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Phylogenetic Evolution of Egyptian Goat Breeds "*Capra Hircus*" Based on Mitochondrial DNA D-Loop Sequence

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Abstract: The population genetic and molecular evidence of the Arabian goat breed's phylogenetic standing (*Capra hircus*) is misunderstood, and little is known about genetic diversity in this species. Despite their importance in producing meat and milk, as well as their capacity to endure difficult conditions with little access to food and water, goats are the most common animal in Arab countries. We investigated the phylogenetic history and demographics of domestic goats by sequencing a hypervariable segment of the mtDNA reference region for Baladi, Demuscous, and Zaraibi breeds, as well as sequences retrieved from 16 Arab countries. Three significantly divergent lineages associated with Africa and the Middle East were identified through phylogenetic analysis of the investigated strains of Egyptian goats' mtDNA D-loop region. All Egyptian goats were allocated to lineage A, which is made up of 445 different haplotypes, including Egyptian and Arabian goats. Diversity in haplotypes and in nucleotides for each Arabian goat population was calculated separately and estimated to be 0.99835 and 0.02576, respectively. Finally, we suggest that all goat lineages descend from a single clan. The ancient world's migration and travel may have influenced goat diversity, implying that goats had multiple matrilineal forebears.

1 Introduction

The best source of micronutrients and high-protein foods for human nutrition and health is a livestock (Ahmed et al 2020). The goat (*Capra hircus*) was the first animal that humans domesticated and are the world's most prevalent domestic species (Araújo et al 2010). Goats play a major economic role in developing nations (Araújo et al 2010, Mahrous et al 2013). It underwent substantial natural and artificial selection because of extensive-domestication, adaptation to many settings, and important levels of phenotypic variability (Wang et al 2016). Goat breeds in the Near East and North Africa (NENA) region have valuable and unique genetic diversity due to production environments and numerous harsh environments, such as high temperatures and arid conditions. Domesticated goats are a major source of milk and meat in Egypt (Galal et al 2005). They are found all over the country but are particularly prevalent in the Nile Valley and Delta region. They can be divided into many breeds based on their size, color and

other morphological characteristics, such as Baladi (BA), Demuscous (DA), and Zaraibi (ZA). The famed goat breeds known as Baladi are raised all over Egypt, particularly in the Delta and the Nile Valley, and are important for marginal farmers and landless laborers in rural Egypt. Damascus goats (known as Shami goats) are a native breed of Syria, Iraq, and particularly in the Kurdistan region; they are important for the provision of meat and milk due to their high milk yield and prolificacy. Zaraibi goats, known as Egyptian Nubian goats, were discovered in Upper Egypt's Nile Delta's northeast (Alkass and Juma 2005, Galal 2005, Agha et al 2008). To adapt to the various habitats, most of the goat breeds in Egypt lack molecular characterization and genetic variety, which are required for developing animal production. Two of the main factors used to determine the domestication zones are nucleotide and haplotype diversity (Kul and Ertugrul 2011). Our study aims to understand the phylogeny of domestic goats based on mtDNA D-loop sequence analysis. The Nile Delta, the Nile Valley, and the northwestern coastal region are home to three breeds of native Egyptian goats, on which we concentrated in this study. We sequenced the Baladi, Demuscous, and Zaraibi goats' first hypervariable (HV) segments of the mtDNA regulatory region to calculate the date of the mtDNA lineages' split.

2 Material and Methods

2.1 Blood sampling and DNA extraction

There were fifteen blood samples taken in all. Utilizing the jugular vein to administer vacutainer tubes (Agha et al 2008) for three Egyptian healthy goat breeds (Baladi, Demuscous, and Zaraibi). Samples of the EG breed were obtained from the Sakha Experimental Station, which is a part of the Agriculture Research Centre's (ARC) Animal Production Research Institute (APRI). Genomic DNA was extracted from filter paper hallmarks making use of an *Easy Pure*® Blood Genomic DNA Kit (code EE121-01) (TransGen Biotech Co., Beijing) following the recommended manufacturer's instructions, although we made some modifications to this method.

