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The Complete Mitochondrial Genome from Iraqi Meriz Goats and the Maternal Lineage Using Whole Genome Sequencing Data

Research Article

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ABSTRACT

Meriz goat is a native goat breed found along the northern boundary of the Iraqi Kurdistan region near the center of species diversity and domestication. This economically important breed is distinguished by its production of fine hair, high persistence, and ability to thrive in harsh environmental conditions. Although the phenotype and productive traits of the Meriz goat have been described, the complete mitochondrial genome, maternal lineage, and genetic diversity of the breed have yet to be identified. Therefore, the whole genome sequencing data and bioinformatics analysis were used to assemble the complete mitochondrial genome, generate a maternal phylogeny, and identify some mitogenomic diversity features of Meriz goats from the Iraqi Kurdistan region. The complete mitochondrial genome of the two individuals was assembled with lengths of 16641 and 16639 bp, respectively (MH165338 and MH165339). The mitogenome comprises13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA), 22 transfer RNA (tRNA) genes and one non-coding control region. In addition, our data revealed that the mitogenome copy number is greater in female goats than in males. Integration into a phylogenetic tree with other goat breeds showed that Meriz goats belong to the most predominant maternal haplogroup A ($_{HPG}$A). Furthermore, nucleotide diversity and mitogenomic analysis indicated that Meriz goats have a high level of mitogenomic similarity to Chinese Cashmere goats and Turkish Angora goats within the same maternal lineage. The molecular data reported here provide useful insights into the evolutionary relationships and mitogenomic diversity of domestic and wild goats from the center of diversity of animal species in the Middle East.

KEY WORDS capra, genetic diversity, mitogenome copy number, next generation sequencing, phylogeny.

INTRODUCTION

The domestic goat (*Capra hircus*) is in the *Caprini tribe*, *Caprinae subfamily*, of the Bovidae family in the order Artiodactyla. The domestic goat was among the first group of the oldest domesticated species of livestock (Zeder, 2008). Zooarchaelogical data and evidence indicated that the farming process (domestication) of goat was started around 10000-11000 years before the present (YBP) in the Fertile Crescent area, in particular in the long range of Zagros mountains, covering parts of Western Asia, Iran, North-East Iraq and southeastern Turkey (Naderi *et al.* 2008; Zeder, 2008; Colli *et al.* 2015). According to the molecular genetics data, Daly *et al.* (2018) demonstrated that the current genetically and geographically distinct goat populations were domesticated from multiple divergent wild goat sources. At present, the population of domestic goats exceeds 1 billion which are distributed across geographically different regions worldwide (reviewed in Amills *et al.* 2017). In Iraq, the native goats are mainly raised for milk and meat production, while their hair is of secondary significance. In 1999, the populations of the native goats were

about 1.3 million head (FAO, 2000). Recent data indicated that about 43.3%, 44.2% and 12.5% of native goats are distributed in the northern, central and southern parts of Iraq (Alkass and Jurna, 2005). Meriz (Maraz or Cheer) is one of the indigenous goat breeds found in the mountainous altitude regions (with poor grazing habitats) of the Kurdistan region of Iraq in the Fertile Crescent area, and is raised for meat, milk and fiber production. The economically important breed produces valuable fine outercoat fibers which are mainly used for manufacture of Kurdish traditional costume (Alkass and Juma, 2005).

Cashmere producing goats are found in several countries such as China, Iran, Afghanistan, Mongolia and India in which China has highest production of Cashmere than any other country (Shakyawar *et al.* 2013; Seki *et al.* 2011; Wang *et al.* 2013). The evolutionary biology of mitochondrial DNA (mtDNA) fragments have been widely investigated and used as markers for documentation of many species (Tillmar *et al.* 2013; Yang *et al.* 2014; Lv *et al.* 2015; Harley *et al.* 2016; Mannen *et al.* 2020). Phylogenetic relationships based on the diversity of maternal lineages can be used to revolve ancestry and origins of different domesticated animals by studying nucleotide polymorphisms within mitochondrial DNA sequences (Meadows *et al.* 2007; Achilli *et al.* 2009; Lippold *et al.* 2011; Osman *et al.* 2021).

