. Furthermore, the number of sheep breeding enterprises has decreased in Russia [35], and this has led to an economic decline in domestic sheep breeding. In 35 regions of the Russian Federation, 43 sheep breeds are bred, including 15 fine (34.9%), 12 semi-fine (27.9%), 2 semi-coarse (4.7%), and 14 coarse (32.5%) wool breeds [36].

Despite the problems with domestic sheep breeding, it has begun to recover. Currently, improving the potential meat productivity of raised breeds is considered promising for increasing the economic efficiency of the sheep breeding industry. This is due to a significant difference between the economic importance of wool (5% of the total income) and mutton (95%). Due to market reorientation, the share of wool breeds has decreased significantly from 90.0% in 1990 to 55.2% in 2020, whereas that of meat breeds have increased from 10.0 to 44.8% [37, 38]. These changes have had serious consequences. Some woolly breeds are on the verge of extinction. Most fine and semi-fine wool breeds were developed using native ewes as maternal forms, with sires of highly productive foreign breeds [39]. Local coarse wool breeds were created based on the genetic resources of native sheep and their history of origin, which has not yet been fully elucidated.

In this study, we analyzed the polymorphism in the complete sequence of *Cytb* in Russian breeds of sheep of various origins. For the first time, the haplogroups of sheep from 25 Russian breeds were established, and haplotype relationships between coarse, fine, and semi-fine wool breeds were determined. The characteristics of maternal variability in local sheep breeds were compared with those of transboundary breeds. Our goal was to determine the genetic diversity, haplotype variability, and haplogroup assignments of Russian local sheep breeds based on *Cytb* sequences.

Materials and methods. The study was performed on 25 Russian sheep breeds between 2020 and 2021. Tissue samples (ear notches) were obtained from the biocollection "Bank of genetic material of domestic and wild species of animals and birds" (registered by the Ministry of Education and Science of the Russian Federation No. 498808). It is established and maintained by the Ernst Federal Research Center for Animal Husbandry. The final dataset included nine fine wool breeds: Baikal fine-fleeced (BAKL, n = 3), Dagestan Mountain (DGMT, n = 4), Groznensk (GRZY, n = 5), Kulundin (KLND, n = 5), Manych Merino (MNCM, n = 5), Salsky (SLSK, n = 5), Soviet Merino (SVTM, n = 3), Stavropol (STVP, n = 5), and Volgograd (VLGD, n = 5); five semi-fine wool breeds: Altai Mountain (ALTM, n = 5), Kuibyshev (KBSV, n = 1), North Caucasian (NCCS, n = 5), Russian Longhaired (RSLH, n = 3), and Tsigai (TSIG, n = 2); and eleven coarse wool breeds: Romanov (RMNV, n = 3), Andean (ANDB, n = 5), Buubei (BUBI, n = 5), Karakul (KRKL, n = 3), Karachaev (KRCV, n = 5), Kuchugur (KHGR, n = 3), Lezgin (LZGN, n = 5), Tushin (TSHN, n = 5), Tuva short-fat-tailed (TUVA, n = 4), Edilbaev (EDLB, n = 5), and Kalmyk (KLMY, n = 5).

DNA was extracted using a DNA-Extran-2 kit (OOO Sintol, Russia) according to the manufacturer's recommendations. Quality control of the obtained DNA solutions was performed in two stages. In the first stage, the concentration was measured (DNA from 15 to 50 ng/μl was included) using a Qubit 4.0 fluorimeter (Invitrogen/Life Technologies, USA). In the second stage, the ratio of the degree of absorption OD_{260}/OD_{280} (DNA with a ratio ≥ 1.8 was included) was measured using a NanoDrop8000 spectrophotometer (Thermo Fisher Scientific, USA). Complete sequences of the *Cytb* gene of the sheep breeds were sequenced using next-generation sequencing technology. For this purpose, three overlapping mtDNA fragments (overlapping region > 290 bp), 6500, 5700, and 6700 bp long, were amplified using the following primer pairs: F1 5′-GTCCTTCGCCCTAATC-CTCTC-3′, R1 3′-AGGGTGCCGATATCTTTGTG-5′; F2 5′-ACCCAAAACTCTTCGTGCTC-3′, R2 3′-GGAAGTCAGAATGC-GATGGT-5′; and F3 5′-AC-ACCAAACCCACGCTTATC-3′, R3 3′-GG-GTGTTGATAGTGGGGCTA-5′. Reactions were performed at a final volume of 25 μl: 10 μl reaction buffer (2.5× HF Reaction buffer), 10.25 μl Milli-Q Water H_2O, 2.5 μl dNTPs, 1 μl primer mix, 0.25 μl SmartTaq HF-FuZZ DNA polymerase (Dialat, Russia), and 1 μl of DNA. After initial denaturation (2 min at 94 °C), amplification was performed on an Applied Biosystems SimpliAmp thermal cycler (Thermo Fisher Scientific, USA) using the following temperature-time regime: 30 s at 94 °C (1 cycle); 30 s at 61 °C, 6.5 min at 70 °C (10 cycles); 30 s at 94 °C, 30 s at 60 °C, 3.5 min at 70 °C (25 cycles); and the final stage for 10 min at 72 °C.

