



Original Article

Conservation Genetics and Phylogenetic Analysis of Wild Goats in The Barzan District of The Kurdistan Region of Iraq

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ABSTRACT

The genetic assessment of *Capra aegagrus* in Barzan district, Kurdistan region, north of Iraq, was investigated. Thirteen blood samples were collected from twelve different locations. Partial mitochondrial D-loop region targeting 756 bp was amplified through a set of specific primers. Several genetic tests were performed to assess the phylogenetic and genetic variation among them and neighborhood-studied samples. In total, 47 sequences were used for phylogenetic tree construction, including 13 studied samples and 34 neighborhood sequences. These sequences were put into seven distinct clades in the phylogenetic tree in which the Barzan sequences were found in the clade I and II. These clades included Turkish and Iranian sequences. Genetic differentiation (F_{st}) showed that Barzan sequences were closely related to wild goats in Iran and Turkey, being 0.21193 and 0.27451, respectively. There was a clear gene flow (N_m) of Barzan sequences with Iranian (0.93) and Turkish (0.66) wild goats. There was a medium rate of genetic diversity among the Barzan sequences. The nucleotide diversity (π) was 0.0423, and average number of nucleotide differences (K) was 24.026. The highest pairwise distance was found between Silke and the Turkish sequence (EF989489), being 0.1611, and the lowest value was recorded between Shkafta Valley Two and the Iranian sequence (EF989610), being 0.0278. There was a clear genetic distance among Barzan sequences in which the highest pairwise distance was between Alka and Dore (0.0825), and the lowest one was between Bedaron and Zrara (0.00). This study indicates a close relationship among wild goats in Barzan, Iran, and Turkey.

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Keywords: D-loop DNA, phylogenetic tree, gene flow, genetic distance, PCR.

1 Introduction

A number of studies were focused on the evolutionary history and genetic divergence of the *Capra* genus, which includes wild goats like the markhor, wild ibex, and bezoar goat. Early researchers, such as [1] and [2], used nuclear markers and mitochondrial DNA to create a phylogenetic tree of *Capra* species, showing that the bezoar goat is the closest ancestor of domesticated goats. This phylogenetic structure indicates that goats were domesticated from wild populations in the Near East around 10,000 years ago, with the divergence of the wild ibex and domesticated lineages occurring two to three million years ago, during the Pleistocene.

The study of [3, 4] support this gradual divergence through mitochondrial analysis, suggesting that domestication was a gradual process rather than an abrupt event. Since the Neolithic agricultural revolution, domestic goats (*C. hircus*) were become

integral to agriculture, the economy, and culture [5], with the first domestication of goats occurring in the Fertile Crescent from the bezoar goat.

Recent studies were further explored the genetic structuring of wild goat populations, especially the bezoar goat. For example, [6] examined genetic variation among bezoar goats across the Middle East and Central Asia, identifying significant genetic differences due to geographic isolation during the Pleistocene. These findings highlight the role of environmental factors [7], such as glaciations and climate shifts, in shaping genetic diversity. Similarly, [8] analyzed genetic relationships among various *Capra* species, including the Nubian ibex and the bezoar goat, finding that geographic barriers and climate change were influenced the evolutionary history of these species.

The classification and phylogeny of wild goats were the subject of extensive study, with different authors proposing various taxonomic classifications. Some researchers recognize up to thirteen species in the *Capra* genus, while others group certain

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populations under a single species^[9]. The Studies reveal the use of different genetic material regions including the control region (D-loop), ribosomal genes, and protein-coding genes of the entire mitogenomes of domestic and wild goats for differentiating their primary maternal lineages^[10]. This author obtained such results confirming the beneficial use of partial D-loop region in performing the genetics of the *Capra* genus.

The Kurdistan region of Iraq is home to some of the most genetically diverse populations of *Capra aegagrus* in the country. Recent research^[11] showed that wild goats in this region differ genetically from those in the rest of Iraq. The isolated populations in Kurdistan were evolved distinct genetic lineages that are important for both the conservation of wild goats and the understanding of goat domestication. Studies using microsatellite markers and mitochondrial DNA were provided insight into the population diversity and structure in this area. These populations represent a large reserve of genetic material that is crucial for the broader goat gene pool and domestication history. The genetic evidence underscores Kurdistan's significance as a potential origin for the domestication of goats and highlights the importance of protecting these populations for future conservation efforts. Despite challenges like habitat loss and poaching, the conservation of these wild goat populations is essential to preserve their genetic diversity for future generations.

