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Original Research Article

GENETIC DIVERSITY AMONG TWO COMMON POPULATIONS OF *CANIS LUPUS FAMILIARIS* IN EGYPT BY USING MITOCHONDRIAL DNA HVR1 SEQUENCE

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Abstract: The current study aimed to investigate the variation of mtDNA hypervariable region 1 (*HVR1*) among Egyptian Baladi and German shepherd dogs in Egypt with respect to their phylogenetic origin. Blood samples were obtained from two dog breeds; Egyptian Baladi (n = 46) and German shepherd (n = 42) and used for genomic DNA extraction and PCR amplification of the mtDNA *HVR1* using primers H15360 and L16106. The determined haplotypes were aligned to the sequences of the first published dog mitochondrial genome (Accession No. U96639). We identified 22 different haplotypes from 34 single nucleotide polymorphisms (SNPs) including 2 insertion-deletion polymorphisms among the Egyptian Baladi dogs and 12 haplotypes from 22 SNPs among the German shepherd dogs. Four haplogroups (A, B, C, and D) were identified in the two breeds, their distribution includes 78% of Egyptian Baladi dogs and 76% of German shepherd dogs, respectively were located in the haplogroup A. While 19 % of the German shepherd and 15% of Egyptian Baladi dogs were found in the haplogroup B. 5% of the detected haplotypes of the two breeds were belonged to haplogroup C. 2% of the detected haplotypes of Egyptian Baladi dogs were classified to a haplogroup D. High haplotype and nucleotide diversities were found in the two breeds indicating a lack of genetic differentiation and a recent population growth. The later was confirmed in the Egyptian dogs with the negative values of the neutrality tests and their clustering in the same clade within the phylogenetic tree.

Key words: Egyptian Baladi dogs; German shepherd dogs; HVR1; mtDNA; genetic diversity

Introduction

Dogs (*Canis lupus familiaris)* participate in human life in plentiful ways as such, they protect human from enemies and often could act as ‘‘silent witnesses’’ in forensic casework (1).

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They also, assist with herding and guarding livestock by protecting domestic animals from predators (2). The origin and evolution of the domestic dog are depending on many factors including the place of origin (3). Historically, their domestication occurred when the primitive dogs were obtained from their wild ancestor, the gray wolves (*Canis lupus lupus*) which was further selected to form many dog breeds with specialized characteristics (3, 4). After domestication, several breeds of dogs have been recognized all over the world as a result of the selective breeding either by inbreeding from the same ancestral lines, or by mixing dogs from very different lines (5).

In Egypt, dogs are well known, popular and highly regarded. They were probably domesticated in Egypt in the Pre-Dynastic eras" and served as hunters and as companions for the Egyptians (6). There are many well-known dog breeds such as Egyptian Baladi and German shepherd dogs. However, there is a lack of accurate breed identification for these animals despite the numerous efforts to study dog phylogeny. Several reasons have been listed for this limitation and for the infrequent use of canine evidence in forensic investigations. The absences of validated microsatellite markers or short tandem repeats (STRs), low yield of canine DNA and accurate canine databases are the main causes (7). However, the isolation and analysis of canine mitochondrial DNA (mtDNA) can dramatically improve the identification of the genetic diversity among dog breeds.

The mtDNA is highly informative because of its characteristics such as small molecular weight, high variability and maternal transmission (8-10). The canine mtDNA is approximately 16,728 bp, which consists of 13 protein-coding genes, 22 tRNAs, 2 rRNAs, and polymorphic non-coding regions referred to as hypervariable regions (HVR1 and HVR2) (11). HVR1 is highly polymorphic and can be effectively used in forensic investigations because it can be successfully amplified from limited or severely degraded DNA (7, 12). Besides, HVRI is more effective than HVR2 in studying molecular evolution of animals because the HVR2 is associated with mtDNA replication and translation with limited mutations making it less polymorphic (13).

In Egypt, there is a lack of works on dog breeds diversity and phylogenetic studies among the existence native and foreign breeds and the only conducted researches include DNA polymorphism among pure-breed dogs. Moreover, genetic differences between dogs in Egypt and those in other countries are currently unknown. We therefore investigated mtDNA *HVR1* diversity among 46 Egyptian Baladi dogs and 42 German shepherd dogs in Egypt to establish a genetic database for these two dog breeds, screening for DNA polymorphisms and phylogenetic analysis searching for their origin and relationship.

