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A Mitochondrial DNA-based Molecular Phylogenetics Study of the Mahalli Goat as a New Animal Genetic Resource in Southern Anatolia in Turkey

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ABSTRACT

Background: The Karacadağ Mountains are of extinct volcanic origin and cover a large area in the triangle between Diyarbakır (Center, Nar and Ergani districts), Şanlıurfa (Siverek and Viransehir districts) and Mardin (Derik district). Agricultural activities are limited due to the stony structure of the region; therefore, small animal husbandry is intensively practiced in the villages of the region. Mahalli goats, which are morphologically new domestic goats, are distinguished from other domestic goats by their spiral horn structure. They are not only resistant to cold weather conditions, but also resistant to diseases. Therefore, present study aimed to determine the genetic diversity and phylogenetic relationships of Mahalli goats in Diyarbakır region.

Methods: The study was carried out at Şanlıurfa, Harran University of Agricultural Faculty, Laboratory of Animal Biotechnology and Molecular Genetics, in 2019-2020. Out of 24 Mahalli goats, forward and reverse primers were used to amplify the 598 bp region over the mtDNA D-loop region encompassing the 481 bp most variable region (HVR1). Analysis of goat D-loop sequences revealed 481 regions of 0.378 G+C, 97 polymorphic regions and 19 haplotypes. The phylogenetic analysis of Mahalli goats was carried out using molecular genetic techniques.

Result: As a result of data analysis, both haplotype diversity (HD) and nucleotide diversity (ND) were calculated to be 1.000±0.0039 and 0.0375±0.00209, respectively. The genetic distance between the haplotypes of Mahalli goat ranged from 0.003 to 0.087 and the genetic distance between the haplotypes of Mahalli goat and other domestic goat breeds in Turkey ranged from 0.016 to 0.027. All 19 haplotypes used in this study were represented in the lineage A phylogenetic tree.

Key words: Genetic resources, Indigenous Mahalli goat, mtDNA, Phylogenetic.

INTRODUCTION

Goats have been closely associated with humans since the beginnings of agriculture and the domestication of animals. They are considered a socio-economically very important animal as they provide many products (such as meat, milk, fiber and hair) and services to people worldwide, especially in developing countries (Bhardwaj *et al*., 2018). In Turkey, three breeds are called domestic goat breeds, namely the hair goat, the Angora goat and the Kilis goat (Kaymakçı and Aşkın, 1997). Although hair goats are common in all regions, they are most intensively bred in the Mediterranean region (26.5%), the southeast (25.6%) and the Aegean region (20.3%).

Karacadağ is an extinct volcanic mountain in the triangle of Diyarbakır (Center, Çınar, Ergani districts), Şanlıurfa (Siverek and Viransehir districts) and Mardin (Derik district) that occupies a very large area. The land is covered with basal rocks formed by volcanic eruptions. Due to the stony nature of the region, agricultural activities are limited and small livestock farming is practiced intensively in the villages of the region. Mahalli goats are bred in and around the Gümüştaş village of Çınar district of Diyarbakır province in the Karacadağ region and are distinct from the hair goats bred in Diyarbakir. Morphologically, Mahalli goats, one of the indigenous goat breeds, are distinguished from other goats by their horn structure, which spirals to the side and upward. The hair color is black, brown and beige and the percentage of cashmere hair is high. All males and females have beards and the forehead is furrowed. It is resistant to cold weather conditions and diseases. The foot and leg structure is developed and strong according to the stony ground. It can run long distances. The grazing ability is good (Fig 1 and 2).

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The goat mitochondrial genome (mtDNA) consists of 13 protein-coding regions and is 16616 base pairs long. In addition to cytochrome C oxidase complexes I, II and III, ATPase complexes 6 and 8 and NADH dehydrogenases 1, 2, 3, 4L, 4, 5 and 6, mitochondrial genomes contain ribosomal RNA regions (12S and 16S), control regions (D-loops) and 22 tRNAs (Parma *et al.*, 2003).