The obtained polymerase chain reaction products were purified using a Cleanup Standard kit for DNA purification from agarose gel and reaction mixtures (ZAO Evrogen, Russia) and used to prepare libraries, which were then sequenced using 300 bp paired-end sequencing on a MiSeq device (Illumina, Inc., USA). The *Cytb* sequence was reconstructed from the complete mtDNA sequence after alignment was performed using the MUSCLE algorithm [40] in MEGA 7.0.26 software [41]. A median-joining haplotype network [42] was constructed using PopART 1.7 software [43]. The best evolutionary models were determined in PartitionFinder 2 [44], using the adjusted Akaike information criterion [45]. The evolutionary models HKY and HKY + I were found to be optimal. An analysis of molecular variance (AMOVA] was performed using Arlequin 3.5.2.2 [46]. The Bayesian phylogenetic tree was constructed using MrBayes 3.2.7 [47] with subsequent visualization in FigTree 1.4.3 [48]. The *Cytb* sequence

of snow sheep (*O. nivicola*; GenBank accession number NC_039431.1) was used as the outgroup [49]. A Markov chain Monte Carlo search was performed using four chains with 10,000,000 steps, with trees sampled every 500 generations (the first 25% of the trees were discarded as burn-in). In the DnaSP 6.12.01 program [50], the following parameters of genetic diversity were calculated: the number of polymorphic sites (S), the average number of nucleotide differences (K), the number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), and the standard error of the mean (±SEM).

Results. A total of 82 haplotypes were identified from 106 domestic sheep. All the individuals from the Tuva short-fat-tailed breed group had an identical haplotype. The highest haplotypic diversity (Hd = 1.000) was observed in the Baikal fine-fleeced, Kalmyk, Karakul, Lezgin, Russian Longhaired, Stavropol, Tsigai, Volgograd, and Manych Merino breeds (Table 1). The North Caucasian meat-wool and Tushin breeds showed the lowest haplotype diversity (Hd = 0.400). The lowest values of nucleotide diversity and smallest average number of nucleotide differences were recorded in the Soviet Merino breed (π = 0.00058, K = 0.667). The Baikal fine-fleeced breed was characterized by the highest values for these indicators (π = 0.00760, K = 8.667).

1. Indices of genetic diversity in populations of 25 Russian local breeds of domestic sheep (*Ovis aries*), based on the nucleotide sequence of the mitochondrial gene cytochrome B (Ernst Federal Research Center for Animal Husbandry, Moscow region, 2020–2021)

Population	*n*	S	K	H	Hd±SEM	π±SEM
ALTM	5	8	4.000	4	0.900±0.161	0.00351±0.00069
ANDB	5	5	2.600	4	0.900±0.161	0.00228±0.00049
BAKL	3	13	8.667	3	1.000±0.272	0.00760±0.00308
BUBI	5	13	5.800	3	0.800±0.164	0.00509±0.00208
DGMT	4	14	7.333	3	0.833±0.222	0.00643±0.00240
EDLB	5	6	2.800	4	0.900±0.161	0.00246±0.00064
GRZY	5	4	1.600	4	0.900±0.161	0.00140±0.00042
KBSV	3	2	1.333	2	0.667±0.314	0.00117±0.00055
KHGR	3	4	2.667	2	0.667±0.314	0.00234±0.00110
KLMY	5	8	3.600	5	1.000±0.126	0.00316±0.00065
KLND	5	5	2.000	4	0.900±0.161	0.00175±0.00051
KRCV	5	13	5.600	4	0.900±0.161	0.00491±0.00220
KRKL	3	6	4.000	3	1.000±0.272	0.00351±0.00141
LZGN	5	15	8.000	5	1.000±0.126	0.00702±0.00160
MNCM	5	7	2.800	5	1.000±0.126	0.00246±0.00051
NCCS	5	3	1.200	2	0.400±0.400	0.00105±0.00062
RMNV	3	2	1.333	2	0.667±0.314	0.00117±0.00055
RSLH	3	4	2.667	3	1.000±0.272	0.00234±0.00068
SLSK	5	6	2.400	4	0.900±0.161	0.00211±0.00065
STVP	5	18	7.800	5	1.000±0.126	0.00684±0.00212
SVTM	3	1	0.667	2	0.667±0.314	0.00058±0.00028
TSHN	5	2	0.800	2	0.400±0.237	0.00070±0.00042
TSIG	2	1	1.000	2	1.000±0.500	0.00088±0.00044
TUVA	4	0	0.000	1	0.000±0.000	0.00000±0.00000
VLGD	5	18	7.600	5	1.000±0.126	0.00667±0.00183

