

Genetic diversity and phylogenetic position of Manavli goat based on mitochondrial DNA (D-loop) region

Aykut Asım Akbaş^{1,a}, Müge Doğan^{2,b}, Mustafa Saatci^{3,c}, Özkan Elmaz^{1,d}, Can Metin Yazıcı^{1,e}

¹Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Animal Science, Burdur, Türkiye

²Konya Veterinary Control Institute, Republic of Türkiye Ministry of Agriculture and Forestry, Konya, Türkiye

³Muğla Sıtkı Koçman University, Fethiye Faculty of Agriculture, Department of Animal Science, Muğla, Türkiye

ORCID: ^a0000 0003-2235-9439; ^b0000-0002-0593-5476; ^c0000-0003-3697-8804; ^d0000-0002-2599-0907; ^e0000-0002-6771-0977

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Abstract

This study investigates the maternal genetic diversity and phylogeographic relationships of the Manavli goat population, a native genotype of Anatolia, using mitochondrial DNA (mtDNA) D-loop sequences. A total of 17 Manavli goats were analysed and compared with reference sequences from other Anatolian goat breeds as well as several global goat breeds. Sequence analyses revealed 30 polymorphic sites defining eight different haplotypes within the Manavli goat population. The haplotype diversity ($H_d = 0.824$) and nucleotide diversity ($\pi = 0.0128$) values indicate a moderate level of genetic variation. Results of the analysis of molecular variance (AMOVA) indicated that approximately 10.18% of the total genetic variation was due to differences among populations ($F_{st} = 0.1018$, $P < 0.01$), while 89.82% of the variation occurred within populations. In the unweighted pair group method with arithmetic mean (UPGMA) dendrogram constructed with other indigenous Anatolian goat breeds, clustering based on genetic distance revealed a closer genetic relationship between the Manavli and Ankara goats. All phylogenetic and haplotype network analyses demonstrated that the Manavli goat population predominantly clusters within the G and A haplogroups, possessing unique maternal lineages. These findings emphasize the genetic distinctiveness of the Manavli goat and highlight its importance as a local genetic resource for conservation and sustainable breeding programs in Anatolia. To further validate and explore the observed genetic data, it is strongly recommended that future studies include larger sample sizes.

Caprine, Anatolian breeds, haplotype, phylogeography, sustainability

Many countries, including Türkiye, signed the ‘Convention on Biological Diversity’ during the 1992 United Nations Rio Summit, committing to contribute to the preservation of native genetic resources. The Food and Agriculture Organization (FAO) requested to assess current status and take measures to conserve local genetic resources (Çanta and Oğuz 2004). It is known that Türkiye has experienced losses in its native farm animal genetic resources over the past 50 years and that some breeds have gone extinct before they could be molecularly identified (Ertuğrul et al. 2010). Therefore, there is an urgent need to strengthen efforts for the conservation of farm animal genetic resources in Türkiye. If there are genotypes that have not yet been registered, the necessary molecular and morphological characterisation studies should be conducted as soon as possible to enable their identification and registration. In the 2024–2028 Strategic Action Plan prepared by the Ministry of Agriculture and Forestry of the Republic of Türkiye, General Directorate of Agricultural Research and Policies (GDARP), the lack of long-term performance records is identified as a deficiency, resulting in insufficient knowledge of genetic variation. The plan emphasises the need to collect and maintain data on animal performance and genetic variation (GDARP 2024).

Address for correspondence:

Aykut Asım Akbaş
Department of Animal Science
Faculty of Veterinary Medicine
Burdur Mehmet Akif Ersoy University, Burdur, Türkiye

E-mail: icould_akbas@hotmail.com
<http://actavet.vfu.cz/>

For each region where breeding is undertaken, its own genetic resources are of great importance (Akçay et al. 2025). One of the main activities related to the conservation of farm animal genetic resources is the implementation of studies involving the characterization of the population's phenotypic traits, performance, and genetic similarity (Álvarez et al. 2009). Studies on genetic characterization provide valuable information not only for the conservation of genetic diversity but also for understanding variation within and between races (Álvarez et al. 2004; Tapio et al. 2010; Calvo et al. 2011).