2.2 Amplification and Sequencing

We amplified a portion of the partial HV1 region in the D-loop to examine the genetic diversity and phylogenetic studies of EG (positions 15,579 to 16,555 on the complete goat mitochondrial sequence of reference NC_ 005044) employing a series of primers from 15 animals, which were obtained from previous research papers **(Table 1)**. A 25 µl reaction mixture including the PCR amplifications was used, along with 6.25 µl of master-mix *Easy Taq*® DNA Polymerase (TransGen Biotech Co., Ltd.), 2.0 µl genomic DNA (20 ng/µl), 2.0 µl of each primer (10 µM), and 6.5 µl distilled water to adjust the PCR product's final volume. All PCRs were run in a thermal cycler C1000 equipment with negative controls (without DNA) (Bio-Rad laboratories Inc., Hercules, CA, USA), under the following conditions: denaturation at 3 min at 94°C, followed by 30 cycles of 30 s at 94°C, annealing at 15s at 55-60°C annealing, extension at 45s at 72°C, and 5 min at 72°C. All the obtained amplicons were run on a 1.5% (w/v) agarose gel with 120 volts for 30 minutes. All PCR products were purified and analyzed using the Sanger DNA sequencing technique (Macrogen, Korea).

2.3 Data assembly and Analysis

Fifteen different sequences totaling three healthy Egyptian goat (EG) populations from this research were combined with the previously described sequences and aligned and trimmed by utilizing BioEdit v.7.2.5 (Hall 1999). We created an NJ (neighbor joining) tree (using 500 bootstraps to determine evolutionary distances) by using MEGA_ver.11.0.13 software (Kumar et al 2008, Kumar et al 2016) to examine EG sequences. Moreover, we used another 481 sequences of references for 17 breeds from different geographic regions in Africa and the Middle East to understand the phylogeny of EG (Supplementary **Table 1**). Additionally, we represented sequences A, B, C, D, F, and G, the six caprine haplogroups previously identified (accession numbers of all reference sequences in Supplementary **Table 1**). The level of the specific population, DNA Sequence Polymorphism v5.10.01 (Librado and Rozas 2009). The number of haplotypes (h), haplotype diversity (Hd), number of segregating sites (S), nucleotide diversity (Pi), and average number of nucleotide differences were used to calculate the diversity parameters of mtDNA (K). Pairwise Fst was calculated with the same programs.

3 Results and Discussion

3.1 Haplotype of EG breeds and variation of mtDNA

Depending on the amount of genetic variation, the degree of polymorphism is high as a result of the high mutation rate observed in the D-loop control region, which is widely used to investigate the structure of populations. This is due to interspecies diversity,

Table 1. Summarizes the used Specific primers and their nucleotide sequences

Region	Primer	Sequence, 5'→ 3'	Ta	bp	Ref.
D-loop	gdloop-F	CAC AAA CTT CCC ACT CCA CA	60ºC	1000bp	Ganbold et al (2020)
	gdloop-R	AGC GTG TTT AAA ACG GTG GT			
	CAPT-F	CTGATTAGTCATTAGTCCATC	55ºC	700bp	Awotunde et al (2015)
	CAP-R	CGTGTATGCAAGTACATTAC			

connections across populations, and identifying the contributions of many ancestral stocks throughout domestication (Naderi et al 2007, 2008, Colli et al 2015).

In this study, we found the amplified region size of gdloop, and CAP markers in EG breeds to be 1000 bp and 700 bp, respectively. Those markers have low polymorphism between three different breeds, which indicates that they may have had the same common ancestor (**Fig 1 A**). In addition, a comparison of the HVI sequences of five EG populations and three different populations revealed high polymorphism. Polymorphism in 109 sites was found over 452 bp, while 16494 and 16430 insertion/deletion (InDel) mutations were detected in five EG populations and three populations, respectively. The gdloop marker showed higher polymorphic sites in Damascus and Zaraibi than in the Baladi breed **(Fig 1 B).**

The Hd and π were calculated for each population **(Table 2)** and were 0.99835 and 0.02576, respectively. Seven populations (Algerian goats, Demuscous, Iraqi goats, Nigerian goats, Senegalese goats, Syrian goats, Tunisian goats, and Zaraibi) exhibited a high Hd value (1.000), and the Somalian goats had a high π value (0.036), while it ranged from 0.01121 to 0.02814 (overall, π = 0.02576). Moreover, the Gondar population had a high K-value (11.16842) (overall, K = 11.9023) (**Table 1**).