Material and methods

Sampling and DNA extraction

Forty six Egyptian Baladi dogs and 42 German shepherd dogs were randomly chosen because they are the most common dog breeds in Egypt. About 1.5 ml blood was collected from the forelimb (cephalic vein), and transferred into potassium EDTA containing tubes and then preserved in ice tank till transferred to the Lab. The samples obtained from German dogs were collected from private clinics in Cairo and Mansoura cities after obtaining written consent from the owners. Samples from Baladi dogs were collected at Faculty of Veterinary Medicine, Kafrelsheikh University. Total genomic DNA was extracted from the collected samples using QIAamp DNA Blood Mini Kit (QIAGEN GmbH - Germany) according to the manufacturer instructions. The quality of the extracted DNA was tested for any fragmentations using 1% agarose gel electrophoresis stained with ethadium bromide.

PCR amplification and DNA sequencing

HVR1 region was amplified with specific primers H15360 and L16106 listed in the Table 1. The target fragment corresponded to the sequence at position 15,458–16,727 in the complete annotated nucleotide sequence of the do-

mestic dog (*Canis lupus familiaris*) mitochondrial genome (14). Total volume of PCR mixture was 25 µL contained 1 µl DNA, 0.5 µL of each primer (20 µM each), 2.5 µL of 10x PCR buffer, 0.5 µl Taq polymerase, 2.5 µl dNTPs mix and 17.5 µl milliQ water. PCR amplification was carried out under the conditions previously described by (15). After amplification, The PCR products were purified using ethanol precipitation method depending on the previous method described by Ausubel et al., (16). Sequencing of all samples, in both directions using cycle sequencing ready reaction kit (Applied Biosystems, Foster City,CA) was then done in a BigDye™ Terminator Ver.1.1 using specific primers for sequencing (Table 1**)**.

Statistical and phylogenetic analysis

All the obtained sequences of HVR1 were edited and aligned with the dog mtDNA genome I. D: U96639 (14) as a reference using MEGA software version X (17). Each sequence was first trimmed to approximately 653-bp (from15459–16112 bp of the reference dog mtDNA genome (U96639). Ugene software version 1.26 (18) was used to determine the haplotypes. The sequence of each HVR1 mtDNA haplotype in this sequenced region was deposited in National Center for Biotechnology Information (NCBI) GenBank database under accession numbers (MK050465 to MK050498). Power of discrimination (PD) of the dog mtDNA HVR1 haplotypes were calculated according to the Tamura and Nei model of evolution (19). The statistical quantities for the DNA sequences, including number of haplotypes, nucleotide diversity and Fu and Li's D and F test were performed by using DnaSP 5.10.1(20). Maximum likelihood phylogenetic trees were constructed among the Egyptian Baladi dog and German shepherd dogs and the dog mtDNA genome (U96639) using MEGA software version X (17). Also, the genetic relationship of the identified haplotypes was graphically presented by median-joining network using the network program NETWORK version 5.0.1.0 (21).

Results

Genetic diversity and differentiation

A 653-bp sequence spanning the *HVR1* region was successfully amplified from forty six Egyptian Baladi and forty two German shepherd dogs. The nucleotide sequences of this *HVR1* segment were aligned and clustered into 22 unique haplotypes, represented by 46.83% in Egyptian Baladi dogs (EGYBD1-EGYBD22) while only 12 haplotypes, represented by 26.19% were determined in the case of German shepherd dogs (GYSD01-GYSD12) (Table 2 and 3). Each sequence of these haplotypes covered from 15459–16112 bp of the reference dog mtDNA genome (U96639) (14). The nucleotide sequences of all haplotypes of the two dog breeds were deposited into the GenBank database under accession numbers listed in (Table 2 and 3). The frequencies of these haplotypes among the Egyptian Baladi and German shepherd dogs were calculated and mentioned in Table 2 and 3. The most frequent haplotype among the Egyptian Baladi dogs was EGYBD1 which was detected in 10 individuals from the 46 dogs with a frequency of 0.217 followed by EGYBD18 and EGYBD22 with a frequency 0.109 (Table 2). Among the German shepherd dog, the GYSD11 was the most frequent with a frequency 0.262 followed by GYSD12 and GYSD04 with frequencies 0.190 and 0.143, respectively (Table3).