Note. *n* — number of samples, S — number of polymorphic sites, K — average number of nucleotide differences, H — number of haplotypes, Hd — haplotype diversity, π — nucleotide diversity. ALTM — Altai Mountain, ANDB — Andean, BAKL — Baikal fine-fleeced, BUBI — Buubei, DGMT — Dagestan Mountain, EDLB — Edilbaev, GRZY — Groznensk, KBSV — Kuibyshev, KHGR — Kuchugur, KLMY — Kalmyk, KLND — Kulundin, KRCV — Karachaev, KRKL — Karakul, LZGN — Lezgin, MNCM — Manych Merino, NCCS — North Caucasian, RMNV — Romanov, RSLH — Russian Longhaired, SLSK — Salsky, STVP — Stavropol, SVTM — Soviet Merino, TSHN — Tushin, TSIG — Tsigai, TUVA — Tuva short-fat-tailed, VLGD — Volgograd.

The coarse wool sheep breeds (Fig. 1, A) formed three clusters corresponding to haplogroups A, B, and C. These breeds were characterized by high genetic diversity. The exception was the Tuva short-fat-tailed breed, all the study individuals of which belonged to the same haplogroup A. Animals of the other breeds belonged to different haplogroups, which may indicate that these populations are of a mixed origin. The fine wool sheep breeds (Fig. 1, B) also had a

high haplotype diversity. As in the case of coarse wool sheep, animals of the same breed clustered into different haplogroups, with the exception of the Salsky breed. In contrast to Tuva short-fat-tailed sheep, sheep from the Salsky breed were characterized by higher nucleotide diversity and had different haplotypes within haplogroup B, which was the most numerous one among the fine wool breeds.

Sheep assigned to haplogroup C differed from those belonging to haplogroup A by nine nucleotide substitutions. Among the fine wool sheep, one individual of the Volgograd breed, which differed by eight nucleotide substitutions from haplogroups A and C, formed a separate cluster, haplogroup D.

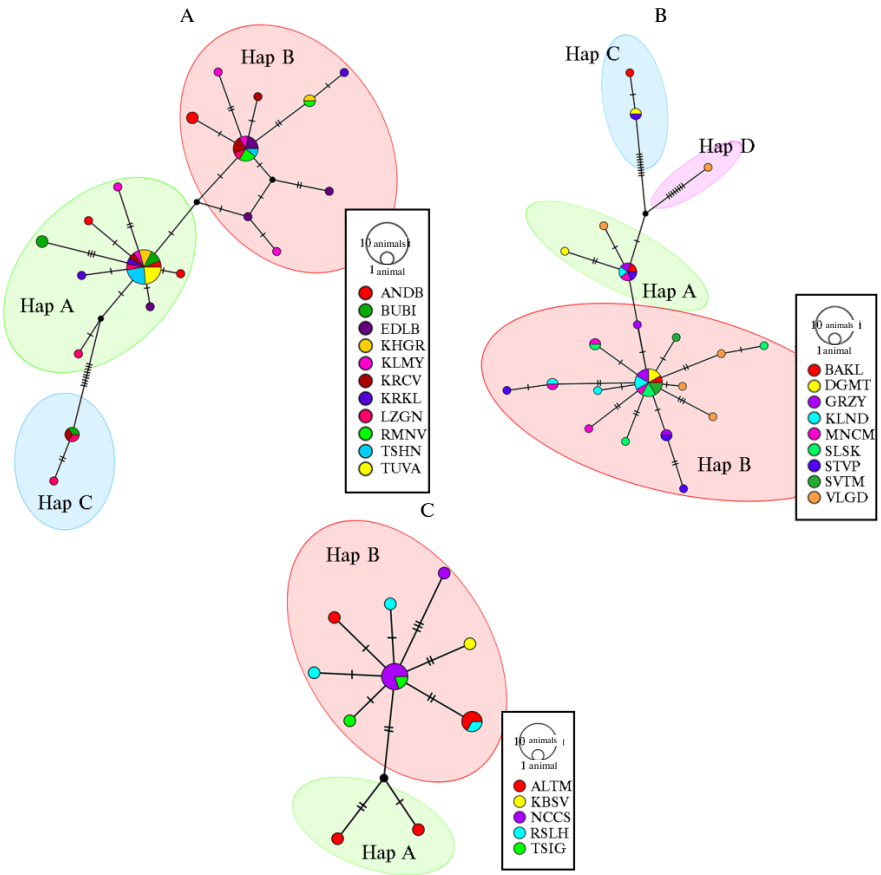

Fig. 1. Median-joining haplotype network displaying the relationships among haplotypes identified in 25 Russian local breeds of domestic sheep (*Ovis aries*), based on the mitochondrial gene Cytb (Ernst Federal Research Center for Animal Husbandry, Moscow region, 2020-2021).