In goats, phylogenetic analyses based on either control region sequences or complete mitochondrial genome (mitogenome) classified multiple maternal lineages and represented in the six major monophyletic mtDNA haplogroups ($_{HPG}$); $_{HPG}$A, $_{HPG}$B, $_{HPG}$C, $_{HPG}$D, $_{HPG}$F, and $_{HPG}$G; of which $_{HPG}$A was predominant and widespread in goats worldwide (Naderi *et al.* 2007; Colli *et al.* 2015).

Despite the central location of Iraq in the Fertile Crescent, it is notable that there has not been any record regarding mitogenomic diversity and phylogenetic position in Meriz goats. Therefore, in this study, we aimed to assemble the complete mitochondrial genome (mitogenome), identify maternal phylogeny and estimate mitogenomic diversity of the Iraqi Meriz goats using Next Generation Sequencing (NGS) data.

MATERIALS AND METHODS

Sampling and isolation of genomic dna

The whole blood was sampled with K2E Vacutainers (Becton Dickinson, Franklin Lakes, NJ, United States) from the jugular vein of Meriz goats (1 male and 1 female) from the flocks of animal project, College of Agricultural Engineering Sciences, University of Duhok, Duhok (geographic coordinates, latitude 36.8679 and longitude, 42.9488), Iraq. Total genomic DNA was isolated from whole blood using the Wizard Genomic DNA Purification kit (Promega, Southampton, UK).

Sequencing and assembly of complete mitogenomes

Two samples of genomic DNA of Meriz goats (male and female) (Table 1) were sequenced commercially using Illumina HiSeq-PE150 reads by Novogene Company Limited, Hong Kong, China. Approximately 70-76 million reads of total sequence were given for each genomic DNA sample (Table 1). For each DNA sample, paired end reads were mapped against the complete mitochondrial genome of domestic Dazu black goat (GenBank: KP271023; Guang-Xin *et al.* 2016) as a reference. After assembling, the two complete mitogenomes of Meriz goats were then annotated. Geneious 8.0 software (Kearse *et al.* 2012; http://www.geneious.com) was used for bioinformatics analysis (setting paired end reads, mapping and annotation). The annotated complete mitogenomes are available in GenBank under accession numbers MH165338 and MH165339.

Data analysis and phylogenetic relationships

To build a phylogenetic tree, the two complete mitogenomes of Meriz goats were aligned with published mitogenomes from domestic and wild goat species representing the six maternal haplogroups $_{HPG}$A, $_{HPG}$B, $_{HPG}$C, $_{HPG}$D, $_{HPG}$F, and $_{HPG}$G (Table 2).

To find the best model of mitochondrial sequence alignments, MEGA X (Kumar *et al.* 2018) was used with setting the maximum likelihood criteria. Thereafter, the phylogenetic position of the assembled mitogenomes of Meriz goats was established by building a phylogenetic tree with the Tamura-Nei (genetic distance model) and neighbour-joining (NJ) method of 1000 bootstrap replicates using Geneious software (Kearse *et al.* 2012; http://www.geneious.com). The mitogenome of *Capra ibex* (NCBI ID: FJ207526) was used as out-group.

Estimation of mitogenomic diversity

Following multiple alignments of the whole sequences of the mitogenome, the mtDNA sequences were analyzed for nucleotide diversity and polymorphic sites. Evolutionary distances between sequences were estimated by calculating the proportion of nucleotide differences (pairwise differences) between each pair of sequences using MEGA X (Kumar *et al.* 2018). Additionally, variable sites (polymorphic sites) were calculated using Geneious software (Kearse *et al.* 2012). The results of nucleotide differences and variable sites were merged together and illustrated in Tables 3 and 4.