The analysis of the 653-bp of Egyptian Baladi dog *HVR1* (Table 4) by DNAsp software (20) resulted in the presence of 33 polymorphic sites with 34 single nucleotide polymorphisms (SNPs) including 2 insertion-deletion polymorphisms in the 46 *HVR1* sequences. These polymorphic sites contained 13 singleton variable sites with two variants (the presence of a different nucleotide in only one sequence) at these positions 2, 17, 99, 134, 172, 178, 252, 292, 356, 390, 409, 501, and 581. While there were no singleton variable sites with three variants detected. Besides, they contained 19 parsimony informative sites with two variants (the presence of different nucleotide repeated at least in 2 sequences) at 50, 68, 137, 153, 154, 162, 167,

169, 174, 185, 192, 194, 342, 357, 454, 497, 545, 567, and 625. In addition, one parsimony informative site with three variants was found at position 181. Moreover, eight transitional pairs (si) were reported through the 653-bp sequences without transversional pairs (sv) and the transitional transversional ratio (R = si/sv) was 31.1. Likewise, the analysis of the German shepherd dog sequences (Table 4) resulted in presence of 21 polymorphic sites with 22 SNPs. Among them, six singleton variable sites at position 1, 3, 4, 11, 13, and 15 were noticed. Total numbers of 14 two variants parsimony informative sites were found at position 2, 5, 6, 7, 8, 10, 12, 14, 16, 17, 18, 19, 20, and 21. On the other hand, one three variants parsimony site was noted at 9. The transitional transversional ratio was 24.00 due to presence of 7 transitional pairs without transversional nucleotides were reported. Moreover, the power of discrimination was calculated to be 0.908318 for the Egyptian dogs and 0.851474 for the German shepherd one, respectively.

The nucleotide (π_n) and haplotype (H) diversity coefficients were calculated for the Egyptian Baladi and German shepherd dogs (Table 5). The German shepherd dogs showed higher haplotype diversity compared to the Egyptian ones hence 1 ± 0.034 compared to 0.987 ± 0.018 were reported, respectively. The two dog breeds showed, to some extent, a similar nucleotide diversity which was 0.01299 in the case of Egyptian Baladi dogs and 0.01158 for the German shepherd. The average number of pairwise differences (π) between haplotypes within the dog populations varied from 8.472 in the case of Egyptian Baladi dogs to 7.561 for the German shepherd dogs. Moreover, the Fu and Li's D and Fu and Li's F tests indicated that the Egyptian Baladi dogs showed the lowest values; -0.55934 and -0.58080 compared to 0.38302 and 0.37188 for the German shepherd dogs, respectively.

Phylogenetic analysis

The maximum likelihood analysis, based on kimura-2 parameter model (+G+I) with 1000 bootstraps replicates of the 22 haplotypes of Egyptian Baladi dogs and the 12 haplotypes of German shepherd dogs included in the current study resulted in the phylogenetic tree shown in Fig. 1. Two main clades were observed A, and B. Within the clade A and B, two main subclades; A1 and A2 and B1 and B2 were noticed, respectively. These subclades were further divided into lower subclades. Through the tree, there were no demarcations between the Egyptian Baladi and German shepherd haplotypes. Most of the Egyptian haplotypes were located in the clade A. The EGYBD07 haplotype was located in a separate subclade (A2). These clades were supported 1 and 97 bootstraps. Likewise, the ML analysis of the 22 Egyptian Baladi dogs' haplotypes, the 12 ones of the German shepherd dogs and the reference dog mtDNA genome (U96639) (14) was done using MEGA software version X (17), the resulted phylogenetic tree was shown in Fig. 2. The evolutionary analysis by Maximum Likelihood method showed a tree with the highest log likelihood (-23500.98). Two main clades, A, and B were observed which further subdivided into many subclades. These subclades were also, split into lower subclades. Through the tree, reference dog mtDNA genome (U96639) was shown to be the most probable ancestor. Most of the haplotypes were located in the clade A. The EGYBD07 haplotype was located in a separate subclade (A2). These clades were supported 16 and 95 bootstraps.