A — median-joining haplotype network, constructed for coarse wool sheep breeds: ANDB — Andean (n = 5), BUBI — Buubei (n = 5), EDLB — Edilbaev (n = 5), KHGR — Kuchugur (n = 3), KLMY — Kalmyk (n = 5), KRCV — Karachaev (n = 5), KRKL — Karakul (n = 3), LZGN — Lezgin (n = 5), RMNV — Romanov (n = 3), TSHN — Tushin (n = 5), TUVA — Tuva short-fat-tailed (n = 4).

B — median-joining haplotype network, constructed for fine wool sheep breeds: BAKL — Baikal fine-fleeced (n = 3), DGMT — Dagestan Mountain (n = 4), GRZY — Groznensk (n = 5), KLND — Kulundin (n = 5), MNCM — Manych Merino (n = 5), SLSK — Salsky (n = 5), STVP — Stavropol (n = 5), SVTM — Soviet Merino (n = 3), VLGD — Volgograd (n = 5).

C — median-joining haplotype network, constructed for semi-fine wool sheep breeds: ALTM − Altai Mountain (n = 5), KBSV — Kuibyshev (n = 3), NCCS — North Caucasian (n = 5), RSLH — Russian Longhaired (n = 3), TSIG — Tsigai (n = 2).

Haplogroups: Hap A — haplogroup A, Hap B — haplogroup B, Hap C — haplogroup C, Hap D — haplogroup D. The diameter of each circle corresponds to the number of individuals belonging to a given haplotype. The number of transverse lines indicates the number of nucleotide substitutions. The black circles at network branching points indicate hypothetical haplotypes.

Most sheep of semi-fine wool breeds (Fig. 1, C) belonged to haplogroup B. Two animals of the Altai Mountain breed were clustered separately into haplogroup A.

Similar conclusions to those mentioned above were drawn based on the analysis of the Bayesian phylogenetic tree (Fig. 2). The largest number of animals was assigned to haplogroup B and two clusters, corresponding to haplogroups C and D, were separated from haplogroup A. The results of the AMOVA performed for the three groups of sheep (coarse, fine, and semi-fine wool breeds) confirmed the presence of genetic differentiation within the breeds, which corresponded to 90.55% of the variability (Table 2). The inter-breed difference was only 3.77%, and the genetic variation between groups was 5.68%.

2. The results of an analysis of molecular variance on populations of 25 Russian local breeds of domestic sheep (*Ovis aries*), based on the nucleotide sequence of the mitochondrial gene *Cytb* (n = 106, Ernst Federal Research Center for Animal Husbandry, Moscow, 2020–2021).

Source of variation	Degrees of freedom, d.f.	Sum of squares, SS	Variance components, VS	Percentage of variation, V%
Intergroup differences	2	12.052	0.11615	5.68
Interbreed differences within the group	22	47.906	0.7706	3.77
Intrabreed differences	81	150.033	1.85226	90.55
Total	105	209.991	2.04547	

Domestic sheep are a traditional and significant type of farm animal in Russia, providing for the needs of the population with food and raw materials for light industry [1]. However, previous studies that have been conducted on the genetic resources of Russian sheep populations are characterized to a greater extent by nuclear molecular genetic markers such as SNPs [51] and microsatellites [52]. In this regard, our study will serve as the basis for accumulating knowledge about maternal variability and genetic diversity based on mtDNA *Cytb* polymorphism in Russian sheep breeds.

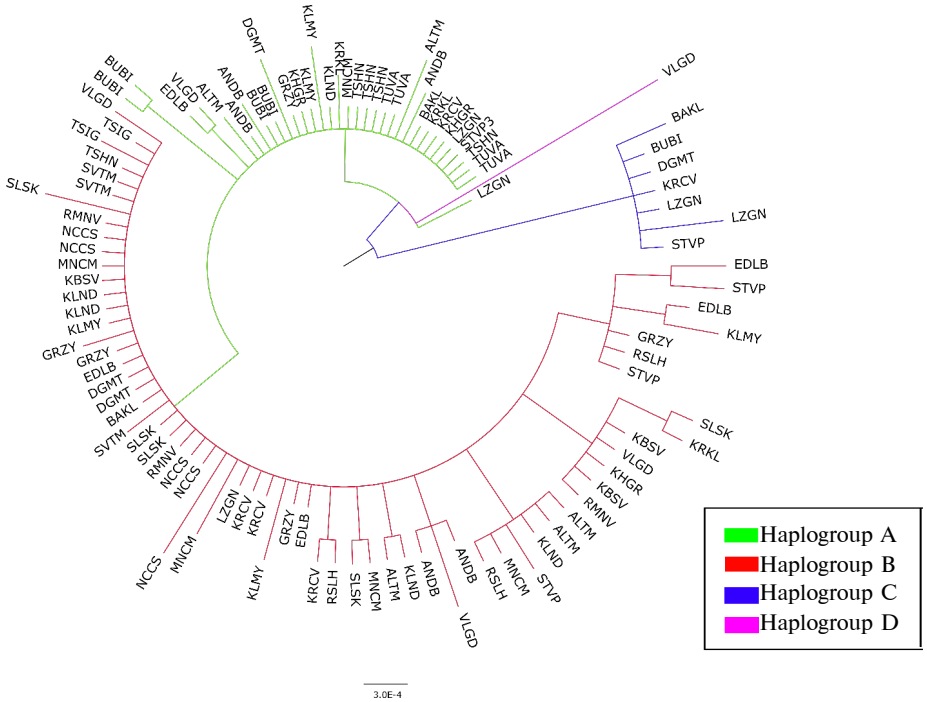

Fig. 2. Bayesian phylogenetic tree reflecting the genetic relationships of 25 Russian local breeds of