Capra aegagrus, particularly in the Kurdistan region, holds immense ecological and genetic significance. The genetic studies in Iraq and neighboring regions, such as Iran and Turkey, reveal unique genetic traits in the wild goat populations, shaped by isolation and environmental conditions^[12]. The preservation of these genetic resources is crucial for both the survival of the species and for understanding the evolutionary history of

domestic goats. Conservation programs in Kurdistan, focused on safeguarding native habitats and protecting these unique genetic lineages, are essential for maintaining genetic diversity and ensuring the long-term viability of wild goat populations. The objectives of this study were to explore the origin and genetic diversity of *Capra aegagrus* through targeting a partial D-loop region of the mitochondrial DNA. This study would not only improve our knowledge of the wild goat's origins but may also support conservation efforts to protect its genetic diversity.

2 Methods and Materials

2.1 Animal ethics

This research was conducted following the Animal ethics regulations in College of Agricultural Engineering Sciences, University of Duhok under the accession number: AEC 16052025.

2.2. Study area

This study was conducted in the Barzan District, located on the northern banks of the Great Zab River in the Erbil Governorate of the Kurdistan Region in Iraq. Barzan is located at coordinates (longitude: 43.774403 to 44.414705; latitude: 36.750910 to 37.152746) on the western foothills of the Zagros Mountains (altitude: 504-2300m a.s.l.). The habitat of this area is characterized by a sophisticated landscape consisting of deep valleys, large limestone formations, and high mountains that offer different microhabitats for a diverse range of living organisms, including wild goats. The climate of this region is distinguished by cold, wet winters and hot, dry summers, which affect the seasonal migration and habitat preferability of wild goats.

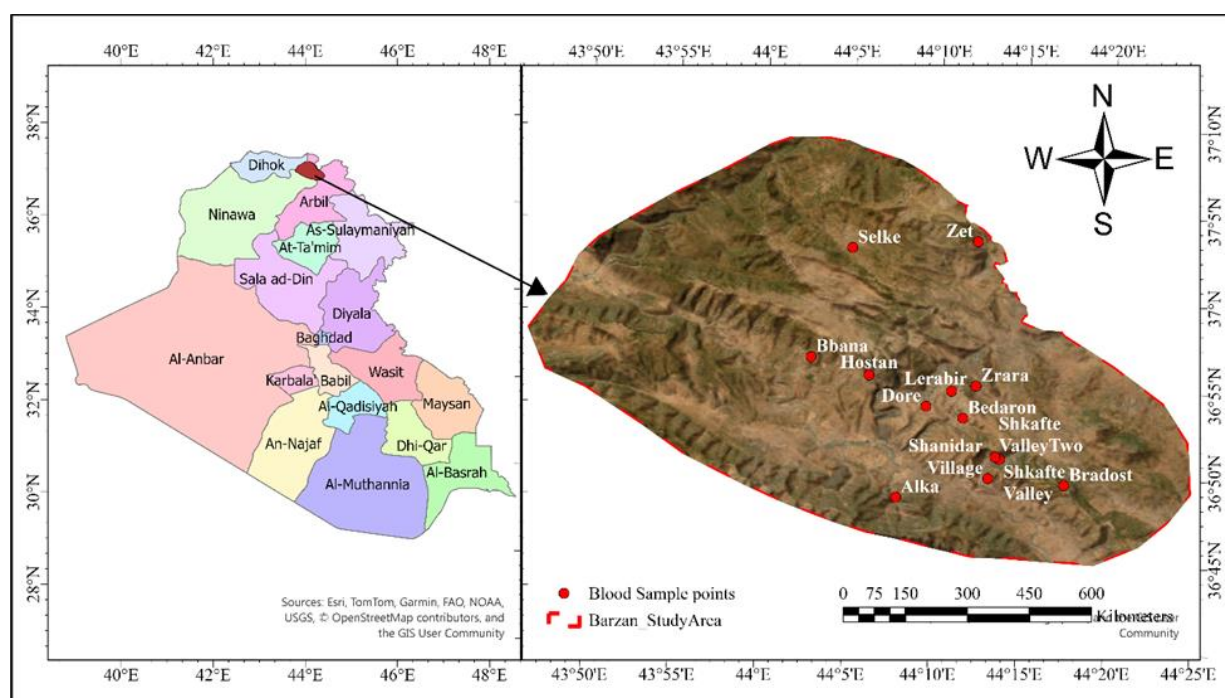


Figure 1: Locations of taking *Capra aegagrus* blood samples.



Figure 2: Field observations of wild goats (*Capra aegagrus*) in diverse mountainous habitats.

2. 3 Blood Sample Collection

A total of thirteen blood samples in various populations and locations were obtained from wild goats (buck and doe) in the Barzan area between September 1, 2024, and February 29, 2025 (Fig. 1 and 2). Two methods were used to draw blood from the wild goats: first, a dart gun was used to anesthetize the animal with 0.22 mg/kg of Xylazine. Additionally, the sick and injured

wild goats were brought for medication by the “Barzan Environmental Protection Battalion's” aide, under the veterinarian's monitoring. Blood is drawn from the jugular veins using a 10 ml syringe (18G x 25 mm). The area where the blood was drawn was sanitized with alcohol and cotton. Two milliliters of the blood are then placed in EDTA tubes and then sent in a cold box to the GenomeVet laboratory (Duhok, Iraq) for molecular analysis.