The establishment of genetic databases based on morphological traits and molecular genetic markers is extremely important for the development of a bioinformatics infrastructure specific to animal breeds that form part of cultural heritage. Among the effective methods that can be used to support this bioinformatics infrastructure and enable more efficient conservation strategies, molecular genetic data-based approaches, and particularly microsatellite markers, are prioritised (Bowling 2001). Mitochondrial DNA (mtDNA) is a widely used tool for assessing genetic diversity and evolutionary relationships among species (Gissi et al. 2008). The control region of mtDNA (the D-loop region) has been shown to be particularly informative in elucidating the origins of many animal species (Bruford et al. 2003). Therefore, mtDNA, and specifically the D-loop region, is frequently preferred as a marker for determining maternal lineage and genetic diversity in goats, as well as for phylogenetically revealing genetic relationships between breeds or populations (Manceau et al. 1999; Luikart et al. 2001; Mannen et al. 2001; Sultana et al. 2003; Sultana and Mannen 2004; Joshi et al. 2004; Chen et al. 2005; Pereira et al. 2005; Sardina et al. 2006; Fernandez et al. 2006; Naderi et al. 2007).

Luikart et al. (2001) conducted the first study aimed at characterising mtDNA diversity in goats and identified three mitochondrial haplogroups (A, B, and C) in domestic goats, suggesting either multiple maternal origins or haplotype introgression following initial domestication. Subsequently, studies investigating goat genetic diversity in various countries, including Pakistan (Sultana et al. 2003), Spain (Amills et al. 2004; Azor et al. 2005), India (Joshi et al. 2004), China (Chen et al. 2005), Portugal (Pereira et al. 2005), Sicily (Sardina et al. 2006), and South Korea (Odahara et al. 2006), reported the presence of three additional haplogroups (D, E, and F). However, the most comprehensive analysis of mtDNA diversity in goats was carried out by Naderi et al. (2007). That study aimed to resolve ambiguities in earlier research by establishing a standardised nomenclature for goat mitochondrial haplogroups and by providing a robust global framework for interpreting goat genetic diversity. Six mitochondrial haplogroups (A, B, C, D, F, and G) were identified in domestic goats. The phylogenetic analysis included goat breeds from a wide range of countries, including Italy, Iran, Egypt, Nigeria, Libya, Jordan, Kazakhstan, Syria, Mongolia, Azerbaijan, France, Albania, Austria, Portugal, India, China, Korea, Switzerland, Spain, Romania, South Africa, and Pakistan. Haplogroup A is the most widespread and dominant lineage, while haplogroups B and C are more common in Central and East Asia. Haplogroup G is primarily associated with goat populations in the Near East and Anatolia. The phylogeographic structure of these haplogroups provides valuable information about domestication, migration routes, and regional adaptation patterns. Therefore, it provides powerful phylogeographic assessments useful for evaluating native Turkish breeds, tracing their maternal origins, understanding historical demographic dynamics, and assessing conservation priorities.

This study aimed to perform preliminary molecular characterization to phylogenetically determine the maternal diversity of the Manavli goat and to reveal its relationships with indigenous Anatolian goat breeds.

Materials and Methods

Ethical approval

The study was approved by the Burdur Mehmet Akif Ersoy University Local Ethics Committee on Animal Experiments (20.05.2020, resolution number: 647).

Blood samples collection and DNA extraction

Blood samples from 17 unrelated goats belonging to 3 Manavli goat flocks located within the boundaries of Denizli province were used as the material for this study. From each individual, 10 ml of blood was collected into EDTA-coated tubes and stored at 4 °C. DNA extraction was performed using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germany), following the manufacturer's protocol. The quality and concentration of the extracted DNA were measured using a Denovix DS-11 spectrophotometer, and all samples were diluted to a final concentration of 50 ng/μl. The integrity of the isolated DNA samples was checked by electrophoresis on a 0.5% agarose gel at 130 V/h, stained with GelRed, and visualised using the Azure Biosystems 280 agarose gel imaging system.