domestic sheep (*Ovis aries*) based on the nucleotide sequence of the mitochondrial gene *Cytb*: ALTM — Altai Mountain, ANDB — Andean, BAKL — Baikal fine-fleeced, BUBI — Buubei, DGMT — Dagestan Moun-tain, EDLB — Edilbaev, GRZY — Groznensk, KBSV — Kuibyshev, KHGR — Kuchugur, KLMY — Kalmyk, KLND — Kulundin, KRCV — Karachaev, KRKL — Karakul, LZGN — Lezgin, MNCM — Manych Merino, NCCS — North Cauca-sian, RMNV — Romanov, RSLH — Russian Longhaired, SLSK — Salsky, STVP — Stavropol, SVTM — Soviet Merino, TSHN — Tushin, TSIG — Tsigai, TUVA — Tuva short-fat-tailed, VLGD — Volgograd (Ernst Federal Research Center for Animal Husbandry, Moscow region, 2020–2021)

The haplotype diversity in Russian sheep populations (Hd = 0.400–1.000) was comparable to the values obtained in other studies on Tibetan (Hd = 0.464–1.000) [31] and Moroccan sheep (Hd = 0.963–0.996) [53]. Nucleotide diversity (π = 0.0000–0.00760) was slightly lower than that of Mexican (π = 0.00041–0.90000) [54] and Moroccan sheep (π = 0.01330–0.02260) [53], and close to the values obtained for Tibetan sheep (π = 0.00100–0.00600) [31]. Consequently, the genetic and nucleotide diversity of Russian sheep did not differ significantly from previously reported values, which supports the adequacy of our approach for calculating genetic indicators. According to the results of the AMOVA analysis, genetic diversity was mainly determined by intrabreed differences (90.55%). Similar results were obtained by Oliveira et al. [55], who reported that 91.54% of the genetic variation was due to intrabreed differences in Brazilian sheep raised in the state of Mato Grosso do Sul.

Four sheep haplogroups have been identified based on mtDNA nucleotide sequences: A, B, C, and D [18-21, 23]. Haplogroups B and A, which are typical for sheep of European and Asian origin, were found to be the most common among the Russian local sheep breeds. This result was expected and is consistent with the data obtained earlier by Wood et al. [19], Hiendleder et al. [20], and Meadows et al. [25]. Wood et al. [19] identified two haplogroups (A and B) in domestic sheep from New Zealand, with haplogroup A predominant in Asian populations. These haplogroups were characterized by Hiendleder et al. [20] as being of Asian (haplogroup A) and European (haplogroup B) origin because haplogroup B was prevalent among European breeds but was a minority in East Asia. Meadows et al. [25] obtained similar results, with haplogroups A and B being the most common (approximately 89%). Haplogroup A had a high frequency of occurrence (approximately 77%) in the Indian subcontinent, whereas in Europe, its frequency was less than 10%. In contrast, lineage B was mainly found in Europe, with the highest frequency (> 90%) in southwestern Europe. In our study, haplogroup C was also found in Russian local sheep breeds. Similar to earlier studies [22, 23, 26], haplogroup C was less common, and only a small number of individuals were identified in Asia and Europe. In addition, one animal from the Volgograd region clustered with haplogroup D. Tapio et al. [23] found haplogroup D in one animal of the Karachaev breed from the North Caucasus, indicating the presence of this maternal type in Russia.

Thus, our analysis of mtDNA *Cytb* polymorphism in domestic sheep showed that there is high genetic diversity in Russian sheep breeds. Four haplogroups (A, B, C, and D) were identified, which can be explained by the wide habitat of the study animals. Moreover, the diversity of the presented haplogroups, including the presence of an Asian and European phylogenetic root, could indicate that the processes of migration of domestic sheep in Eurasia, including the Russian Federation, took place in two directions.