2. 4 Molecular characterization

2. 4. 1 Total genomic DNA extraction

The total genomic DNA was extracted from whole blood using DNeasy® Blood & Tissue Kit (QIAGEN, Germany). The quality and quantity of the extracted DNA were measured by NanoDrop™ 2000/2000c (Thermo Fisher Scientific, USA) and agarose gel electrophoresis. Then, the DNA samples were saved at -20 °C for further use.

2. 4. 2 Partial mitochondrial D-loop amplification

The most polymorphic region of mitochondrial DNA (D-loop

region) was targeted for amplification. A set of primers (Table 1), which was previously designed by ^[10], that synthesized by the Macrogen company from South Korea and used in this study to amplify 756 bp of the D-loop region. A total of 50 µl reaction was set up using AddTaq Master Mix (Add Bio, South Korea) containing all required components for PCR amplification. The PCR program was performed in a GeneAmp PCR thermal cycler system (Applied Biosystems 9700, USA) as follows: one cycle of initial denaturation at 95 °C for 5 minutes followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 59 °C for seconds, extension at 72 °C for 60 seconds, and one cycle of final extension of 5 minutes at 72 °C. Finally, the results were confirmed in 2% agarose gel electrophoresis.

Table 1: Primer information for partial D-loop amplification.

Primer Name	Sequences	Size (bp)	Amplicon (bp)
D-loop F	5' CAACACAACTTCCCACTCCAC 3'	22	756
D-loop R	5' AGATGGCCCTGAAGAAAGAACC 3'	22	

2. 4. 3 DNA Sequencing and Alignments

The PCR products for partial D-loop region of mitochondrial DNA of goats were sent for sequencing to Macrogen, South of Korea using Sanger sequencing method. The sequencing was performed for these amplicons in both directions (forward and revers). The data were cleaned and consensus files were aligned

using forward and revers templates for each sample using Molecular Evolutionary genetic analysis (MEGA 12.0.10). For sequence confirmation 34 accession number of genomic data of *Capra aegagrus* were obtained from genbank, NCBI. Moreover, the studied DNA sequences of local wild goats were submitted to NCBI GenBank as a prerequisite for reliability and provided with accession numbers (PV754607- PV754619) as shown in (Table 2).

Table 2: GenBank accession numbers, wild goat species, and country of complete mitogenomes used in the bioinformatics analysis of present study.

No.	GenBank ID	Species	Maternal Haplogroups	country
1.	EF989194	<i>Capra aegagrus</i>		Iran
2.	EF989195	<i>Capra aegagrus</i>		Iran
3.	EF989196	<i>Capra aegagrus</i>		Iran
4.	EF989198	<i>Capra aegagrus</i>		Iran
5.	EF989197	<i>Capra aegagrus</i>		Iran
6.	EF989610	<i>Capra aegagrus</i>		Iran
7.	EF989200	<i>Capra aegagrus</i>		Turkey
8.	EF989201	<i>Capra aegagrus</i>		Turkey
9.	EF989184	<i>Capra aegagrus</i>		Turkey
10.	EF989187	<i>Capra aegagrus</i>		Turkey
11.	EF989190	<i>Capra aegagrus</i>		Turkey
12.	EF989163	<i>Capra aegagrus</i>		Iran
13.	EF989168	<i>Capra aegagrus</i>		Iran
14.	EF989191	<i>Capra aegagrus</i>		Turkey
15.	EF989171	<i>Capra aegagrus</i>		Iran
16.	EF989176	<i>Capra aegagrus</i>		Iran
17.	EF989169	<i>Capra aegagrus</i>		Iran
18.	EF989170	<i>Capra aegagrus</i>		Iran