Amplification and sequencing

For the sequence analysis of the caprine mtDNA control (D-loop) region, a 1002-bp fragment was amplified by polymerase chain reaction (PCR) using the forward and reverse primers previously described by Sultana et al. (2003). The PCR reaction was prepared according to the kit protocol (Taq DNA Polymerase; Thermo Scientific, Germany) and performed in a Bio-Rad PCR system (T100 Thermal Cycler; Singapore) under the following conditions: initial denaturation at 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 30 s, and extension at 72 °C for 2 min. PCR products were visualised by gel electrophoresis on a 2% agarose gel at 150 V for 40 min. The primers used for this purpose are listed in Table 1.

Table 1. Primers used to amplify the goat mtDNA D-loop region.

Primer	Primer sequence; 5'→3'	Reference
Forward primer: 2F	5'-CCTCACTATCAGCACCCAAAGC-3'	Sultana et al. 2003
Reverse primer: 1R	5'-CTACAATTTATGCTCCGGGTGC-3'	

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen), and all amplified fragments were directly sequenced in both directions using an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Raw sequence quality control was evaluated using Chromas Lite 2.6.6 software.

Genetic diversity, population history, and phylogenetic analyses

To determine the haplotypes and haplogroups to which they belong among the indigenous goat breeds of Anatolia, reference sequences obtained from GenBank (Naderi et al. 2007; Akis et al. 2014) were used. The mtDNA data of indigenous goat genotypes, together with the reference sequences from GenBank, were aligned and saved using the Clustal W Multiple Alignment option in BioEdit v.7.1.3.0 (Hall 2001). Based on these aligned sequences, nucleotide diversity, haplotype diversity (Hd), and nucleotide substitution rates were calculated using the DnaSP v6 program (Librado and Rozas 2009). Genetic distance between populations was assessed using Nei's standard genetic distance and Wright's F-statistics (*F_{st}*) (Nei and Kumar 2009; Excoffier and Lischer 2010; Kumar et al. 2016). Based on the genetic distance data obtained between genotypes, a genetic distance tree of the populations was constructed using the Phylip v3.6 program (Felsenstein 2005).

Results

Within the scope of the study, genomic DNA was isolated from blood samples collected from Manavli goats. DNA samples, whose quality and concentration were assessed, were subjected to PCR amplification using primers designed for the mitochondrial D-loop gene regions.

After removing low-quality reads, a final alignment length of 648 bp was obtained. Within this region, 30 polymorphic sites defining 8 haplotypes were detected in the Manavli population. All haplotypes identified in this study are compiled and presented in Fig. 1 (Plate II). Additionally, all 30 polymorphic regions detected in Manavli goats are given in Table 2. The following list is of the Naderi et al. (2007) and Akis et al. (2014) Manavli haplogroups; the reference sequence is AF533441.1.