Table 1 Morphological traits, maternal haplogroups, GenBank accession numbers, mitogenome size, total next generation sequencing (NGS) data and assembled reads of Meriz goats (male and female) using Illumina HiSeq-PE150 reads of total genomic DNA

Sample code	Morphological traits[1]/sex	Maternal haplogroup[1]	GenBank accession	Mitogenome size (bp)	Total NGS reads[2]	Assembled reads (*n*)[3]	Coverage[4]
CHM7	Meriz goat/ male	$_{HPG}$A	MH165338	16641	71796930	27767	250
CHF14	Meriz goat/ female	$_{HPG}$A	MH165339	16639	76632598	31800	287

[1] Haplogroups were identified based on the phylogenetic relation using known haplogroups see Figure 3 and Table 2.
[2] Total NGS reads= total next generation sequencing raw reads used from each sample.
[3] Assembled reads (n)= numbers of assembled NGS raw reads out of total numbers to produce whole mitogenome.
[4] Coverage= number of assembled reads × 150 [average read length] / mitogenome size.

Table 2 Different breeds of domestic and wild goats, their geographical locations, GenBank accession numbers, maternal haplogroups and references used for analysis of phylogenetic tree as shown in Figure 3

GenBank accession numbers	Breed (species)	Place of collection	Haplogroup ($_{HPG}$)	References
MG837555	Tibetan (*C. hircus)*	China	$_{HPG}$A	Liu *et al.* (2018)
GU068049	Inner Mongolia White Cashmere (*C. hircus*)	China	$_{HPG}$A	NCBI-unpublished
KR059195	Skopelos (*C. hircus)*	Greece	$_{HPG}$A	Colli *et al.* (2015)
MF573068	Erlangshan Cashmere (*C. hircus*)	China	$_{HPG}$A	Ma *et al.* (2018)
KR059188	Zaraibi (*C. hircus*)	Egypt	$_{HPG}$A	Colli *et al.* (2015)
KR059189	Kermanshah (*C. hircus*)	Iran	$_{HPG}$A	Colli *et al.* (2015)
KR059183	Baladie (*C. hircus)*	Jordan	$_{HPG}$A	Colli *et al.* (2015)
MH165338	**Meriz (*C. hircus)***	**Iraq**	**$_{HPG}$A**	**Present study**
MH165339	**Meriz (*C. hircus)***	**Iraq**	**$_{HPG}$A**	**Present study**
KR059200	Angora (*C. hircus)*	Turkey	$_{HPG}$A	Colli *et al.* (2015)
KR059210	Bezoar (*C. aegagrus)*	Iran	$_{HPG}$D	Colli *et al.* (2015)
KR059212	Kyrgyzstan (*C. hircus)*	Kyrgyzstan	$_{HPG}$D	Colli *et al.* (2015)
KR059215	Hair (*C. hircus)*	Turkey	$_{HPG}$G	Colli *et al.* (2015)
KR059218	Khalkhali (*C. hircus)*	Iran	$_{HPG}$G	Colli *et al.* (2015)
KR059220	Malaysia (*C. hircus)*	Malaysia	$_{HPG}$B	Colli *et al.* (2015)
KF952601	Yunnan Black (*C. hircus*)	China	$_{HPG}$B	Colli *et al.* (2015)
KR059225	Payoya (*C. hircus*)	Spain	$_{HPG}$C	Colli *et al.* (2015)
KR059224	Swiss Alpine (*C. hircus)*	Switzerland	$_{HPG}$C	Colli *et al.* (2015)
KR059226	Bezoar (*C. aegagrus)*	Iran	$_{HPG}$F	Colli *et al.* (2015)
FJ207526	*Capra ibex* (Wild goat)	-	Outgroup	Hassanin *et al.* (2009)

Table 3 Nucleotide differences (pairwise differences) and variable sites (polymorphic sites) between mitogenome of Meriz goats and and other domestic goat breeds within the same maternal haplogroup (HPGA)

Goats breed ($_{HPG}$A)	MH165338 Meriz_Goat male		MH165339 Meriz_Goat female	
	Nucleotide differences	Variable sits	Nucleotide differences	Variable sits
MG837555 (*C. hircus*) Tibetan G. $_{HPG}$A	0.00133	32	0.00217	48
GU068049 (*C. hircus*) Inner Mongolia Cashmere G. $_{HPG}$A	0.00151	28	0.00223	40
KR059189 (*C. hircus*) Kermanshah G. $_{HPG}$A	0.00157	27	0.00217	39
KR059200 (*C. hircus*) Angora G. $_{HPG}$A	0.00139	24	0.00199	34
MF573068 (*C. hircus*) Erlangshan Cashmere G. $_{HPG}$A	0.00139	24	0.00212	36
KR059188 (*C. hircus*) Zaraibi G. $_{HPG}$A	0.00157	26	0.00242	42
KR059183 (*C. hircus*) Baladie G. $_{HPG}$A	0.00139	24	0.00211	38
KR059195 (*C. hircus*) Skopelos G. $_{HPG}$A	0.00121	21	0.00181	33