3.2 Phylogenetic Analysis of Egyptian Goats and Arabian Goats

Based on the highly variable D-loop region in mtDNA, phylogenetic relationships of goat populations within five Egyptian breeds and between 19 Arabian breeds (including EG) were initially studied in this study. It demonstrated that domestic goats from the Arabian region have lineage A genetic diversity, the same as a previous study by Naderi et al (2007). Phylogenetic analysis of all tested Arabian goat sequences revealed three distinct haplogroups: A, F, and G **(Table 2** and **Fig 2).** All goat populations showed evidence of haplogroup A, with an average frequency of 92.5% (445), which is like previous studies. Moreover, haplogroup F was only discovered in one goat (34% of the Demuscous), and haplogroup G represented 7.48% (36) of the total samples and was predominant in the Iran native goat **(Table 2** and **Fig 2)**, Turkey goat, and Gondar as mentioned by Deniskova et al (2020). The Middle East and Northern Africa are the origins of haplogroup G, as first identified by Naderi et al (2007). Among the investigated populations, the goats from Egypt are mostly in haplogroup A, with an average frequency of 13.93%. These observations point to a particular group as the source of the local goat breeds.

The phylogenetic tree revealed that the sequences of the Zaraibi goat were intricately connected to Saudi Arabian goats, and then Algerian goats. According to some evidence, the Algerian goat, the Zaraibi goat, and the Arabian Saudi goat may have shared an ancestor. Demuscous populations are close to Iranian goat, Jordanian goat, and wild-type populations. Al-Araimi et al (2017) found DNA research evidence of domestic goat migrations from Saudi Arabia through Egypt to North Africa, towards Morocco via Tunisia and Algeria; the other migration path went from Egypt directly to Sudan.

3.3 Genetic Distance and Population Structure

We also estimated Wright's F-statistic (pairwise Fst) between different populations for comparison among all 18 populations **(Table 3).** The low genetic difference was evident from the pairwise Fst values among 12 EG populations, which ranged between -0.0002 and 0.9627. The De breed was genetically closely related to the Moroccan goat (MoG), Arabian goat (ArG), Syrian goat (SyG), Iraqi goat (IqG), and Jordanian goat (JoG) populations (-0.0019, -0.0002), (-0.0115, -0.0026), and (-0.0058), respectively. The Za breed had a greater distance value from the Iran native goat (ING) population. The Ba breed was closely related genetically to Senegalese goats (Se), SyG, and JoG populations (-0.0510, -0.0549, and -0.0514, respectively). Similarly, little genetic difference between the goats from several Arab nations was demonstrated by the pairwise FST values (Al-Araimi et al 2017).