Hap_1: 9 [Manavli_1 Manavli_3 Manavli_5 Manavli_6
 Manavli_8 Manavli_9 Manavli_13 KC574105
 KC574101]
 Hap_2: 1 [Manavli_2]
 Hap_3: 2 [Manavli_4 Manavli_11]
 Hap_4: 1 [Manavli_7]
 Hap_5: 3 [Manavli_10 Manavli_14 KC574182]
 Hap_6: 2 [Manavli_12 Manavli_17]
 Hap_7: 1 [Manavli_15]
 Hap_8: 1 [Manavli_16]
 Hap_9: 1 [HQ996625]
 Hap_10: 1 [HQ996624]
 Hap_11: 2 [HQ996623 HQ996620]
 Hap_12: 1 [HQ996622]
 Hap_13: 1 [HQ996610]
 Hap_14: 1 [HQ996621]
 Hap_15: 1 [HQ996619]
 Hap_16: 1 [HQ996618]
 Hap_17: 1 [HQ996617]
 Hap_18: 1 [HQ996616]
 Hap_19: 1 [HQ996615]
 Hap_20: 1 [HQ996614]
 Hap_21: 1 [HQ996613]
 Hap_22: 1 [HQ996612]
 Hap_23: 1 [HQ996611]
 Hap_24: 2 [EF618505 EF618529]
 Hap_25: 1 [EF618506]
 Hap_26: 3 [EF618492 EF618507 EF618508]
 Hap_27: 1 [EF618493]
 Hap_28: 1 [EF618494]
 Hap_29: 1 [EF618495]
 Hap_30: 1 [EF618496]
 Hap_31: 1 [EF618509]
 Hap_32: 1 [EF618510]
 Hap_33: 1 [EF618511]
 Hap_34: 1 [EF618512]
 Hap_35: 1 [EF618513]
 Hap_36: 1 [EF618514]
 Hap_37: 1 [EF618497]
 Hap_38: 1 [EF618515]
 Hap_39: 1 [EF618516]
 Hap_40: 1 [EF618517]
 Hap_41: 1 [EF618518]
 Hap_42: 1 [EF618519]
 Hap_43: 1 [EF618520]
 Hap_44: 1 [EF618521]
 Hap_45: 1 [EF618522]
 Hap_46: 4 [EF618536 EF618537 EF618538 EF618535]
 Hap_47: 2 [EF618523 EF618524]
 Hap_48: 2 [EF618525 EF618498]
 Hap_49: 1 [EF618526]
 Hap_50: 1 [EF618539]
 Hap_51: 1 [EF618499]
 Hap_52: 1 [EF618500]
 Hap_53: 1 [EF618501]
 Hap_54: 1 [EF618502]
 Hap_55: 1 [EF618503]
 Hap_56: 1 [EF618504]
 Hap_57: 2 [EF618527 EF618528]
 Hap_58: 1 [EF618530]
 Hap_59: 1 [EF618531]
 Hap_60: 1 [EF618532]
 Hap_61: 1 [EF618533]
 Hap_62: 1 [EF618534]
 Hap_63: 2 [KC574380 KC574372]
 Hap_64: 1 [KC574378]
 Hap_65: 1 [KC574369]
 Hap_66: 1 [KC574345]
 Hap_67: 3 [KC574343 KC574323 KC574296]
 Hap_68: 1 [KC574324]
 Hap_69: 1 [KC574308]
 Hap_70: 2 [KC574284 KC574283]
 Hap_71: 1 [KC574282]
 Hap_72: 2 [KC574281 KC574280]
 Hap_73: 1 [KC574279]
 Hap_74: 1 [KC574278]
 Hap_75: 1 [KC574277]
 Hap_76: 1 [KC574276]
 Hap_77: 1 [KC574275]
 Hap_78: 1 [KC574274]
 Hap_79: 1 [KC574273]
 Hap_80: 3 [KC574185 KC574186 KC574188]
 Hap_81: 1 [KC574241]
 Hap_82: 1 [KC574184]
 Hap_83: 1 [KC574086]
 Hap_84: 1 [KC574183]
 Hap_85: 1 [KC574181]
 Hap_86: 1 [KC574180]
 Hap_87: 1 [KC574179]
 Hap_88: 1 [KC574178]
 Hap_89: 1 [KC574177]
 Hap_90: 1 [KC574176]
 Hap_91: 1 [KC574174]
 Hap_92: 1 [KC574173]
 Hap_93: 1 [KC574163]
 Hap_94: 1 [KC574129]
 Hap_95: 1 [KC574118]
 Hap_96: 1 [KC574106]
 Hap_97: 1 [KC574160]
 Hap_98: 1 [KC574155]

The agarose gel image of the PCR products is presented in Fig. 2 (Plate II).

Haplotype diversity and nucleotide diversity in Manavli goats were calculated as 0.824 and 0.01281, respectively. The Tajima's D value, used to evaluate the neutral evolution hypothesis within the population, was calculated as -0.54286 and found to be non-significant ($P > 0.05$). Haplotype assignment revealed that all Manavli goat individuals clustered within mtDNA haplogroup G, with only rare representation of haplogroup A (Table 2). No individuals belonged to haplogroups B, C, D, or F (Naderi et al. 2007).