For the genetic linkage of the detected haplotypes with the reference dog mtDNA genome (U96639), the genetic network (Fig. 3) was drawn using the NETWORK software version 5.0.1.0. The GYSD12 and EGYBD22 haplotypes (node H_1) were directly connected to U96639 ancestor (node H_1). While, GYSD10 and EGYBD15 (Node H_3), GYSD09 (node H_4), GYSD08 and EGYBD08 (node H_5), GYSD05 (node H_8), GYSD02 (node H_11) and EGYBD02 (node H_27) could be linked to the ancestral node (H_1). Also, it was shown that the most frequent haplotypes (EGYBD01 for the Egyptian Baladi dogs and GYSD11 for German shepherd dogs) were unlinked to the U96639 ancestor.

Four haplogroups (A, B, C, and D) were identified in the studied two dog breeds (Fig. 4,

A and B). Their distribution in the two breeds was 78% of Egyptian Baladi dogs and 76% of German shepherd dogs, respectively were located in the haplogroup A. Besides, 19 % of German shepherd and 15 % of Egyptian Baladi dogs were found in the haplogroup B. While, the two dog breeds shared the same percentage (5 %) for haplogroup C. Haplogroup D was noticed in 2 % of the Egyptian Baladi dogs only.

Table1: List of primers used for PCR amplification and sequencing of *HVR1* in dogs

Primer name	Primer sequence (5'-3')	Purpose	Reference
H15360	ATTACCTTGGTCTTGTAAACC	PCR amplification & sequencing	(7, 15)
L16106	AAACTATATGTCCTGAAACC	PCR amplification & sequencing	(7, 15)
H15422	CTCTTGCTCCACCATCAGC	Sequencing	(15)
H15840	TACTCCAATCCTACTAATTC	Sequencing	(15)
L16102	AACTATATGTCCTGAAACCATTG	Sequencing	(15)

Table 2: Sequence variations, GenBank accession No. and frequencies of the 22 haplotypes detected in 46 Egyptian Baladi dogs

	15464	**15475**	**15508**	**15526**	**15557**	**15592**	**15595**	**15611**	**15612**	**15620**	**15625**	**15627**	**15630**	**15632**	**15636**	**15639**	**15643**	**15650**	**15652**	**15710**	**15750**	**15800**	**15814**	**15815**	**15848**	**15867**	**15912**	**15938**	**15955**	**15959**	**16003**	**16025**	**16039**	**16083**	**GenBank Ac. No.**		
Haplotype	.	T	C	C	T	T	C	T	T	T	T	A	C	C	T	T	A	T	G	C	C	T	C	T	G	A	C	G	C	C	A	T	T	A	U96639	No.	F
EGYBD1	.	.	.	.	.	.	.	.	.	.	.	G	.	T	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	MK050465	10	0.2173913
EGYBD2	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	MK050466	1	0.0217391
EGYBD3	.	.	.	.	.	.	.	.	.	.	C	G	.	T	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	MK050467	1	0.0217391
EGYBD4	.	.	.	.T	.	.	T	.	C	.	C	.	.	T	.	G	G	.	A	.	.	C	T	C	.	.	T	.	T	.	G	.	.	G	MK050468	3	0.0652174
EGYBD5	.	.	.	.T	.	.	T	.	C	.	.	.	.	T	.	G	G	.	A	T	.	C	T	C	.	.	T	.	T	.	G	.	.	G	MK050469	2	0.0434783
EGYBD6	.1C	.	.	T	.	.	.	.	C	.	.	.	.	T	.	G	G	.	A	.	.	C	T	C	.	.	T	.	T	.	G	.	.	G	MK050470	1	0.0217391
EGYBD7	.	.	.	.	.	C	.	.	.	.	C	.	T	T	C	A	.	.	.	.	.	C	T	C	A	.	T	.	.	T	G	.	C	G	MK050471	1	0.0217391
EGYBD8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	MK050472	2	0.0434783
EGYBD9	.	.	.	.	.	.	.	.	.	C	.	G	.	.	.	A	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	MK050473	2	0.0434783
EGYBD10	.	.	.	.	.	.	.	.	.	.	.	G	.	T	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	MK050474	1	0.0217391
EGYBD11	.	.	T	T	.	.	.	C	.	.	.	.	.	.	.	G	.	C	.	.	.	C	T	.	.	.	T	.	T	.	G	.	.	.	MK050475	1	0.0217391
EGYBD12	.	.	T	T	.	.	.	C	.	.	.	.	.	.	.	G	.	C	.	.	.	C	T	.	.	.	T	D	T	.	G	.	.	.	MK050476	1	0.0217391
EGYBD13	.	.	.	.	.	.	.	.	.	.	.	G	.	T	.	A	.	.	.	.	.	.	T	.	.	G	.	.	.	.	.	C	.	.	MK050477	1	0.0217391
EGYBD14	.	.	.	.	.	.	.	.	.	.	.	G	.	T	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	G	MK050478	1	0.0217391
EGYBD15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	MK050479	1	0.0217391
EGYBD16	.	.	.	.	.	.	.	.	.	C	.	G	.	.	.	A	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	MK050480	3	0.0652174
EGYBD17	.	.	.	.	.	.	.	.	.	.	.	G	.	T	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	G	MK050481	1	0.0217391
EGYBD18	.	.	.	.	.	.	.	.	.	.	.	G	.	T	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	MK050482	5	0.1086957
EGYBD19	.	C	.	.	.	.	.	.	.	.	.	G	.	T	.	A	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	C	.	.	MK050483	1	0.0217391
EGYBD20	.	.	.	T	.	.	T	.	C	.	.	.	.	T	.	G	G	.	A	.	.	C	T	C	.	.	T	.	T	.	G	.	.	G	MK050484	1	0.0217391
EGYBD21	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	A	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	G	MK050485	1	0.0217391
EGYBD22	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MK050486	5	0.1086957