19.	EF989408	<i>Capra aegagrus</i>		Iran
20.	EF989211	<i>Capra aegagrus</i>		Iran
21.	EF989270	<i>Capra aegagrus</i>		Iran
22.	EF989420	<i>Capra aegagrus</i>		Iran
23.	EF989642	<i>Capra aegagrus</i>		Pakistan
24.	EF989644	<i>Capra aegagrus</i>		Pakistan
25.	AB110590	<i>Capra aegagrus</i>		Pakistan
26.	EF989538	<i>Capra aegagrus</i>		Iran
27.	EF989495	<i>Capra aegagrus</i>		Turkey
28.	EF989489	<i>Capra aegagrus</i>		Turkey
29.	EF989493	<i>Capra aegagrus</i>		Turkey
30.	EF989573	<i>Capra aegagrus</i>		Turkey
31.	EF989629	<i>Capra aegagrus</i>		Turkey
32.	EF989612	<i>Capra aegagrus</i>		Iran
33.	EF989636	<i>Capra aegagrus</i>		Daghestan
34.	FJ207526	<i>Capra ibex</i>	Outgroup	European countries
35.	Shkafe Valley Two_PV754607	<i>Capra aegagrus</i>		Iraq
36.	Bedaron_PV754608	<i>Capra aegagrus</i>		Iraq
37.	Bbana_PV754609	<i>Capra aegagrus</i>		Iraq
38.	Alka_PV754610	<i>Capra aegagrus</i>		Iraq
39.	Skafe Valley_PV754611	<i>Capra aegagrus</i>		Iraq
40.	Shanidar Village_PV754612	<i>Capra aegagrus</i>		Iraq
41.	Selke_PV754613	<i>Capra aegagrus</i>		Iraq
42.	Lerabir_PV754614	<i>Capra aegagrus</i>		Iraq
43.	Zet_PV754615	<i>Capra aegagrus</i>		Iraq
44.	Bradost_PV754616	<i>Capra aegagrus</i>		Iraq
45.	Zrara_PV754617	<i>Capra aegagrus</i>		Iraq
46.	Hostan_PV754618	<i>Capra aegagrus</i>		Iraq
47.	Dore_PV754619	<i>Capra aegagrus</i>		Iraq

2. 4. 4 Phylogenetic tree construction

To illustrate the evolutionary relationships within and between populations of wild goats, Mega Software (version 12.0.10) was used to construct the phylogenetic tree. Mitochondrial genes from the D-loop regions were gathered from new DNA samples of 13 wild goats and 34 DNA sequences from NCBI GenBank. The ClustalW of MEGA's integrated alignment tools was used to align the DNA sequences to ensure the homologous comparison. The Akaike Information Criterion (AIC) is a statistical criterion in MEGA's model selection function used to identify the best-fit substitution model after alignment. The phylogenetic tree was constructed through the Maximum Likelihood (ML) evaluated by bootstrap analysis as a statistical support.

2. 4. 5 Gene flow and Genetic differentiation

These sequences were put into seven groups including Barzan district in Kurdistan region of Iraq, Iran, Turkey, Daghestan,

Azerbaijan, and Pakistan. The gene flow and genetic differentiation was performed using the DNA Sequence Polymorphism (DnaSP version: 6.12.03) to measure the haplotypes number, haplotype diversity, average number of nucleotide differences, and nucleotide diversity (π) among studied sequences (13 sequences) and closely related sequences from phylogenetic tree (Iran and Turkey).

3 Results

3. 1 DNA extraction and quantification

The used kit for DNA extraction was successful in isolating the pure and good quality nucleic acids from whole blood samples that were collected for this study. The overall 260/280 score in the NanoDrop spectrophotometer was 1.78-2 with the concentration from to indicating the good quality DNA samples. The DNA bands in the agarose gel electrophoresis were bright and clean.

3. 2 Partial D-loop mitochondrial DNA amplification

The primer set were collected from the literature was successful in amplifying the targeted region of D-loop region of mitochondrial DNA (756 bp) from studied sample.

3. 3 Phylogenetic relationships based on the of partial mitochondrial genome

A phylogenetic tree was constructed from partial mitochondrial DNA (D-loop) sequences to investigate the genetic relationships between *Capra aegagrus* individuals. The final dataset consisted of 47 sequences including both under study and NCBI sequences. The best-fit model was Tamura 3-parameter. The 1,000 bootstrap repetitions were used to assess the clade's supporting strength by Maximum Likelihood methods.

Phylogenetic studies were assigned seven main clades within *Capra aegagrus* (Fig. 3). Clade I comprised 16 samples, including the Barzan and Iran (bootstrap=73). Clade II consists of 15 sequences from the Barzan, Iran, and Turkey (bootstrap=100). The other sequences were clustered in the remaining clades with different bootstraps. Generally, all of the investigated sequences in this study were aggregated in clades I and II. Overall, the supporting bootstrap percentages among the

nodes constructing the phylogenetic tree ranged from 52% to 100%.

Therefore, the last four clades exhibited significant diversity, indicating that the clades were weaker relationships with the current samples under study. The long branch connecting clade I to clade II indicates a deep evolutionary divergence between them. The clade I, including Dore, Shkafte Valley, Shkafte Valley Two, Zet, and Lerabir, was very close to the *Capra aegagrus* in Iran, and Bbana, Hostan, and Shanidar Village had a close relationship with the *Capra aegagrus* in Turkey. While in clade II the Zrara, Bedaron, Selke, Bradost, and Alka had a relationship with wild goats from Iran and Turkey.