In addition, mtDNA is used as a molecular marker (especially the D-loop) to identify populations, determine the origin of populations and species, identify haplotypes within subspecies, calculate genetic variation within/between populations and determine phylogenetic relationships based on genetic similarities or differences between populations. In addition, the origin of the domestic goat was studied in detail using mtDNA. Using molecular phylogenetic analysis, it was demonstrated that there are six maternal lineages in goats, namely A, B1, B2, C, D, F and G (Naderi *et al.*, 2007). Lineage A is the predominant one and has spread to all continents. Many studies have supported the assumption that domestic goats have multiple maternal lineages.

To date, no studies have been conducted to describe the genetic characteristics of these goats. In order to make a scientific contribution to goat phylogenetic studies, animal breeding, biodiversity and genome conservation, this study aimed to determine the genetic diversity and phylogenetic relationships of Mahalli goats in Diyarbakir region.



Fig 1: Mahalli goat (Photo by M. Emin Vural).



Fig 2: Mahalli goat (Photo by M. Emin Vural).

MATERIALS AND METHODS

Animal material and sampling

The animal material for the study consisted of Mahalli goats (n=24) kept in Diyarbakır region. Hair samples were collected from goat herds for DNA isolation. Hair samples were collected by pulling from the upper back and neck of the animals with gloves. Molecular and phylogenetic analyses were conducted for this study at Şanlıurfa Harran University's Faculty of Agriculture, Department of Animal Science, Laboratory of Animal Biotechnology and Molecular Genetics. Total genomic DNA was isolated from hair samples by modifying the phenol/chloroform+Proteinase K method (Sambrook *et al.*, 1989).

Molecular genetic analyzes

Molecular genetic analyzes: PCR amplification of the 598-bp region was performed with forward and reverse primers (Naderi *et al.*, 2007) over the mtDNA D-loop region containing the most variable 481-base region (HVR1). Forward and reverse primers were used to amplify the 598 bp region over the 481 bp region of the D-loop region of mtDNA (HVR1). Forward primer: 5′CGTGTATGCAAGTA CATTAC′3 and reverse primer: 5′CTGATTAGTCATTAGT CCATC′3. As described by Kiraz *et al.* (2021) a PCR. Samples with PCR amplification of the D-loop gene regions were selected for gene sequencing and the forward (F) and reverse chain (R) samples were prepared into 50 µl (25 µl PCR product+25 µl ddH_2O) and sent for sequencing. DNA sequencing was performed by Iontek (Istanbul). Here, the peaks were analyzed in the sequence chromatographs and sequence samples with clear and high quality peak signals were evaluated.

Phylogenetic analyzes

Nucleotide sites (S), HD and HD were estimated using DNA Sequence Polymorphism (DnaSP) version 5.1 (Librado and Rozas, 2009). Based on the Kimura-2 parameter model (Kimura, 1980) in MEGA 6.0 (Tamura *et al.*, 2013), the genetic relationships associated with the D-loop gene region and haplotypes were determined by applying the UPGMA method with the Kimura-2 parameter + gamma distribution model (K2P+G) based on the Neighbor-Joining method (NJ) (Saitou and Nei, 1987). The value of the gamma distribution was assumed to be α=0.28 in the construction of the phylogenetic trees (Naderi *et al.*, 2007). To determine whether the nodes (tree branches) were reliable, a bootstrap test was performed (1000 replicates) (Nei and Kumar, 2000).

RESULTS AND DISCUSSION

The D-loop region, which is 1212 bp long in domestic goats, was amplified using D-loop region primers and a 598 bp long segment thereof. As a result of gene sequence analysis and editing, 481 bp of sequence information was obtained for all samples. The gene sequence information and DNA polymorphism features were determined in goats

(Table 1). In goats, 97 polymorphic sequences and 19 haplotypes were detected in the D-loop region. Haplotype and nucleotide diversity (HD and ND) in goats were 1.000±0.0039 and 0.0375±0.00209, respectively. Of the goat haplotypes identified, the rooted UPGMA tree formed downstream of the D-loop region is shown in Graph 1, the N-J tree is shown in Graph 2 and the rootless N-J tree is shown in Graph 3.