Table 3: Sequence variations, GenBank accession No. and frequencies of the 12 haplotypes detected in 42 German shepherd dogs

	15508	**15526**	**15553**	**15595**	**15612**	**15620**	**15627**	**15632**	**15636**	**15639**	**15643**	**15650**	**15652**	**15665**	**15800**	**15814**	**15815**	**15912**	**15955**	**16003**	**16025**	**16083**	**GenBank Ac. No.**		
Haplotype	C	C	A	C	T	T	A	C	T	T	A	T	G	T	T	C	T	C	C	A	T	A	U96639	No	F
GYSD01	.	T	.	.	C	.	.	T	.	G	G	.	A	.	C	T	C	T	T	G	.	G	MK050487	1	0.0238095
GYSD02	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	MK050488	1	0.0238095
GYSD03	.	.	.	.	.	C	G	.	.	A	.	.	.	.	.	T	.	.	T	.	.	G	MK050489	2	0.047619
GYSD04	.	T	.	T	C	.	.	T	.	G	G	.	A	.	C	T	C	T	T	G	.	G	MK050490	6	0.1428571
GYSD05	.	.	G	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	MK050491	2	0.047619
GYSD06	.	T	.	.	C	.	.	T	.	G	G	.	.	.	C	T	C	T	T	G	.	G	MK050492	1	0.0238095
GYSD07	T	T	.	.	C	.	.	.	.	G	.	C	.	.	C	T	.	T	T	G	.	.	MK050493	2	0.047619
GYSD08	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	MK050494	3	0.0714286
GYSD09	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	T	.	.	.	.	C	.	MK050495	1	0.0238095
GYSD10	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	C	.	MK050496	4	0.0952381
GYSD11	.	.	.	.	.	C	G	.	.	A	.	.	.	.	.	T	.	.	T	.	.	.	MK050497	11	0.2619048
GYSD12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	MK050498	8	0.1904762

Table 4: Haplotype diversity (H), nucleotide (πn) diversity, mean number of pair-wise differences (π) between haplotypes within populations, polymorphic sites (p.s.), number of haplotypes (Hn), Fu and Li's D and F tests, and Tajima's D in two dog breeds

Breed	H ± SD	(π_n)	π	p.s.	Fu and Li's D	Fu and Li's F	Tajima's D
Egyptian Baladi	0.987 ± 0.018	0.01299	8.472	33	-0.55934 (P > 0.10)	-0.58080 (P > 0.10)	-0.35475 (P > 0.10)
German Shepherd	1 ± 0.034	0.01158	7.561	21	0.38302 (P > 0.10)	0.37188 (P > 0.10)	0.16720 (P > 0.10)