REFERENCES

1. Chessa B., Pereira F., Arnaud F., Amorim A., Goyache F., Mainland I., Kao R.R., Pemberton J.M., Beraldi D., Stear M.J., Alberti A., Pittau M., Iannuzzi L., Banabazi M.H., Kazwala R.R., Zhang Y.P., Arranz J.J., Ali B.A., Wang Z., Uzun M., Dione M.M., Olsaker I., Holm

L.E., Saarma U., Ahmad S., Marzanov N., Eythorsdottir E., Holland M.J., Ajmone-Marsan P., Bruford M.W., Kantanen J., Spencer T.E., Palmarini M. Revealing the history of sheep domestication using retrovirus integrations. *Science*, 2009, 324(5926): 532-536 (doi: 10.1126/science.1170587).
2. Zeder M.A. Domestication and early agriculture in the Mediterranean Basin: origins, diffusion, and impact. *Proceedings of the National Academy of Sciences*, 2008, 105(33): 11597-11604 (doi: 10.1073/pnas.0801317105).
3. Diamond J. Evolution, consequences and future of plant and animal domestication. *Nature*, 2002, 418(6898): 700-707 (doi: 10.1038/nature01019).
4. Sheriff O., Alemayehu K. Genetic diversity studies using microsatellite markers and their contribution in supporting sustainable sheep breeding programs: a review. *Cogent Food & Agriculture*, 2018, 4(1): 1459062 (doi: 10.1080/23311932.2018.1459062).
5. Zhong Y., Tang Z., Huang L., Wang D., Lu Z. Genetic diversity of *Procambarus clarkii* populations based on mitochondrial DNA and microsatellite markers in different areas of Guangxi, China. *Mitochondrial DNA. Part A*, 2020, 31(2): 48-56 (doi: 10.1080/24701394.2020.1721484).
6. FAO. *The state of food and agriculture: climate change, agriculture, and food security*. Food and Agriculture Organization of the United Nations, Rome, 2016.
7. Wei C., Wang H., Liu G., Wu M., Cao J., Liu Z., Liu R., Zhao F., Zhang L., Lu J., Liu C., Du L. Genome-wide analysis reveals population structure and selection in Chinese indigenous sheep breeds. *BMC Genomics*, 2015, 16(1): 194 (doi: 10.1186/s12864-015-1384-9).
8. Wang H., Zhang L., Cao J., Wu M., Ma X., Liu Z., Liu R., Zhao F., Wei C., Du L. Genome-wide specific selection in three domestic sheep breeds. *PLoS ONE*, 2015, 10(6): e0128688. (doi: 10.1371/journal.pone.0128688).
9. Fariello M.-I., Servin B., Tosser-Klopp G., Rupp R., Moreno C., International Sheep Genomics Consortium, San Cristobal M., Boitard S. Selection signatures in worldwide sheep populations. *PLoS ONE*, 2014, 9(8): e103813 (doi: 10.1371/journal.pone.0103813).
10. Deniskova T.E., Dotsev A.V., Fornara M.S., Sermyagin A.A., Reyer H., Wimmers K., Brem G., Zinov'eva N.A. The genomic architecture of the Russian population of Saanen goats in comparison with worldwide Saanen gene pool from five countries. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2020, 55(2): 285-294 (doi: 10.15389/agrobiology.2020.2.285eng).
11. Kostyunina O.V., Abdel'manova A.S., Martynova E.U., Zinov'eva N.A. Search for genomic regions carrying the lethal genetic variants in the Duroc pigs. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2020, 55(2): 275-284 (doi: 10.15389/agrobiology.2020.2.275eng).
12. Sulimova G.E., Stolpovskii Yu.A., Ruzina M.N., Zakharov-Gezekhus I.A. V sbornike: *Bioraznoobrazie i dinamika genofondov* [In: Biodiversity and gene pool dynamics]. Moscow, 2008: 211-214 (in Russ.).
13. Qiao G., Zhang H., Zhu S., Yuan C., Zhao H., Han M., Yue Y., Yang B. The complete mitochondrial genome sequence and phylogenetic analysis of Alpine Merino sheep (*Ovis aries*). *Mitochondrial DNA. Part B, Resources*, 2020, 5(1): 990-991 (doi: 10.1080/23802359.2020.1720536).
14. Doyle J.J., Gaut B.S. Evolution of genes and taxa: a primer. *Plant Molecular Biology*, 2000, 42(1): 1-23.
15. Knowlton N., Weigt L. New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 1998, 265: 2257-2263 (doi: 10.1098/rspb.1998.0568).
16. Cox A.J., Hebert P.D. Colonization, extinction, and phylogeographic patterning in a freshwater crustacean. *Molecular Ecology*, 2001, 10(2): 371-386 (doi: 10.1046/j.1365-294x.2001.01188.x).
17. Wares J.P., Cunningham C.W. Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution*, 2001, 55(12): 2455-2469 (doi: 10.1111/j.0014-3820.2001.tb00760.x).
18. Guo J., Du L.-X., Ma Y.-H., Guan W.-J., Li H.-B., Zhao Q.-J., Li X., Rao S.-Q. A novel maternal lineage revealed in sheep (*Ovis aries*). *Animal Genetics*, 2005, 36(4): 331-336 (doi: 10.1111/j.1365-2052.2005.01310.x).
19. Wood N.J., Phua S.H. Variation in the control region sequence of the sheep mitochondrial genome. *Animal Genetics*, 1996, 27(1): 25-33 (doi: 10.1111/j.1365-2052.1996.tb01173.x).
20. Hiendleder S., Mainz K., Plante Y., Lewalski H. Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: no evidence for contributions from urial and argali sheep. *The Journal of Heredity*, 1998, 89(2): 113-120 (doi: 10.1093/jhered/89.2.113).
21. Pedrosa S., Uzun M., Arranz J.J., Gutiérrez-Gil B., San Primitivo F., Bayón Y. Evidence of three maternal lineages in Near Eastern sheep supporting multiple domestication events. *Proceedings of the Royal Society B: Biological Sciences*, 2005, 272(1577): 2211-2217 (doi: 10.1098/rspb.2005.3204).
22. Pereira F., Davis S.J., Pereira L., McEvoy B., Bradley D.G., Amorim A. Genetic signatures of a Mediterranean influence in Iberian Peninsula sheep husbandry. *Molecular Biology and Evolution*, 2006, 23(7): 1420-1426 (doi: 10.1093/molbev/msl007).
23. Tapio M., Marzanov N., Ozerov M., Cinkulov M., Gonzarenko G., Kiselyova T., Murawski