Interestingly, the clade II included the sample from Hostan, despite its closer geographic proximity to clade I populations, this may indicate a mislabeling or an ancient gene flow event. As well as, despite Zet being clustered with the clade I, which was very close to wild goats in Iran, geographically it lies on the border with Turkey. On the other hand, Selke, which was grouped with clade II and connected to wild goats in Iran and Turkey. The average number of substitutions at each site due to an evolutionary divergence was 0.091.

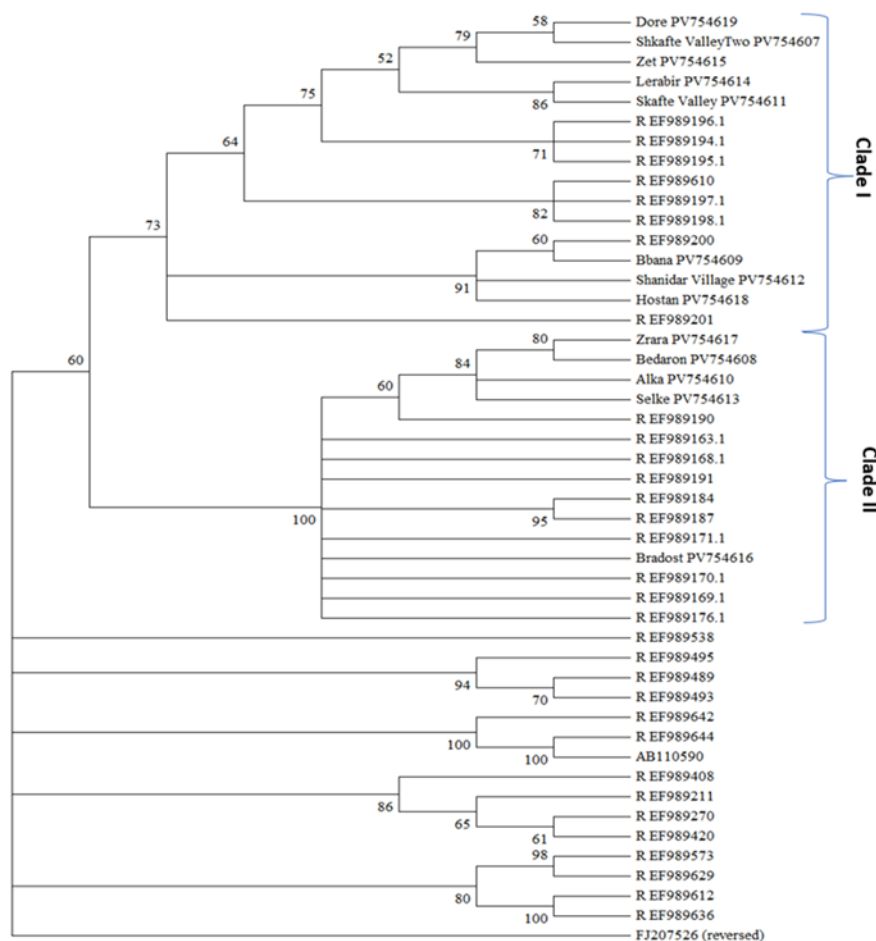


Figure 3: Phylogenetic tree of *Capra aegagrus* based on mitochondrial D-loop sequences, showing genetic relationships among populations from Barzan, Turkey, Iran, Daghestan, Pakistan, and Azerbaijan. Bootstrap values are indicated at the nodes.

3. 4 Genetic Differentiation and Gene Flow Among *Capra aegagrus* Populations

DnaSP (version: 6.12.03) was used to estimate the pairwise genetic differentiation (F_{st}) and gene flow (N_m) between the *Capra aegagrus* population in Barzan district, Kurdistan region of Iraq with 5 different populations from different countries including, Iran, Turkey, Daghestan, Azerbejan, and Pakistan. The genetic differentiation was ranged from moderate to very high between the wild goats in Barzan district and other populations depending on the obtained results. The F_{st} values varied from 0.21193 (Barzan-Iran) to 0.57617 (Barzan-Daghestan),

indicating considerable population structure. Gene flow (N_m) values were estimated, the highest value was between Barzan and Iran (0.93), and the lowest gene flow was between Barzan and Daghestan ($N_m = 0.18$). (Fig. 4). The limited gene flow was possibly due to geographic or ecological barriers.

Pairwise genetic differentiation (F_{st}) and gene flow (N_m) were calculated among populations of *Capra aegagrus* within the Barzan district as well. The results showed that there is a significant gene flow between the local populations, which were 0.14929 genetic differentiation (F_{st}) and 1.42 gene flow (N_m).