Table 5: Haplotype diversity and power of discrimination

Parameter	Egyptian Baladi dogs	German Shepherd dogs
Total number of animals	46	42
No. of haplotypes	22	12
Power of discrimination	0.908318	0.851474
SNPs	34	22
Total number of singleton mutations	13	6
Number of parsimony informative sites	20	15
Transitional Pairs (si)	8	7
Transversional Pairs (sv)	0	0
R = si/sv	31.1	24.0

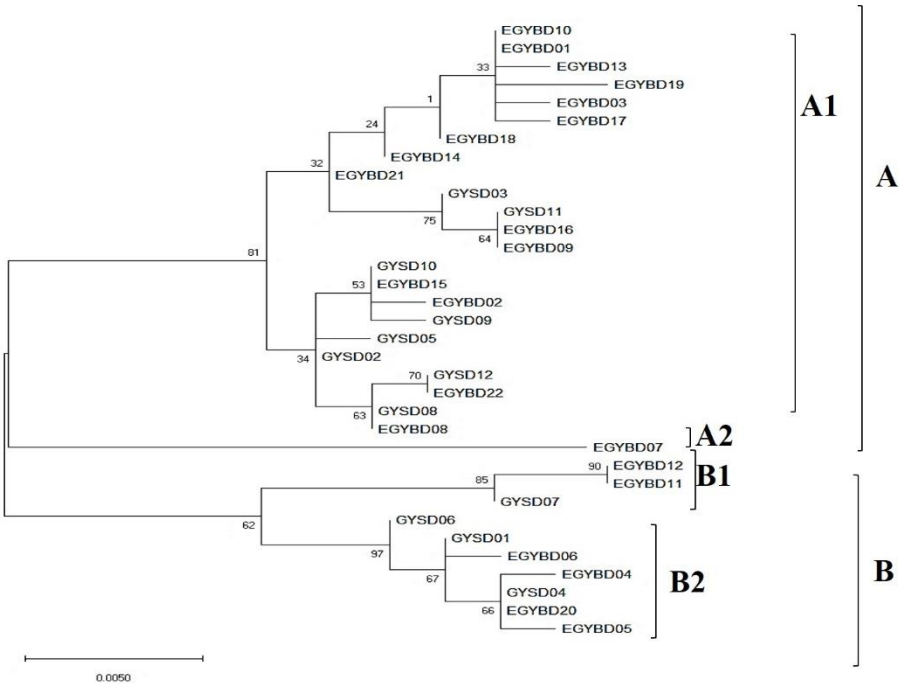

Figure 1: The second Maximum likelihood tree based on kimura-2 parameter method (+G+I) of 34 haplotypes of the two dog breeds included in this study. The number of bootstrap replications=1000. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model. The tree with the highest log likelihood (-23500.98) is shown

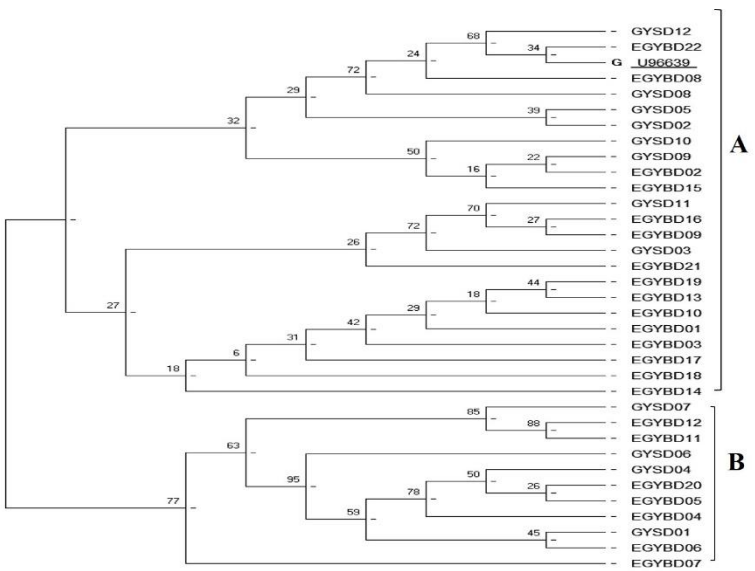

Figure 2: The second Maximum likelihood tree based on kimura-2 parameter method (+G+I) of 34 haplotypes of the two dog breeds and U96639 included in this study. The number of bootstrap replications=1000. U96639 is the first published dog mitochondrial genome. G refers to the most common ancestral line