In this study, ninety-seven polymorphic regions and 19 haplotypes were detected in the D-loop region of goats. Haplotype and nucleotide differences of 1.000±0.0039 and 0.0375±0.00209, respectively, were calculated in goats. Haplotype distances between Mahalli goats were calculated between 0.003 and 0.087. The genetic distance between Mahalli goats and other domestic goat breeds (Kathari, Kilis and Abaza goats) ranged from 0.016 to 0.027 (Table 2). The lowest genetic distance was calculated to be 0.019 between Mahalli goats and Hair goats and the highest genetic distance was 0.027 in Ankara goats. When the phylogenetic trees were examined, complete clustering between the identified goat haplotypes could not be detected because the bootstrap test values were low (<50%). Genetic distances between Mahalli goat haplotypes were found to range from 0.003 to 0.087. In addition, the genetic distances between the indigenous goat breeds in Turkey ranged from 0.016 to 0.027 (Table 2).

Based on the reference sequences of the D-loop gene determined for goat lines A, B, C, D, F and G and the sequences of the haplotypes identified in this study (19 haplotypes), Naderi *et al.* (2007) determined the haplogroups of the Mahalli goat haplotypes. It was also found that all goat haplotypes belonged to lineage A (100%).

In addition, the majority of goats (93.51%) belonged to the A line and were common worldwide, whereas other lines were extremely rare. In this study, the A lineage was also detected to a high degree. In addition, multidimensional evaluations were performed by comparing the D-loop region sequences of the available goat haplotypes with the sequence information of domestic goats from different regions of Turkey. In this context, Naderi *et al.* (2008) found the existence of B, C, D and F strains in Turkish goats, including goats in different regions of Turkey, in addition to previous studies. Similarly, Akis *et al.* (2014) found that haplogroup A is the dominant haplogroup in Anatolian goat breeds. Haplogroup D was observed only in Kilis and Angora goats. Lineage G was found in Ankara and Anatolian Black goat breeds. The Anatolian goat breeds show high genetic diversity and weak phylogeographic structure. Moreover, regarding the *Anatolian peninsula*, Akis *et al.* (2014) figured out that haplogroup A is the dominant haplogroup in Anatolian goat breeds. Anatolian goat breeds show high genetic diversity and weak phylogeographic relationships. When considering domestic goats, it should also be noted that the results in terms of mtDNA polymorphism and phylogenetic characters are different when other regions of the world are considered geographically. Zhong *et al.* (2013) determined Hd and π in Chinese rural goats to be between 0.782±0.079 and 1.000±0.020 and 0.009±0.001 and 0.045±0.006, respectively.

Table 1: DNA polymorphism by D-loop gene sequence in goats.

mt Values	Overall
Total number of region	481
G+C	0.378
Number of polymorphic region (S)	97
Number of Haplotypes (h)	19
Haplotype diversity (Hd)	1.000±0.0039
Nucleotid diversity (Pi)	0.0375±0.00209

Table 2: Genetic distances between goat haplotype groups.

Breeds	Mahalli	Kilis	Angora	Hairy
Kilis	0.021			
Angora	0.027	0.023		
Hairy	0.019	0.016	0.025	
Abaza	0.022	0.021	0.025	0.019