When the maternal ancestry of the Manavli goat among indigenous Anatolian breeds was examined through network analysis (Network v.4.516) (Bandelt et al. 1999) (Plate III, Fig. 3), a large and dense cluster was observed at the centre of the network, with all Manavli individuals positioned within this central cluster. Analysis of molecular variance (AMOVA) statistics (Arlequin v3.5.1.2) (Excoffier and Lischer 2010) were used to determine hierarchical variance components among breeds.

According to the AMOVA results, approximately 10.18% of the total genetic variation was attributed to differences among populations ($F_{st} = 0.1018$, $P < 0.01$), while 89.82% of the variation occurred within populations. In the unweighted pair group method with arithmetic mean (UPGMA) dendrogram constructed using indigenous Anatolian goat breeds (Phylip v3.69) (Felsenstein 2005; Page 1996) (Fig. 4), the Manavli goat was found to be closely related to the Ankara goat based on population-level genetic distances.

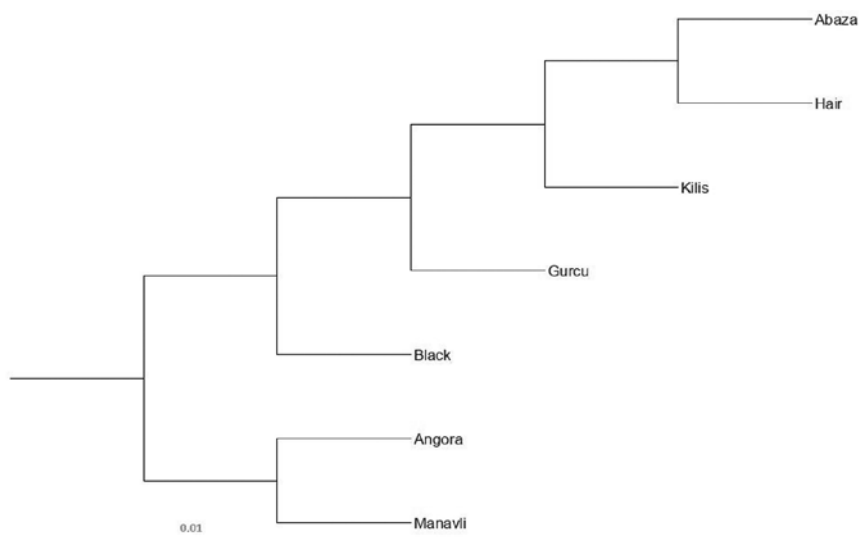


Fig. 4. The unweighted pair group method with arithmetic mean (UPGMA) dendrogram for the mtDNA genetic similarity of Anatolian breeds and Manavli goat on a population basis, scale bar = 0.01 substitutions per site (PHYLIP 3.69 and TreeView)

Discussion

In the present study, 30 polymorphic sites and 8 different haplotypes were detected within the mtDNA D-loop region. Haplotype diversity and nucleotide diversity in Manavli goats were calculated as 0.824 and 0.01281, respectively. Consistent with these findings, Naderi et al. (2007) reported a haplotype diversity of 0.995 in indigenous Turkish goat

breeds based on mtDNA D-loop sequence analyses conducted across seven geographic regions spanning Europe, Asia, and Africa. Similarly, a study investigating phylogenetic relationships in the Sicily region using mitochondrial D-loop sequence data reported an average haplotype diversity of 0.969 and an average nucleotide diversity of 0.0236 (Sardina et al. 2006). Together these results indicate high haplotype diversity accompanied by moderate nucleotide diversity, suggesting that the population has experienced historical processes such as migration or admixture. Comparable patterns have been reported in other populations, where high haplotype diversity combined with moderate nucleotide diversity is generally associated with past migration or admixture events (Tajima 1989; Fu 1997). The Tajima's D value for the Manavli population was -0.54286 ($P > 0.05$). Although the negative value indicates a slight tendency towards population expansion, this result was not significant. These findings indicate that the genetic structure of the population is relatively stable and that there has been no strong selective pressure or recent expansion (Tajima 1989).