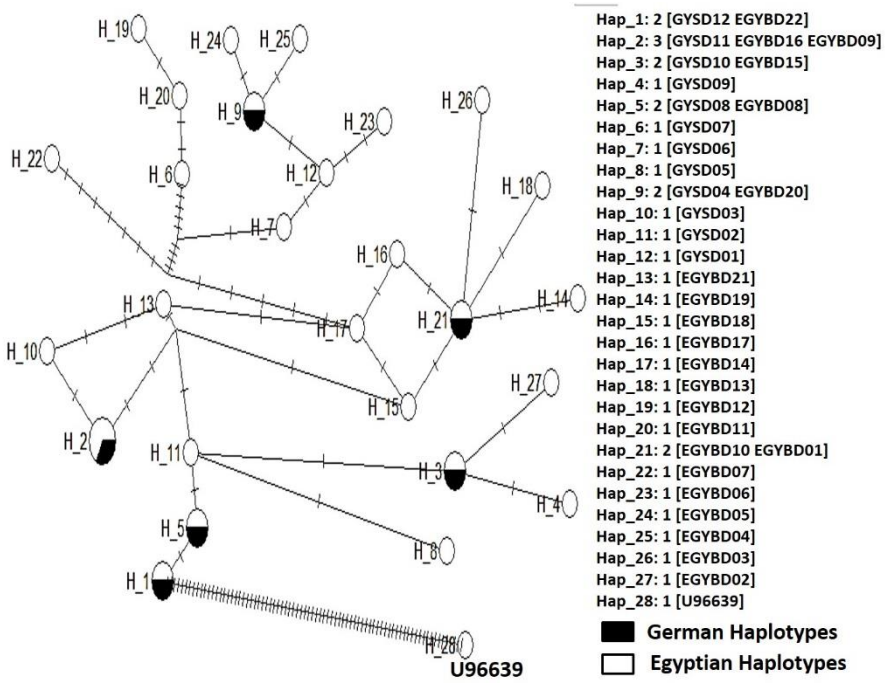

Figure 3: Median-joining network (Ɛ = 0) depicting Genetic relationships among dog breed mtDNA *HVR1* haplotypes from this study using Network v5.0.1.0. Circled areas are proportional to the corresponding haplotype frequency

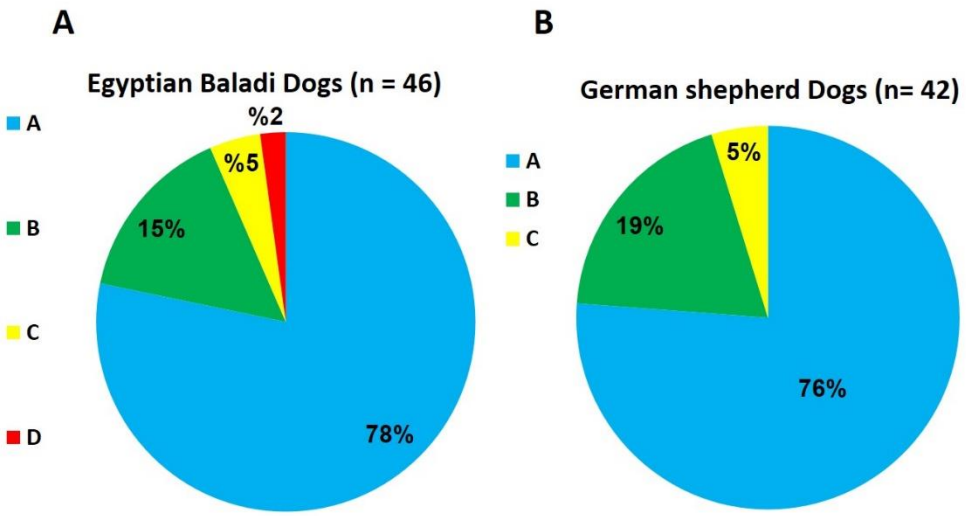

Figure 4: Mitochondrial DNA haplogroup distributions in the most common Egyptian dog breeds

Discussion

German shepherd and Egyptian Baladi are widely distributed and the most common dog breeds in Egypt. These dogs are kept mainly for guarding and have a crucial role in the forensic cases (2). However, up to date, there is missing information on the genetic diversity between these two breeds. Also, there are no effective controlled breeding programs for these breeds. So, for many purposes, such as forensic cases, it is highly recommended to differentiate between these two breeds.