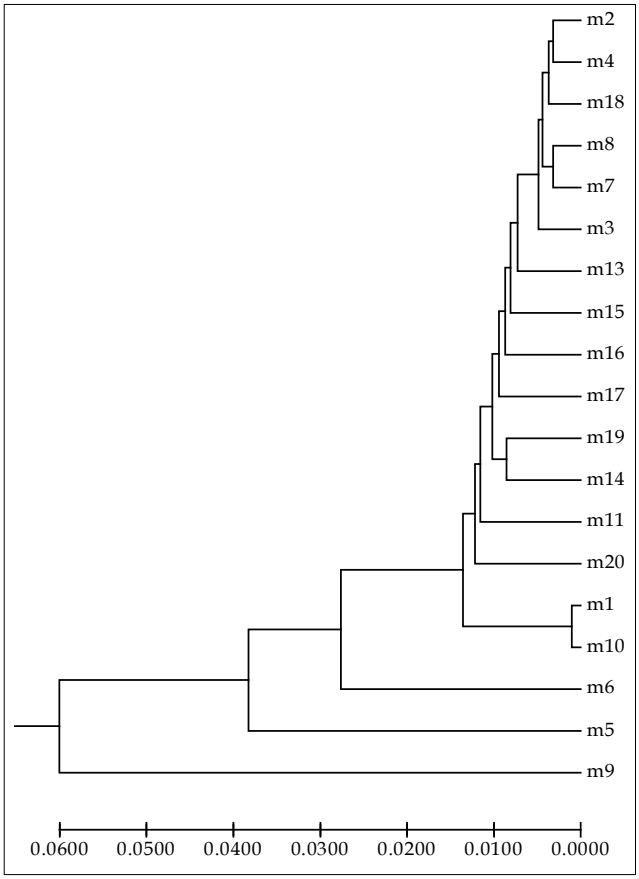
Mahalli: m5, m20, m10, m19, m17, m14, m11, m16, m15, m13, m8, m7, m3, m18, m2, m4