In the study by Naderi et al. (2007), 98 different haplotypes were identified, with a Hd of 0.9920. This finding underscores that the indigenous Anatolian goat populations examined possess extensive genetic variation, reflecting their wide geographic distribution and long-term evolution under diverse selective pressures. When evaluated alongside other Anatolian breeds, these high diversity values further demonstrate that genetic diversity within the Manavli population is comparatively high.

The distribution of haplogroups within the Manavli goat is consistent with earlier reports on Anatolian native breeds, further supporting its phylogenetic position within regional maternal lineages. According to network analysis (Fig. 3) for the maternal ancestry of the Manavli goat among native Anatolian goat breeds, the Manavli goat is positioned within this central cluster. Haplotype assignments based on D-loop variation showed that Manavli goats predominantly belong to Haplogroup G, which is the major haplogroup described by Naderi et al. (2007) in global domestic goat populations. A small number of individuals showing affinity to Haplogroup A were also identified, consistent with patterns reported for Anatolian breeds. The haplogroups referred to in this study as Anatolia G and Anatolia A correspond directly to the standardized global haplogroups A and G defined by Naderi et al. (2007). By positioning within these haplogroups, Manavli goats demonstrate a long-term maternal association with indigenous Anatolian goat lineages (Table 2). This finding also suggests that the Manavli goat shares a long-term common evolutionary history with indigenous goat breeds in Anatolia. Considering these comparisons, the Manavli goat remains strongly clustered within the Anatolian A haplogroup, which reinforces its genetic differences as a native gene resource in Anatolia and can be interpreted as an indication of a long-term association with its geographic region (Kul and Ertuğrul 2011).

In the current study, AMOVA results indicated that 10.18% of the total genetic variation occurred among populations. Consistent with the mtDNA D-loop phylogenetic data, the UPGMA dendrogram showed that the Manavli goat forms a close clade with the Angora (Ankara) goat, indicating a strong maternal genetic relationship that may derive from a shared ancestral haplogroup. Signals of extensive DNA polymorphism and relatively high genetic distance values among haplotypes were also observed. Overall, phylogenetic analyses revealed that Manavli goat populations exhibit strong genetic connections with indigenous Anatolian goat breeds, showing notable genetic similarity with Angora, Abaza, Gürcü, Kil, and Kilis goats.

The preservation of indigenous genetic resources is of critical importance in all geographical regions where animal breeding is practised. Native breeds, having developed adaptive capacities to local environmental and climatic conditions, play a unique and strategic role in national livestock policies by sustaining their own viability while meeting human needs through animal products. Although there has been a notable increase in studies

aimed at identifying indigenous goat genetic resources in Türkiye and describing new breeds, current efforts remain insufficient to fully address the needs of the field. Alongside detailed investigations of recognised goat breeds, it is equally important to identify, document, and promote new indigenous genetic resources, particularly those maintained by local communities and forming an integral part of the country's cultural heritage, which remain under-characterised or undiscovered. Of particular concern are genotypes within widely recognised breeds that face the risk of disappearing before their distinctive genetic characteristics can be adequately identified. This situation makes it essential to intensify molecular genetic characterisation studies, especially in goats and other farm animals, in order to support effective conservation and sustainable utilisation strategies.

This study represents the first investigation addressing the molecular phylogenetic structure of the Manavli goat, an indigenous genetic resource reared under traditional breeder conditions and previously insufficiently characterised. Analyses based on the mtDNA D-loop region revealed that the Manavli goat population is genetically slightly differentiated from other indigenous Anatolian goat breeds, while maintaining substantial genetic diversity, as indicated by high haplotype diversity. In addition, the close genetic relationship observed between Manavli goats and Ankara, Gürcü, and Kilis goats highlights the importance of this genotype for the overall genetic diversity and conservation of regional goat populations. The results of present study do not indicate any signs of genetic erosion in the Manavli population, supporting its preservation as a distinct regional genetic resource.