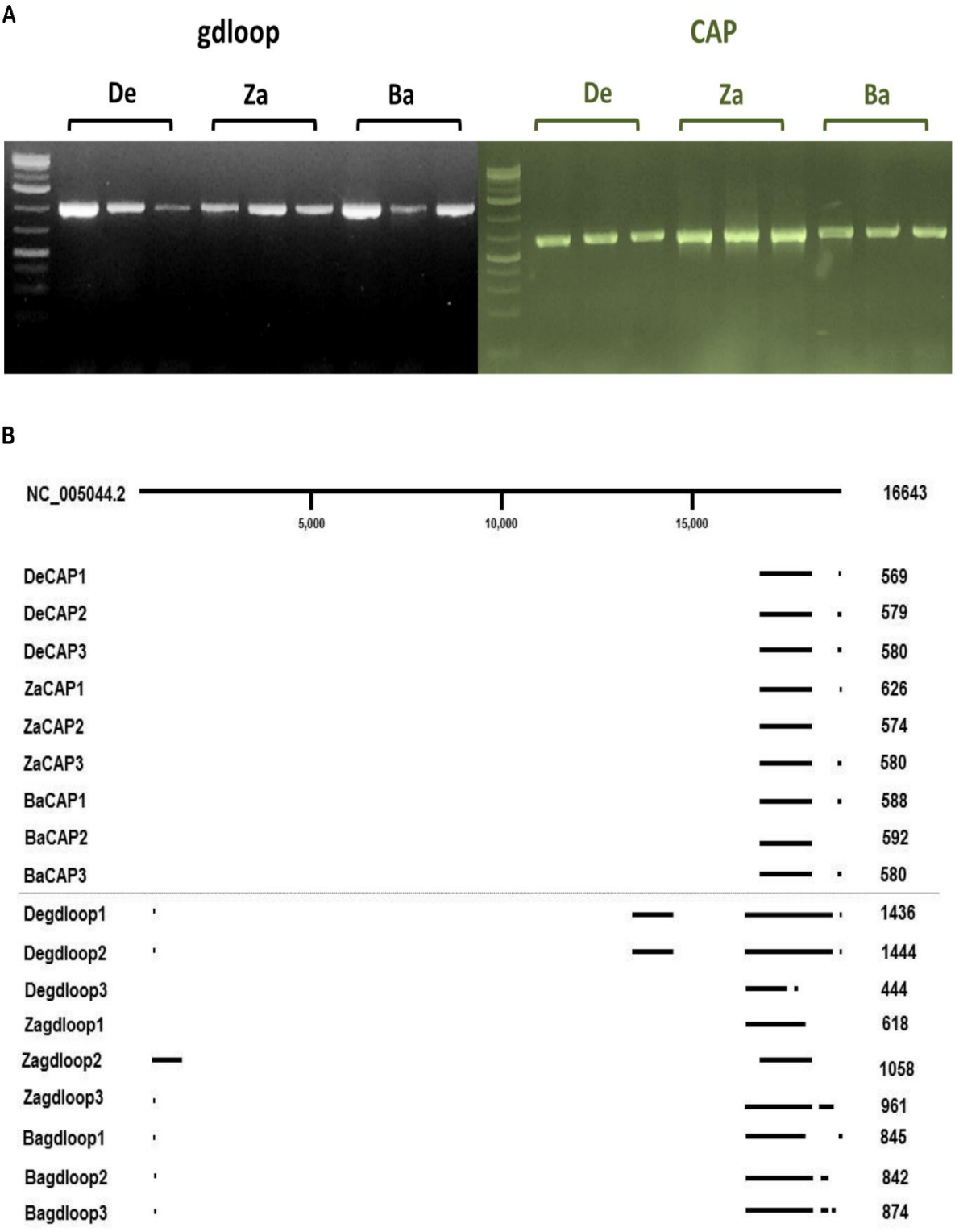

Fig 1. mtDNA variation between three different breeds. A) Band marker for three breads. B) The sequence diversity between different breeds compared to reference sequence.

Table 2. Estimated Genetic Diversity in Populations of Arabian Goats and The Distribution of Their Haplogroups

Populations	ID [1)]	n [2)]	h [3)]	Hd [4)]	S [5)]	Pi [6)]	K [7)]	H(n) [8)]
Algerian goats	AlG	3	3	1.00000	10	0.01443	6.66667	A (3)
Arabian goats	ArG	45	40	0.99495	67	0.01959	9.04848	A (40), G (5)
Baladi	Ba	16	13	0.975	37	0.01696	7.83333	A (17)
Beeshi	Be	22	20	0.99134	36	0.01236	5.70996	A (22)
Demuscous	De	2	2	1.00000	119	0.25758	119	A (1); F (1)
Egyptian goats	EYG	29	24	0.98768	52	0.01984	9.16749	A (27); G (2)
Gondar	GO	20	15	0.95789	35	0.02417	11.16842	A (11); G (9)
Iran goat	IRG	206	153	0.99659	127	0.01864	8.61004	A (206)
Iran native goat	ING	15	12	0.9619	21	0.01121	5.18095	G (15)
Iraqi goats	IqG	7	7	1.00000	26	0.01835	8.47619	A (7)
Jordanian goats	JoG	19	16	0.98246	42	0.01592	7.35673	A (19)
Morocco goats	MoG	6	5	0.93333	14	0.01385	6.4	A (6)
Nigerian goats	NiG	12	12	1.00000	24	0.01354	6.25758	A (12)
Senegalian goats	Se	3	3	1.00000	14	0.0202	9.33333	A (3)
Syrian goats	SyG	2	2	1.00000	13	0.02814	13	A (2)
Tunisian goats	TuG	6	6	1	23	0.02179	10.06667	A (6)
Turkey goat	TG	66	56	0.99301	69	0.01837	8.48625	A (61); G (5)
Zaraibi	Za	2	2	1.00000	13	0.02814	13	A (2)
Overall	18	481	349	0.99835	345	0.02576	11.9023	A (445); F (1); G (36)

[1)] Number of animals. [2)] Number of haplotypes. [3)] Haplotype diversity.