Hairy: KR059152, KR059158, KR059689, HQ996607, EF618532

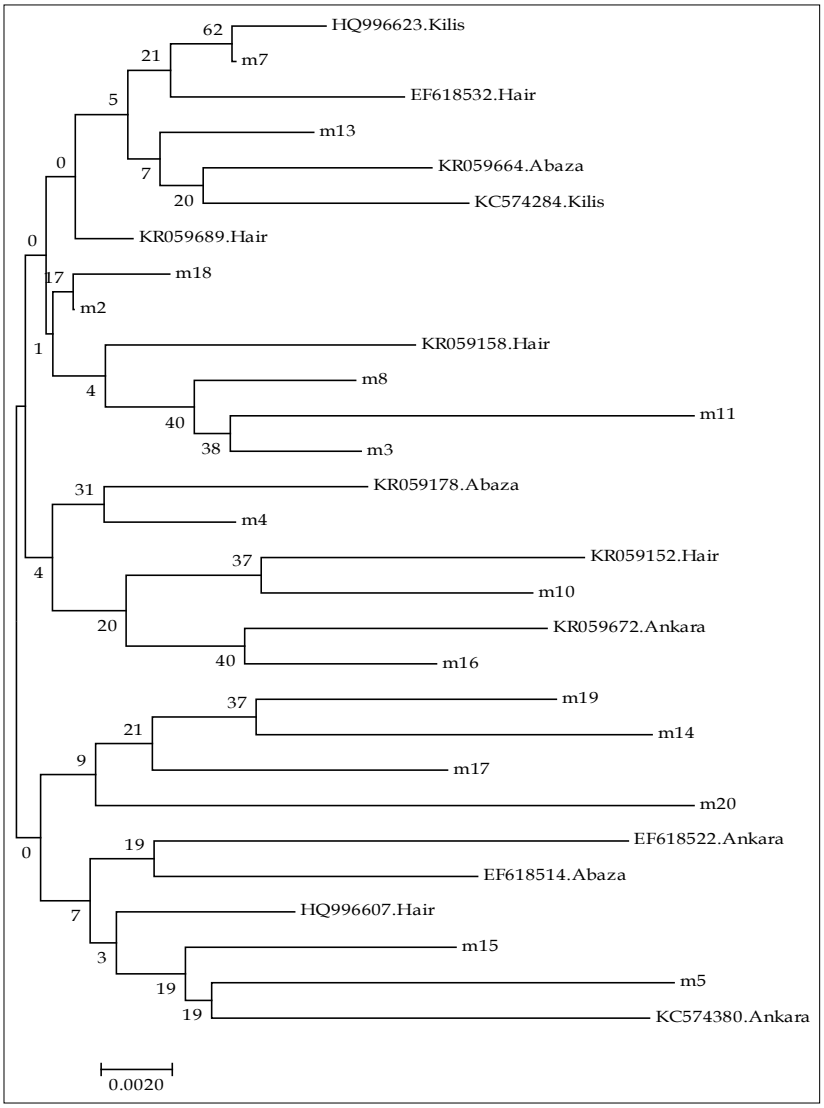
Kilis: HQ996623, KC574284

Angora: KR059672, EF618522, KC574380

Abaza: KR059664, EF618514, KR05917



Graph 1: Mahalli goat haplotypes UPGMA tree.



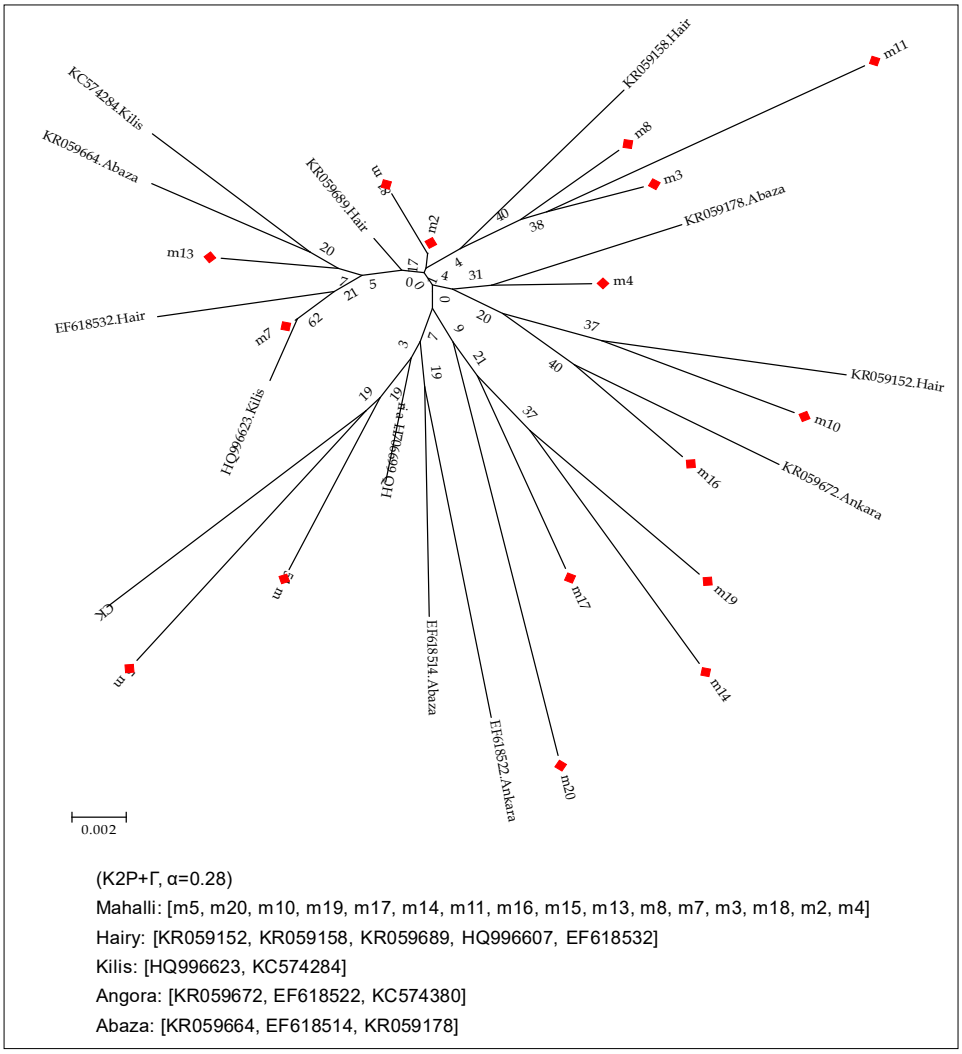
Graph 2: Mahalli goat and other goat haplotypes N-J tree.