Table 4 Nucleotide differences (pairwise differences) and variable sites (polymorphic sites) between mitogenome of Meriz goats and other domestic and wild goat breeds from other maternal haplogroups (HPGB, HPGC, HPGD, HPGF, and HP)

Goats Breed $_{HPG}$F, $_{HPG}$C, $_{HPG}$B, $_{HPG}$G, $_{HPG}$D	MH165338 Meriz_goat male		MH165339 Meriz_goat female	
	Nucleotide differences	Variable sits	Nucleotide differences	Variable sits
FJ207526 (*C. ibex*) Outgroup	0.03845	674	0.03846	674
KR059226 (*C. aegagrus*) Bezoar G. $_{HPG}$F	0.02573	410	0.02627	420
KR059225 (*C. hircus*) Payoya G. $_{HPG}$C	0.00977	163	0.01014	169
KR059224 (*C. hircus*) Swiss Alpine G. $_{HPG}$C	0.00983	164	0.01008	168
KR059220 (*C. hircus*) Malaysia G. $_{HPG}$B	0.0056	93	0.00584	99
KF952601 (*C. hircus*) Yunnan G.$_{HPG}$B	0.00597	98	0.00609	102
KR059218 (*C. hircus*) Khalkhali G.$_{HPG}$G	0.00449	75	0.00486	83
KR05921 (*C. hircus*) Hair G.$_{HPG}$G	0.00449	75	0.00486	83
KR059212 (*C. hircus*) Kyrgyzstan G.$_{HPG}$D	0.00376	62	0.00449	76
KR059210 (*C. aegagrus*) Bezoar G. $_{HPG}$D	0.00357	60	0.00418	72

RESULTS AND DISCUSSION

Previous research (Huang *et al.* 2015) used 22 sets of primers to amplify overlapping segments of the mitogenome from different goat breeds. However, in our study, we used next-generation sequencing (NGS) data to sequence the complete mitogenome of Meriz goats. The blood DNA used in this study was appropriate to generate for whole genome sequencing data and to identify whole mitogenomes. As a result of alignment of the 71796930 and 76632598 million NGS raw reads against a reference complete mitogenome of Dazu black goat (GenBank: KP271023), in total 27767 (250X coverage) and 31800 (287X coverage) of raw reads were assembled and circular DNA molecules of the complete mitogenome of the two individuals of Meriz goat with a length of 16641bp (MH165338; male) and 16639 bp (MH165339; female) were produced, respectively (Table 1 and Figures 1 and 2). Likewise, the complete mitogenomes of the Karadi, Hamdani, and Awassi sheep breeds from Iraq were assembled using whole genome sequencing data (Mustafa *et al*. 2018; Mustafa, 2021).