M., Viinalass H., Kantanen J. Sheep mitochondrial DNA variation in European, Caucasian, and Central Asian areas. *Molecular Biology and Evolution*, 2006, 23(9): 1776-1783 (doi: 10.1093/molbev/msl043).
24. Meadows J.R., Cemal I., Karaca O., Gootwine E., Kijas J.W. Five ovine mitochondrial lineages identified from sheep breeds of the near East. *Genetics*, 2007, 175(3): 1371-1379 (doi: 10.1534/genetics.106.068353).
25. Meadows J.R., Hiendleder S., Kijas J.W. Haplogroup relationships between domestic and wild sheep resolved using a mitogenome panel. *Heredity*, 2011, 106(4): 700-706 (doi: 10.1038/hdy.2010.122).
26. Meadows J.R., Li K., Kantanen J., Tapio M., Sipos W., Pardeshi V., Gupta V., Calvo J.H., Whan V., Norris B., Kijas J.W. Mitochondrial sequence reveals high levels of gene flow between breeds of domestic sheep from Asia and Europe. *The Journal of Heredity*, 2005, 96(5): 494-501 (doi: 10.1093/jhered/esi100).
27. Lv F.-H., Peng W.-F., Yang J., Zhao Y.-X., Li W.-R., Liu M.-J., Ma Y.-H., Zhao Q.-J., Yang G.-L., Wang F., Li J.-Q., Liu Y.-G., Shen Z.-Q., Zhao S.-G., Hehua E., Gorkhali N.A., Farhad Vahidi S.M., Muladno M., Naqvi A.N., Tabell J., Iso-Touru T., Bruford M.-W., Kantanen J., Han J.-L., Li M.-H. Mitogenomic meta-analysis identifies two phases of migration in the history of eastern Eurasian sheep. *Molecular Biology and Evolution*, 2015, 32(10): 2515-2533 (doi: 10.1093/molbev/msv139).
28. Pardeshi V.C., Kadoo N.Y., Sainani M.N., Meadows J.R., Kijas J.W., Gupta V.S. Mitochondrial haplotypes reveal a strong genetic structure for three Indian sheep breeds. *Animal Genetics*, 2007, 38(5): 460-466 (doi: 10.1111/j.1365-2052.2007.01636.x).
29. Zhao E., Yu Q., Zhang N., Kong D., Zhao Y. Mitochondrial DNA diversity and the origin of Chinese indigenous sheep. *Tropical Animal Health and Production*, 2013, 45(8): 1715-1722 (doi: 10.1007/s11250-013-0420-5).
30. Zeder M.A. Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. *Proceedings of the National Academy of Sciences*, 2008, 105(33): 11597-11604 (doi: 10.1073/pnas.0801317105).
31. Liu J., Lu Z., Yuan C., Wang F., Yang B. Phylogeography and phylogenetic evolution in Tibetan sheep based on *MT-CYB* sequences. *Animals*, 2020, 10(7): 117 (doi: 10.3390/ani10071177).
32. Ul'yanov A.N., Kulikova A.Ya. *Ovtsy, kozy, sherstyanoe delo*, 2012, 1: 4-11 (in Russ.).
33. Aboneev V.V. *Dostizheniya nauki i tekhniki v APK*, 2008, 10: 37-39 (in Russ.).
34. Erokhin A.I., Karasev E.A., Erokhin S.A. *Ovtsy, kozy, sherstyanoe delo*, 2019, 3: 3-6 (in Russ.).
35. Aboneev V.V., Kolosov Yu.A. *Ovtsy, kozy, sherstyanoe delo*, 2020, 1: 43-46 (in Russ.).
36. Selionova M.I *Zhivotnovodstvo i kormoproizvodstvo*, 2019, 102(4): 272-276 (in Russ.).
37. Lescheva M., Ivolga A. Current state and perspectives of sheep breeding development in Russian modern economic condition. *Economics of Agriculture*, 2015, 2(62): 467-480.
38. Dunin I.M., Safina G.F., Chernov V.V., Grigoryan L.N., Khatataev S.A., Khmelevskaya G.N., Stepanova N.G., Pavlov M.B. V sbornike: *Ezhegodnik po plemennoi rabote v ovtsevodstve i kozovodstve v khozyaistvakh rossiiskoi federatsii (2019 god)* [In: Yearbook on breeding in sheep and goat on farms of the Russian Federation (2019)]. Moscow, 2020: 3-14 (in Russ.).
39. Ernst L.K., Dmitriev N.G., Paronyan I.A. *Geneticheskie resursy sel'skokhozyaistvennykh zhivotnykh v Rossii i sopredel'nykh stranakh* [Genetic resources of farm animals in Russia and neighboring countries]. St. Petersburg, 1994 (in Russ.).
40. Edgar R.C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 2004, 32(5): 1792-1797 (doi: 10.1093/nar/gkh340).
41. Kumar S., Stecher G., Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 2016, 33(7): 1870-1874 (doi: 10.1093/molbev/msw054).
42. Bandelt H.J., Forster P., Röhl A. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 1999, 16(1): 37-48 (doi: 10.1093/oxfordjournals.molbev.a026036).
43. Leigh J.W., Bryant D. Popart: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 2015, 6(9): 1110-1116 (doi: 10.1111/2041-210X.12410).
44. Lanfear R., Frandsen P.B., Wright A.M., Senfeld T., Calcott B. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 2017, 34(3): 772-773 (doi: 10.1093/molbev/msw260).
45. Akaike H. A new look at the statistical model identification. *IEEE Trans Auto Control*, 1974, 19(6): 716-723 (doi: 10.1109/TAC.1974.1100705).
46. Excoffier L., Lischer H.E. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 2010, 10(3): 564-567 (doi: 10.1111/j.1755-0998.2010.02847.x).
47. Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Hцhna S., Larget B., Liu L., Suchard M.A., Huelsenbeck J.P. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 2012, 61(3): 539-542 (doi: 10.1093/sysbio/sys029).