[4)] Number of segregating sites. [5)] Nucleotide diversity.

[6)] Average number of nucleotide differences.

[7)] Identified haplogroups in each population (number of animals).

[8)] Animals from Luikart et al (2001) as a reference population.

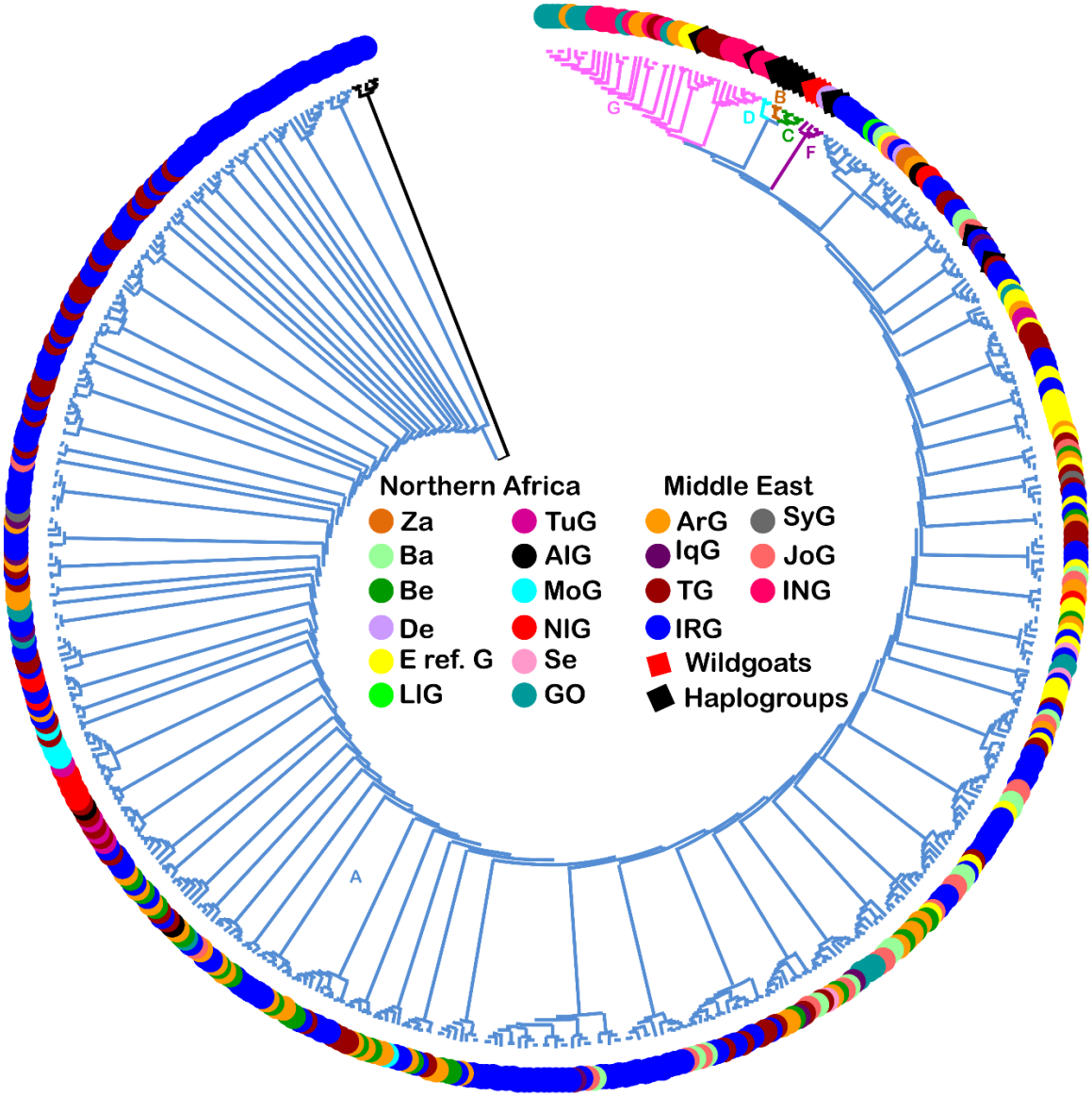

Fig 2. Based on the HV1 region from 485 sequences, a Neighbor-Joining tree of Arabian goats and wild goats (genus Capra) was constructed (6 EG and 481 reference sequences from different regions).

Table 3. Estimates of pairwise Fst among 12 Egyptian goat (*Capra hircus*) populations

	De	Za	Ba	Be	TuG	AlG	MoG	NiG	Se	GO	ArG	SyG	IqG	JoG	TG	ING	IRG
De																	
Za	**0.7417**																
Ba	-0.0027	**0.9569**															
EYG	-0.0067	0.9541	**0.0100**														
Be	0.0009	0.9611	0.0183	**0.0289**													
TuG	0.0173	0.9524	0.1391	0.1037	**0.1616**												
AlG	0.0000	0.9595	0.0387	0.0590	0.0739	**0.1089**											
MoG	-0.0019	0.9596	0.1127	0.0886	0.1100	0.2117	**0.1600**										
NiG	0.0085	0.9600	0.1250	0.0893	0.1065	0.2247	0.1479	**0.0467**									
Se	0.0159	0.9539	-0.0510	-0.0631	-0.0321	0.1046	-0.0141	0.0684	**0.0551**								
GO	0.0344	0.9501	0.2256	0.1465	0.2419	0.2523	0.2443	0.2169	0.2019	**0.1711**							
ArG	-0.0002	0.9543	0.0332	0.0068	0.0049	0.1141	0.0571	0.0706	0.0673	-0.0088	**0.1207**						
SyG	-0.0115	0.9468	-0.0549	-0.0591	-0.0039	0.0773	0.0000	-0.0035	-0.0225	-0.1356	0.0775	**-0.0325**					
IqG	-0.0026	0.9555	0.0158	0.0111	0.0203	0.0662	0.0702	0.0812	0.0778	-0.0275	0.2079	0.0184	**-0.0513**				