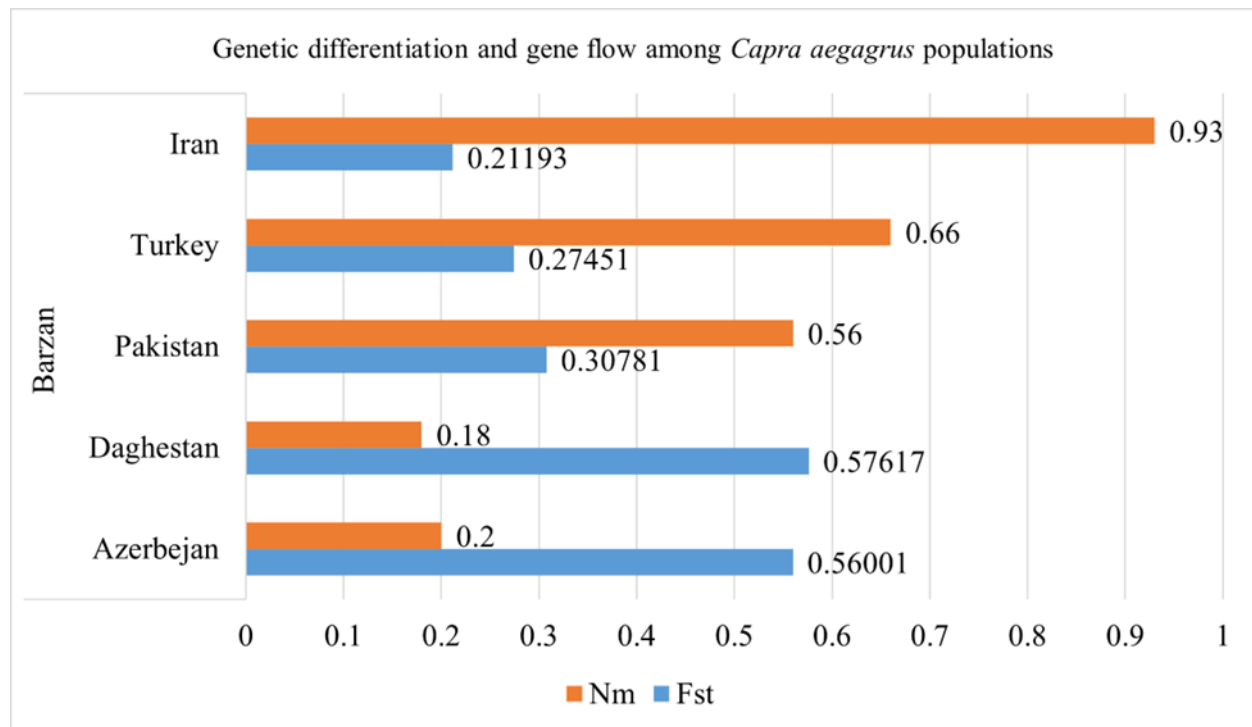


Figure 4: Pairwise genetic differentiation (F_{st}) values and estimated gene flow (N_m) between *Capra aegagrus* populations involving Iraq, calculated using DnaSP v6.

Pairwise genetic differentiation (F_{st}) and gene flow (N_m) have been calculated among populations of *Capra aegagrus* within the Barzan district as well. The results showed that there is a significant gene flow between the local populations, which were 0.14929 genetic differentiation (F_{st}) and 1.42 gene flow (N_m).

3. 5 Genetic Diversity of *Capra aegagrus* Across Its Geographic Range

Analysis of mitochondrial DNA sequence variation from *Capra aegagrus* populations in six different countries showed dramatic variation in levels of genetic diversity. Turkey had the highest nucleotide diversity ($\pi=0.09199$), average number of nucleotide differences ($K=41.212$), a huge number of polymorphic sites ($S=169$) along with 52 haplotypes, suggesting a highly diversified gene pool. In contrast, Azerbaijan had the lowest haplotype diversity (with only three haplotypes) and moderate

high nucleotide diversity ($\pi=0.05142$), indicating some forms of clustering of haplotypes or a rather recent demographic event. Similarly, 129 haplotypes with quite high diversity values ($\pi=0.07107$, $K=33.046$) in Iran, which overall is fairly representative in terms of sample size, document that it was acted as a source center for the genetic wealth of the species. Furthermore, Daghestan and Pakistan also exhibited significant nucleotide diversity ($\pi=0.0563$ and 0.0695 , respectively). Compared with other examined places, the wild goats' populations in Barzan district showed medium rates of genetic variation, the nucleotide diversity (π) was estimated at 0.0423, and the average number of nucleotide differences (K) was 24.026, both being lower than those observed in more genetically rich populations like Turkey and Iran. This indicates that the *C. aegagrus* contains significant genetic structuring throughout its range, where Turkey and Iran might act as two important reservoirs for mitochondrial diversity (Table 3).

Table 3: Genetic diversity indices for *Capra aegagrus* populations based on partial mitochondrial D-loop sequences.