48. *Molecular evolution, phylogenetics and epidemiology*. Available: http://tree.bio.ed.ac.uk/software/figtree. Accessed: 30.07.2021.
49. Dotsev A.V., Kunz E., Shakhin A.V., Petrov S.N., Kostyunina O.V., Okhlopkov I.M., Deniskova T.E., Barbato M., Bagirov V.A., Medvedev D.G., Krebs S., Brem G., Medugorac I., Zinovieva N.A. The first complete mitochondrial genomes of snow sheep (*Ovis nivicola*) and thinhorn sheep (*Ovis dalli*) and their phylogenetic implications for the genus *Ovis*. *Mitochondrial DNA Part B*, 2019, 4(1): 1332-1333 (doi: 10.1080/23802359.2018.1535849).
50. Rozas J., Ferrer-Mata A., Sánchez-DelBarrio J.C., Guirao-Rico S., Librado P., Ramos-Onsins S.E., Sánchez-Gracia A. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, 2017, 34: 3299-3302 (doi: 10.1093/molbev/msx248).
51. Deniskova T.E., Abelmanova A.S., Dotsev A.V., Sambu-Khoo Ch.S., Reyer H., Selionova M.I., Fornara M.S., Wimmers K., Brem G., Zinovieva N.A. PSX-18 High-density genomic description of Russian native sheep breed of the Republic of Tyva. *Journal of Animal Science*, 2020, 98(4): 453-454 (doi: 10.1093/jas/skaa278.789).
52. Deniskova T.E., Selionova M.I., Gladyr' E.A., Dotsev A.V., Bobryshova G.T., Kostyunina O.V., Brem G., Zinov'eva N.A. Variability of microsatellites in sheep breeds raced in Russia. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2016, 51(6): 801-810 (doi: 10.15389/agrobiology.2016.6.801eng).
53. Kandoussi A., Boujenane I., Auger C., Serranito B., Germot A., Piro M., Maftah A., Badaoui B., Petit D. The origin of sheep settlement in Western Mediterranean. *Scientific Reports*, 2020, 10(1): 10225 (doi: 10.1038/s41598-020-67246-5).
54. Alonso R.A., Ulloa-Arvizu R., Gayosso-Vázquez A. Mitochondrial DNA sequence analysis of the Mexican Creole sheep (*Ovis aries*) reveals a narrow Iberian maternal origin. *Mitochondrial DNA. Part A*, 2017, 28(6): 793-800 (doi: 10.1080/24701394.2016.1192613).
55. Oliveira J.A., Egito A.A.D., Crispim B.D.A., Vargas Junior F.M., Seno L.O., Barufatti A. Importance of naturalized breeds as a base for the formation of exotic sheep (*Ovis aries*) breeds in tropical altitude regions. *Genetics and Molecular Biology*, 2020, 43(2): e20190054 (doi: 10.1590/1678-4685-GMB-2019-0054).