JoG	-0.0058	0.9579	-0.0514	0.0195	0.0187	0.1416	0.0276	0.1249	0.1246	-0.0524	0.2418	0.0382	-0.0425	**0.0086**			
TG	0.0085	0.9558	0.0493	0.0311	0.0865	0.0530	0.0415	0.1392	0.1279	-0.0029	0.1797	0.0479	-0.0583	0.0138	**0.0571**		
ING	0.1560	0.9627	0.6473	0.5821	0.6869	0.6013	0.6721	0.6607	0.6565	0.6149	0.3001	0.5652	0.4978	0.6312	0.6629	**0.5937**	
IRG	0.0026	0.9555	0.0276	0.0345	0.0685	0.0790	0.0728	0.1411	0.1423	-0.0153	0.2321	0.0616	-0.0467	-0.0082	0.0341	0.0234	**0.6320**

Fst values exported by DNA Sequence Polymorphism (DnaSP v5.10.01)

4 Conclusions

In this work, we found that different Egyptian goat breeds had different mtDNA D-loop regions. All the Egyptian goats, representing 445 haplotypes, including Egyptian and Arabian goats, were grouped into lineage A, showing that all goat breeds originated from a single population. The phylogenetic analysis indicates the movement of domestic goats across Egypt, Tunisia, and Algeria from Saudi Arabia to Morocco in North Africa and from Egypt directly to Sudan. Zaraibi goats, Arabian Saudi goats, and Algerian goats may have the same origin in one population. The same for the Demuscous goat, which may have the same origin as one population with Iran goat, Jordanian goat, and wild-type populations.

Abbreviations

Egyptian goats (EG), Baladi (BA), Demuscous (DA), and Zaraibi (ZA), hypervariable segment (HVI), Mitochondrial DNA (mtDNA), haplotype diversity (Hd), nucleotide diversity (π), Neighbour-Joining (NJ).

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