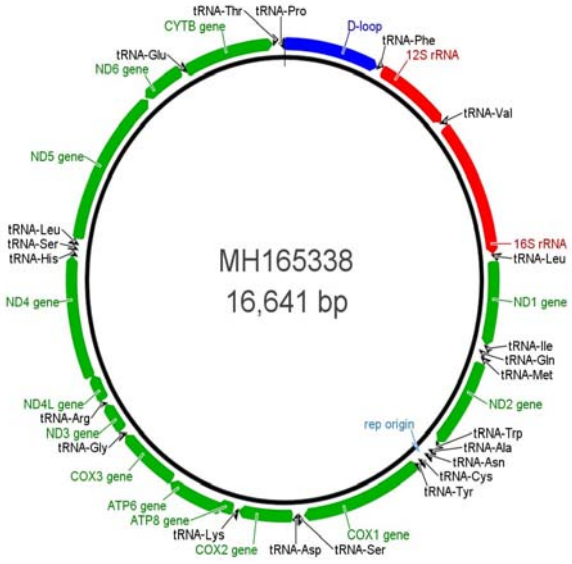

Figure 1 Meriz goat mitogenome map. The assembled mitogenome *Capra hircus*, Meriz_goat_CHM (16,641 bp) (GenBank accession number MH165338) with major features: 13 protein-coding genes (PCGs) (green bars, with the arrow pointing in the transcription directions), 22 transfer RNA tRNA genes (black triangles), the 12S and 16S rRNA genes (red colour) and the D-loop control region (blue). The GC content is 39.2%. For assembly data, see Table 1

The complete record of both mitogenomes of Meriz goats, including gene features (start and stop codons) and other characteristics such as position, sizes and strand distribution of all 13 protein- coding genes (PCGs), 2 ribosomal RNA (rRNA), 22 transfer RNA (tRNA) genes, and non-coding control region are available under the NCBI accession numbers (MH165338 and MH165339). Typical of mammalian mitochondrial genomes with the AT percentages 60.8% was formed. Ten genes in protein-coding regions begin with the ATG (Met) codon, while ND2, ND3 and ND5 genes use the alternative ATA start codon. Our results are consistent with the structure and distribution of the mitogenome of other vertebrates as reported in previous research (Mustafa *et al.* 2018 (sheep); Peng *et al.* 2018 (chicken); De *et al.* 2019 (cattle); Li *et al.* 2019 (fish); Xu *et al.* 2019 (cat)). Moreover, as shown in Table 1, the number of assembled reads (coverage) of the mitogenome is higher in the female Meriz goat in comparison with the male individual. This indicates that the blood cells of female goats have a higher copy number of mitochondria. Similar results were found by Mustafa et al. 2018, in which female sheep were shown to have more copy numbers of mitochondria. In mammalian cells, mitochondria are the key spots of energy production in which their abundance differs according to the organ, age, and physiological condition, such as lactation in female animals (Laubenthal *et al.* 2016).

The phylogenetic position of the assembled mitogenomes of Meriz goats was established by building a phylogenetic tree with the Tamura-Nei (genetic distance model) and neighbour-joining (NJ) method of 1000 bootstrap replicates including published maternal haplogroups of domestic and wild goats (Figure 3 and Table 2). The mitogenome sequences of Meriz goat were positioned on branches with the most predominant maternal haplogroup A ($_{HPG}$A) in goats worldwide (Figure 3).

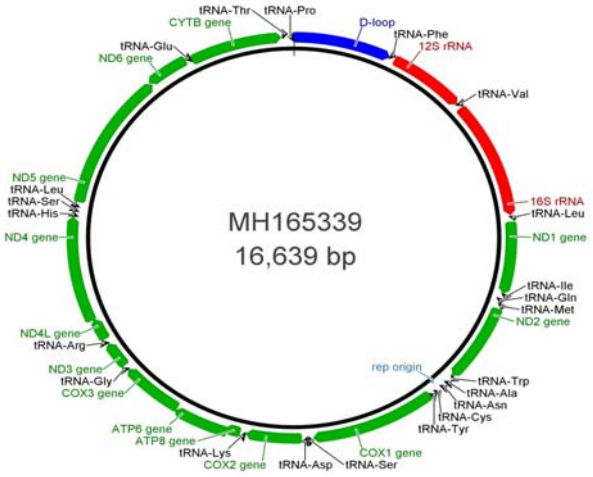

Figure 2 Meriz goat mitogenome map. The assembled mitogenome Capra hircus, Meriz_goat_CHF (16,639 bp) (GenBank accession number MH165339) with major features: 13 protein-coding genes (PCGs) (green bars, with the arrow pointing in the transcription directions), 22 transfer RNA tRNA genes (black triangles), the 12S and 16S rRNA genes (red colour) and the D-loop control region (blue). The GC content is 39.2%. For assembly data, see Table 1