Based on phylogenetic analysis, five haplogroups were identified (A, B1, B2, C and D). Haplogroups A, B1 and B2 were dominant in most breeds, in contrast to C and D, which were found only in northern and northwestern Chinise breeds. Furthermore, in the study by Awotunde *et al.* (2015), mean Hd and π values of 0.982±0.005 and 0.02350± 0.00213, respectively, were calculated for two popular Nigerian goat breeds, West African Dwarf and Red Sokoto and for an exotic breed imported from South Africa, Kalahari Red. Phylogenetic analyzes revealed the existence of two mtDNA strains (A and B), with lineage A predominant. In addition, Ganbold *et al.* (2020) calculated Hd and π in Mongolian goats to be 0.997±0.001 and 0.0283±0.002, respectively. These haplotypes clearly clustered into four lineages (A, B, C and D), with lineage A predominating (90.8%). However, less genetic differentiation was observed in Kazakh, Chinese and Arabian goats (Turkish and Baladi breeds) than in Mongolian goats.

Genetic differences and phylogenetic relationships have been demonstrated based on the sequences of the goat mtDNA control region in different geographical regions, especially in the Asian continent where the goat population is dense. The phylogenetic tree of Chinese domestic goats shows that goats are divided into four mtDNA lineages (A, B, C, D) (Chen *et al.*, 2005; Liu *et al.*, 2006; Fan *et al.*, 2007; Wang *et al.*, 2008; Liu *et al.*, 2009; Wu *et al.*, 2009).

However, haplotype differences in Tibetan, North and South Chinese goats were reported by Chen *et al.* (2005) between 0.712±0.091-0.980±0.0243, Liu *et al.* (2006) between 0.9333-1.000, Hao *et al.* (2008) 0.988±0.003, Wang *et al.* (2008) 0.943-1.000, Liu *et al.* (2009) as 0.989±0.001 and Wu *et al.* (2009) between 0.9111±0.0773-0.9872±0.0354.

In addition, Joshi *et al.* (2004) showed that Indian goats are divided into four lineages (lineages A, B, C and D).



Graph 3: Phylogenetic tree between Mahalli goat and other goat haplotypes.

Odahara *et al.* (2006) have also shown that Korean goats belong to haplogroup A. Similarly, Sardina *et al.* (2006) calculated the mean haplotype difference in Sicilian goats (Girgentana, Maltese, Derivata di Siria) as 0.969±0.007 and the mean nucleotide difference as 0.02359±0.00450. In the phylogenetic tree constructed with the mtDNA sequences of the Sicilian goats and the published sequences of domestic goats from India and Pakistan, it appeared that the majority of the haplotypes belong to the A lineage.

As for the European continent, Azor *et al.* (2005) reported that there is a weak phylogenetic relationship between the Spanish goats (Pirenáica, Moncaína, Blanca Andaluza, Negra Serana, Azpi-Gorri, Blanca Celtibérica) and the Iberian Peninsula goats. They showed that in the phylogenetic tree the individuals of a breed are not grouped in a single cluster.

However, Amills *et al.* (2009) found nucleotide and haplotype differences of 0.020±0.00081 and 0.963±0.0012 in North and South American goats, respectively. They found that South and Central American goats formed a cluster together with Spanish and Portuguese breeds, whereas Bolivia, Chile, Canary Islands and Argentine goats formed distinct clusters in the phylogenetic tree constructed from the sequences of European, Iberian, Atlantic and South and Central American goats. However, all South and Central American goats were assigned to the A lineage, while other lineages were not observed.

As can be seen from the above, many phylogenetic studies have found that lineage A is predominant or unique in goats, with high haplotype diversity and low nucleotide diversity. However, similar results have been found in Mahalli goats.