The canine mtDNA, especially *HVR1*, is highly polymorphic that can be used effectively as a DNA marker for the breed identification (22). However, little is known regarding using *HVR1* in dog breeds identification in Egypt. As the haplotype diversities are informative tools about the history of animals, and for the breed identification. So, the high diversities reported in the current study probably explain the lack of differentiation between the Egyptian Baladi and German shepherd dogs in Egypt (23). These results agreed with the previous studied (11, 24) which reported high haplotype diversities although using different dog breeds and nucleotide numbers. Moreover, the Egyptian Baladi dogs had high haplotype diversity almost, as high as that noticed in the case of German shepherd dogs. This might imply that Egyptian dogs were developed from several breeds over their breeding history. In addition, the high genetic variability among the two studied breeds probably indicates that the dog populations in Egypt have been undergoing a rapid expansion in recent history (25). Plus, an increased effective population size, and a reduced genetic drift might be inferred from the identified high genetic diversity (26). Likewise, the reported high nucleotide diversities for the two breeds might elucidate high genetic differentiation and presence of large differences between haplotypes (27). The rate of genetic diversity is determined effective population size, and rate of mutation. Where, the larger the population size, the higher the genetic diversity (28). So, it is perhaps occurred due to a relatively large long-term effective population size rather than any severe bottleneck during dog evolution (29). Also, the detected higher haplotype and nucleotide diversities probably indicate that the studied populations recently divergent from each other (30).

Additionally, the detected high haplotype and nucleotide diversities could be a signature of a rapid population expansion (31). This was confirmed by the genetic distant from the most frequent haplotypes and the ancestral haplotype (U96639) (25). Another evidence of the rapid population expansion was concluded from calculating the Tajima's D test and Fu's Fs tests that are usually used to find out the population expansion. For the Egyptian Baladi dogs, the Tajima's D test and Fu's Fs values were non-significantly negative. The overall negative values of these neutrality tests perhaps designated an excess of the rare mutations in populations, which might suggest a recent population expansion as well as an evidence of a selective sweep (32). While for the German shepherd dogs, the positive non-significant values of Tajima's D test and Fu's Fs probably implied low levels of both low and high frequency polymorphisms and a balancing selection (32). These non-significant results observed in case of Egyptian and German dogs indicated non-significant variations and the absence of a clear population structure (33).

The phylogenetic analysis of the different haplotypes found in the two dog breeds under study and the reference dog mtDNA genome (U96639), was performed. The analysis revealed the presence of two main clades subdivided to many subclades. Also, most of the Egyptian and German haplotypes as well as the reference dog mtDNA genome (U96639) were clustered in the same clade indicating absence of clear demarcations and a strong relationship between the haplotype sequences of the present study and the reference dog mtDNA genome (U96639). This might indicate that two studied breeds are descended from the same ancestral line, recently originated, and mutations that account for their differences have yet to be reported and become fixed (23). Additionally, similarities among the Egyptian Baladi and German shepherd dogs were revealed from the

haplogroup distribution in which the major haplogroup (A) was identified in the two breeds.

Conclusion

In conclusion, this study aimed to use the mtDNA *HVR1* to differentiate between the most common dog breeds in Egypt; German shepherd and Egyptian Baladi. The calculated high genetic diversity indicates a lake of differentiation between the two breeds. The most frequent haplotypes of the two breeds was belonged to haplogroup A followed by haplogroup B and C. The negative neutrality tests imply recent population growth of the Egyptian Baladi dogs. The results of this study would be helpful in advancement of Egyptian forensics and animal genetic studies.

Disclosure statement

No conflicts of interest, financial, or otherwise, are declared by the authors.

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