Meriz goats from Iraq, near the center of diversity and domestication of animal species, are characterized by having the most abundant maternal lineages within the known mitogenomic diversity. The maternal haplogroup of Meriz goats sampled in our study fitted within the main Capra hircus maternal haplogroups known to be present in different goat breeds from Asia and Europe. Previous studies indicated that the most common maternal haplogroup of most goat breeds worldwide was HPGA. For example, the maternal haplogroup HPGA was predominant and widely distributed in Chinese domestic goats (Zhao *et al.* 2014); Arabian goats in Arab countries (Al-Araimi *et al.* 2017); Ethiopian goats (Tarekegn *et al.* 2018); Indian goats (Diwedi *et al.* 2020); Russian goats (Deniskova *et al.* 2020); Mongolian goats (Ganbold *et al.* 2020) and Nepalese goats (Gorkhali *et al.* 2021). In agreement with the genetic landscape of maternal origin, it can be concluded from the previous studies that the most abundant haplogroup is $_{HPG}$A as seen in the most goats worldwide including Meriz goats from Iraq.Colli *et al.* (2015) suggested in their extensive survey about goat mitogenomes that all modern haplotypes within the haplogroup $_{HPG}$A are possibly derived from a single female of wild goats (*Capra aegagrus*).

Results presented here fill a gap of the previous goat samples in geographical coverage of other countries worldwide identifying multiple haplogroups of maternal lineages of goat $_{HPG}$A, $_{HPG}$B, $_{HPG}$C, $_{HPG}$D, $_{HPG}$F, and , $_{HPG}$G (Naderi *et al.* 2007; Colli *et al.* 2015).

After the mitogenome of Meriz goats was clustered within the maternal haplogroup A, further genetic analysis was performed based on the estimation of nucleotide diversity and polymorphic sites between the mitogenome of Meriz goats and domestic and wild goats of all maternal haplogroups (Table 3 and Table 4).