The findings of this study indicate that genetic diversity should be considered not only at the breed level but also among the subtypes and varieties that comprise a breed. Increasing the sample size of Manavli goat individuals would therefore allow a more precise assessment of whether this genotype represents a genetically homogeneous group within a single lineage or exhibits evidence of admixture. Although the present results support the preservation of the Manavli goat as a distinct genetic variant and its inclusion in sustainable breeding programmes, they also emphasise the need for further, more comprehensive investigation. Identifying potential unique genetic characteristics of the Manavli goat through expanded sampling and deeper genomic analyses would represent a meaningful contribution to national livestock conservation and genetic improvement efforts.

Conflict of interest

The authors declare no conflict of interest.

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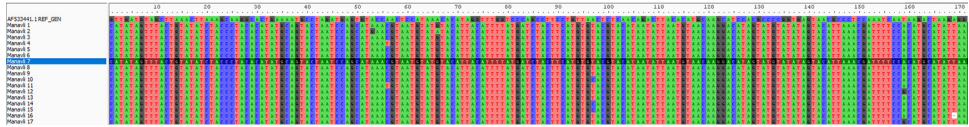


Fig. 1. Distribution of mtDNA D-loop haplotypes identified in Manavli goats and reference sequences from GenBank.

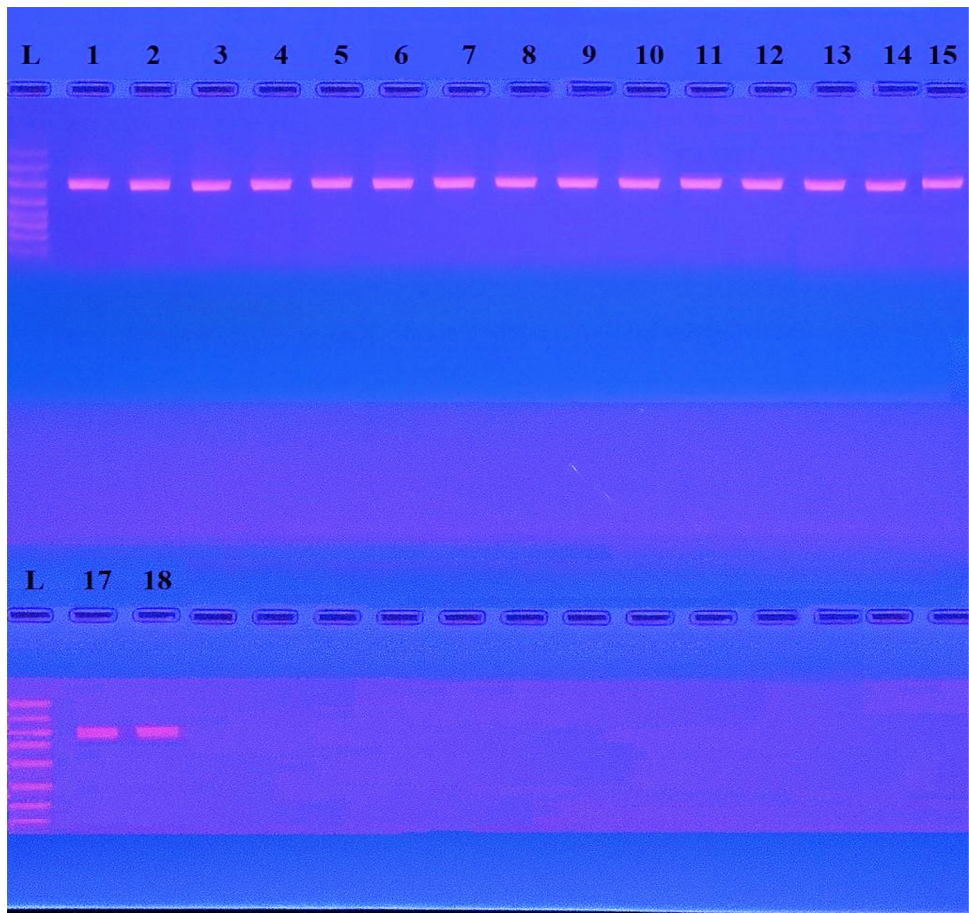


Fig. 2. Agarose gel electrophoresis image of PCR products (1,002 bp)

L: molecular ladder (200 bp DNA marker)