Kamalakkannan *et al.* (2018) studied haplotype diversity in domestic goats in southern India and found that goats in this region belong to haplogroups A, B and D. A study by Tabata *et al.* (2019) examined Kazakh goats and assigned them to four haplogroups: A, C and D, with haplogroup A being the predominant haplogroup with 97%. It is clear that the genetics of Kazakh goats is highly associated with haplogroup A. Moreover, Deniskova *et al.* (2020) found in

their study that most of the indigenous goat breeds in Russia belong to haplogroup A. As Mannen *et al.* (2020) also found in their study of indigenous goat breeds in Indonesia, most Kacang and Marica breeds belong to haplogroup B, suggesting that haplogroup B is widespread in goat populations around the world.

In the study by Nguluma *et al.* (2021), indigenous goat populations in Tanzania were found to have high haplotype and nucleotide diversity, with the majority of haplotypes belonging to haplogroup A, consistent with the global genetic pattern of maternal origin in goat breeds. It was reported that Baenyi Simon *et al.* (2022) conducted a study of three indigenous goat populations in the Democratic Republic of Congo and found that these goat populations were predominantly of haplogroup A.

In addition, Gorkhali *et al.* (2022) studied the genetic diversity of indigenous Nepalese goat breeds, including Khari, Chyangra, Terai and Sinhal. The result of their study was that these breeds have high haplotype diversity divided into four haplogroups (A-D). The complex mtDNA diversity and structure found in indigenous Nepalese goats is explained by gene flow through ancient trade and the current 'free' movement of goats between geographic regions in India and China.

Finally, Guo *et al.* (2022) investigated the genetic diversity and phylogeny of mitochondrial DNA (mtDNA) of goats worldwide. In this comprehensive study, the sequences of the hypervariable region of the mtDNA D_loop of a total of 4,165 individuals from 196 different breeds were analyzed. As a result of the analysis, it was found that the nucleotide diversity (Pi) was 0.03471 and the haplotype diversity (Hd) was 0.9983. Based on the results of phylogenetic analysis, 98.92% of goat haplotypes were classified into six different clusters consistent with the known classification of mitochondrial haplogroups for goats. It is estimated that 86% of these clusters belong to haplogroup A. Phylogenetic analysis of domestic goats and wild goats also suggests that Capra aegagrus was the most likely wild ancestor and played a role in the domestication of the ancestors of haplogroups A, B, C and F. The work of Guo *et al.* (2022) provides valuable insights into understanding the genetic diversity, phylogeny and domestication history of goats.

On the other hand, studies on genetic diversity in goats have also been carried out using microsatellite and single nucleotide polymorhisms (SNP) markers instead of mitochondrial DNA. Some examples of genetic diversity studies conducted with microsatellite markers are Rajkumar and Santpal (2020) and Verma *et al.* (2022) in Indian goats and Pan *et al.* (2023) in Chinese goats, while Mukhina *et al.* (2022) used SNP markers in Mogolian goats.

Taken together, these studies contribute to our understanding of the genetic diversity of goat populations around the world. Different haplogroups are a reflection of geography and historical events and this knowledge is critical to the conservation and management of goat breeds.

CONCLUSION

The results of molecular phylogenetic analysis of native goats will contribute to the study of goat genetics, species diversity and gene conservation. The genetic relationships between morphologically different goat species need to be further investigated. Turkey is not only one of the few countries in the world with high genetic diversity, but based on archeological findings, it is also believed that Turkey is the center of domestication from the past to the present. Considering the decline of genetic diversity in recent years, the protection of Mahalli genetic resources has become an important issue. Obtaining genetic information on breeds and conducting further studies on this subject are of great importance for the conservation of indigenous genetic resources.

Conflict of interest

All authors (I and co-authors) declare that they have no conflict of interest in relation to this publication. As the corresponding author of the article, I confirm that the manuscript has been read and approved for publication by all the authors named. As the corresponding authors of this scientific research article, i solomnly declare that the welfare and safety of the animals and Helsnki Principles were paramount in this study and that I adhere to the ethics of experimentation and safety regulations.

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