Countries	S	h	π (Pi)	K
Barzan district	65	12	0.0423	24.026
Azerbaijan	67	3	0.05142	24.733
Daghestan	74	8	0.0563	26.8
Iran	188	129	0.07107	33.046
Turkey	169	52	0.09199	41.212
Pakistan	111	12	0.06946	31.603

S: number of polymorphic sites, h: number of haplotypes, π : nucleotide diversity, K: average number of nucleotide differences for each country.

3. 6 Genetic Distance Estimation Based on Tamura 3-Parameter Model

Tamura 3-parameter (T92) model were used to find out the pairwise genetic distances between *Capra aegagrus* populations using MEGA Software (version: 12.0.10). The analysis showed that the range of genetic distances is from 0 to 0.18621, indicating that the populations' levels of sequence divergence was differed. The maximum distance obtained during this analyze was 0.18621 which were between R_EF989629_Turkey and R_EF989489_Turkey, referring to a significant divergence among these populations throughout evolution. The populations R_EF989187_Turkey and R_EF989184_Turkey (0.0000) had the smallest distance, indicating a tight genetic connection. Similar results were found between R_EF989196.1_Iran and R_EF989195.1_Iran (0.0000). Genetic distances associating local populations reveal that the highest pairwise distance was between the populations of Selke and R_EF989489_Turkey (0.1611) while the lowest pairwise distance was between Shkafta Valley Two and R_EF989610.1_Iran (0.0278). In terms of the pairwise distances among the targeted local individuals of the study, the highest distance existed between Alka and Dore (0.0825), while the lowest distance occurred between Bedaron and Zrara (0.000); also, Skafta_Valley_6 and Lerabir appeared to be very closely related (0.00452). The overall average of genetic distance among 47 DNA sequences is 0.09141.

4 Discussion

The DNA extraction methodology successfully isolated a high-quality genomic DNA from whole blood sample, evidenced by the 260/280 absorbance ratios (1.78–2.0) and distinct, clear bands on agarose gels. These findings align with research by [13], which underscored the significance of high-purity samples in phylogenetic and demographic analyses.

The primers which were obtained from a previous published study [10] shown to be efficient in amplifying a 756 bp fragment from the mitochondrial D-loop region. Because of its non-recombining nature and rapid mutation rate, mitochondrial DNA is particularly useful for tracking maternal lineages and population patterns [14].

The phylogenetic study of *Capra aegagrus* mitochondrial sequences showed that sampled populations' genesis and

historical divergence require grouping. The tree topology showed well supported clades, one of which connected with significant well-supported clades by area, with Barzan, Daghestan, and Azerbaijan regions being more isolated and Turkey, Iran, and Pakistan are closely related. Mountain ranges and habitat discontinuities might aided regional population diversification, which was influenced by genetic drift and historical isolation [15] Strong evidence for phylogeographic isolation between some lineages supports limited gene flow during evolutionary time, supporting population-level divergence in genetic distance analysis and genetic differentiation, F_{st} [16]. The phylogenetic structure strongly suggests a regional *C. aegagrus* evolutionary history with some populations constituting unique and possibly evolvable important units. Within *Capra aegagrus*, the phylogenetic tree identified three separate clades, which indicates that there is considerable geographic and genetic organization underlying the species. The high bootstrap values, which may reach up to 99%, provide convincing evidence that these divisions are reliable.

Based on findings by [17], Iran is suggested as a likely center of domestication with complex gene flow histories, this statement strongly agree with our results in which the clade I's has a strong connection with Iranian population. Additionally, the clade II's is associated with Turkish and Iranian population. The links between clade I and Iranian populations are very well-established, due to the geographical proximity. However, Zet is grouped in clade I, which is apart geographically from that population and closer to the Turkish border in the northeast of the study area. Thus, it may be due to the connectedness of the landscape and the possibility of unrestricted movement of *C. aegagrus* in the Barzan district. This interconnectedness allows for greater genetic diversity and adaptability among populations. As a result, species can thrive in various environments, leading to a more resilient ecosystem overall. Furthermore, there is a strict conservation strategy in this area, which not only protects their habitats but also promotes biodiversity as a whole. As a result, the blood samples that might coincidentally took from the individual in Zet came from the northwest of the Barzan district near the Bradost mountains. Careful consideration of spatial and demographic sampling strategies is therefore critical in phylogeographic studies to ensure representative and unbiased estimates of population structure [18].

While in clade II, unexpected placement of Bedaron samples was identified. The fact that it is situated approximately at the center of the study area might suggest some degree of historic gene flow or convergence area. Which acts as a link between two separated populations in the Barzan district.

The significant diversity in clade III featured high level of genetic differentiation with the studied samples and may thus represent a group that was diverged more recently or is less diversified.^[19] observed similar results that the wild goat populations in Pakistan appear to were formed isolated clusters.