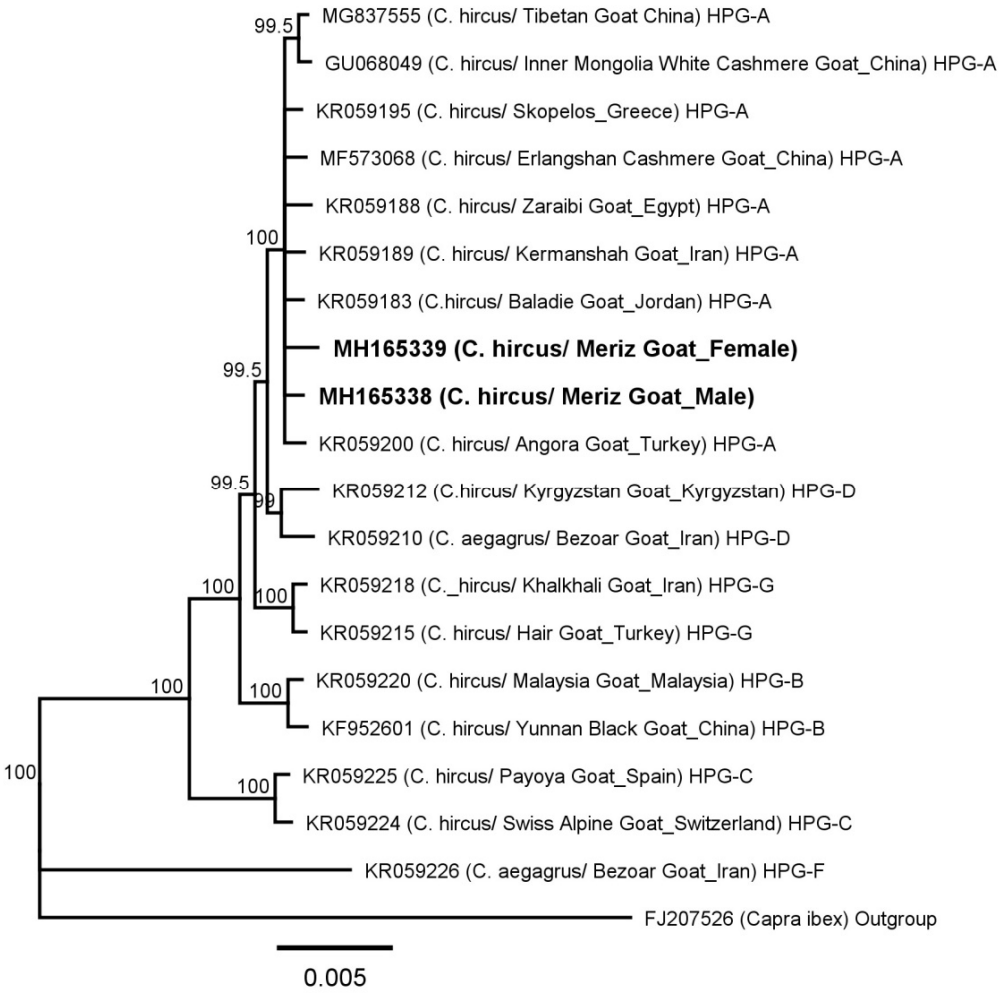

Figure 3 Phylogenetic relationships of Meriz goat mitogenomes. Neighbor-Joining consensus tree showing position of Meriz goats in relation to major haplogroups of domestic goats (*Capra hircus*) and wild goats (*Capra aegagrus*). The tree was derived from genetic distance model (Tamura-Nei) analysis of alignments of the whole mitochondrial genome sequences. Nodes are labelled with consensus support %. Scale range (0.005) and line weight (2) were set. The mitogenome of *Capra ibex* was used as out-group. Table 2 gives accession numbers, place of collection, haplogroups and references for mitogenome sequences used in the tree

The overall value of nucleotide differences (pairwise differences) between Meriz goats and other goat breeds within the same clade of haplogroup $_{HPG}$A was ranged from 0.0012 to 0.0024 and polymorphic sites were about 21 to 48 (Table 3). However, high variation was found between mitogenome of Meriz goats and domestic and wild goats from other haplogroups $_{HPG}$B, $_{HPG}$C, $_{HPG}$D, $_{HPG}$F, and $_{HPG}$G (Table 4). Interestingly, the results of phylogeny and nucleotide differences observed the close genetic distances between Meriz goats and Chinese Cashmere producing goats and Turkish Angora goat (Table 3 and Figure 3). However, in comparison to Iranian Marghoz goats, Seyedabadi *et al.* (2016) analyzed the mitochondrial control region and indicated that all sampled Marghoz goats were classified into maternal haplogroup HPGC. Although both Iraqi Meriz goats and Iranian Markhoz goats have high similarity in terms of phenotype traits and they are present in different morphological colors within Cashmere producing goats (Rashidi *et al.* 2008; Muhammad Salih Al-Barzinj *et al.* 2016), their genetic distance in terms of mtDNA sequences is high as each one belongs to a different maternal haplogroup. As indicated in Table 4, the genetic distance between mitogenome of Meriz goats ($_{HPG}$A) and mitogenomes of goats from $_{HPG}$C was about (0.00977 to 0.01014 pairwise differences and 163 to 169 variable sites). Srirattana *et al.* (2017) concluded that the mtDNA profiles are not always related to the phenotype in cattle. This could be the same case as we found in Meriz goats in comparison to Iranian Marghoz goats, Chinese Cashmere goats and Turkish Angora goats.

CONCLUSION

Phylogenetic analysis of Meriz goats sampled from the center of domestication and diversity of animal species showed they had the most widespread, geographically ubiquitous, maternal haplogroup A (HPGA), one of the six major haplogroups of domestic goats. Nucleotide diversity indicated that Meriz goats have a high level of mitogenomic similarity to Cashmere-producing goats within HPGA, while they are more distant from the rest of the maternal haplogroups. Furthermore, bioinformatics analysis revealed that female goats have more copies of the mitogenome than males. The molecular data described here provides useful knowledge of evolutionary relationships, phylogenetic status and genetic biodiversity of the Meriz goats in the context of breeding and conservation of domestic and wild goats worldwide.

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DATA AVAILABILITY

The annotated mitochondrial DNA sequences are available in GenBank of the NCBI database under accession numbers MH165338 and MH165339. The NGS raw reads that support the results of this study are published in the NCBI database under the BioProject accession number PRJNA797579.

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