Previous studies support the genetic differentiation and little gene flow among *Capra aegagrus* populations in this study.^[5, 20] found that the wild goat populations are highly genetically structured across Iran, the Caucasus, and Central Asia, with genetic differentiation (Fst) values frequently above 0.2, which is in line with the results of this article (0.21193-0.57617). Likewise, according to^[21] geographic and ecological barriers, especially in mountainous landscapes, limit the gene flow ($Nm < 1$), supporting the estimates obtained from the investigated samples (0.18 to 0.93). These levels of differentiation are biologically significant and indicative of restricted movement between populations^[17]. The low gene flow (Nm) of 0.18 recorded in Barzan district, however, corresponded with the threshold values given by^[22], suggesting that less than Nm 1 levels generally cause divergence and reduce connectivity across populations. Using DnaSP v6.12.03 for Fst and Nm estimation is also backed up in^[23], confirming the strongholds of the analytic approach in the present investigation. Collectively, these references emphasize that the populations of *C. aegagrus* in the studied area are genetically differentiated due to restricted gene flow, most probably because of the geographical and ecological barrier effect. Moderate to high genetic diversity appears to exist among *Capra aegagrus* populations. The average distance is 0.09141 and the greatest observed distance is 0.18621 between Turkish individuals. In comparison,^[24] using the same mitochondrial segment, reported an Iranian divergence range from 0.015 to 0.150 for the bezoars. This indicates that the results in this study fall into the prediction count that is anticipated across the world. Probably the largest gap like pairwise distance was between the Selke and Turkish populations being 0.1611, were attributable to historical isolation or slow recent gene flow. Furthermore, the lowest pairwise distance was stated between Shkafta Valley Two and Iranian EF989610.1 (0.0278) because of their geographic proximity to Iran. The estimated genetic distance among the local individuals of *C. aegagrus* in Barzan district demonstrates the highest genetic distance between Alka and Dore (0.0825), and this might be due to the Great Zab River that is splitting the two habitats apart. This geographical barrier could hinder gene flow and increase divergence over time between the populations. Other probability is the limited corridors connecting these two habitats. Nevertheless, the lowest genetic distance that was obtained between Bedaron and Zrara (0.000), and Shkafta Valley_6 and Lerabir (0.00452) is because of no geographical barriers separating these two groups; consequently, there is a possibility for outbreeding between them.

According to the previous studies^[2, 25], Turkey and Iran were the highest mitochondrial diversity regarding their functions as refuges centers of *C. aegagrus*, in agreement with results obtained in this study. Confirming that the areas that have nucleotide diversity greater than ($\pi = 0.07$) and a large number of haplotypes will form an essential gene pool. The investigated analyses in this research indicate that Turkey and Iran were 0.09199 and 0.07107 nucleotide diversity, in addition to the large number of haplotypes (52 and 129), respectively, meaning these two areas have a good gene pool. Based on previous literature, it was ultimately discovered that Turkey and Iran possess the highest mitochondrial diversity, in regard to their functions as refuges center of *C. aegagrus*^[2, 25]. The areas that have π values greater than 0.07 and large numbers of haplotypes will thus form an essential genetic pool.

The population of *Capra aegagrus* in the Barzan district had a moderately high level of nucleotide diversity ($\pi = 0.0423$) and number of haplotypes ($h = 12$) compared to Turkey and Iran. The reasons behind low mitochondrial diversity in Barzan district may be due to the fragmented habitats and isolated populations that motivate inbreeding in these groups. However, it has higher mitochondrial diversity than Azerbaijan and Daghestan. This information would imply that Barzan may be a marginal area for *C. aegagrus*, with some historic separation from the major populations.

The outcomes confirm previous worldwide evaluations of the population structure of *Capra aegagrus*. The reality of several phylogeographic clades, that Barzan's genetic diversity of wild goats is poor and such limited gene flow implies that conservation measures must consider many evolutionarily significant units (ESUs). This is particularly relevant for groups that inhabit in the Barzan area, which were affected mostly by habitat fragmentation or genetic bottlenecks.

5 Conclusion

In conclusion, the obtained data declare the separation of the collected samples into two different clades based on the nucleotide variation in the targeted mitochondrial D-loop region. Their mitochondrial diversity shows the relatedness of these samples to the Iranian and Turkish *C. aegagrus* population. Overall, the average genetic distance in this study across 47 DNA sequences indicated the moderate nucleotide divergence of the studied species. All of these results give an insight into the degree of genetic variation and further authenticate the concept of distinct population clusters within *C. aegagrus*.

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Authors contribution

All authors contributed significantly to this study; A.M. conceived and designed the research, collected and analyzed the data, and drafted the first manuscript. J.A. assisted with data interpretation, provided critical revisions, and contributed to the

final manuscript preparation with supervision. A.Kh. supervised the study, offered conceptual guidance, and approved the final version for publication. All authors read and approved the final manuscript.

Conflict of interests

The authors declare that there is no conflict of interest.

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