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MOLECULAR PHYLOGENETIC RELATIONS OF MAMMALS OF THE GENUS CANIS IN EGYPT

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ABSTRACT

The taxonomic identity of canids of the genus *Canis* in North Africa has been investigated using molecular phylogenetic analysis based on Cytochrome *b* mitochondrial DNA sequence analysis. Tissue samples from 18 *Canis* specimens collected from all known populations/taxa in Egypt were obtained and analyzed. The resulting *Cyt b* mt DNA sequences of current *Canis* taxa, described based morphological characters were compared to each other and to other *Canis* populations/taxa across North Africa and the Middle East. The results showed no detectable sequence differentiation among all populations, suggesting that the separation of the phenotypically differentiated populations of Egypt has been relatively recent. Estimate divergence time between different phylogroups of the genus and the phylogeographic history of the genus was discussed in a regional paleogeographic context.

KEYWORDS Molecular Phylogenetic, mitochondrial DNA, cytochrome b

INTRODUCTION

The Egyptian Wolf *Canis lupaster* Hemprich and Ehrenberg, 1833 is a widespread canid throughout northeast Africa. Its distribution and biology in Egypt have been studied in detail by Osborn and Helmy (1980) [1], and Saleh and Basuony (2014) [2]. The morphology of this canid was described by Anderson and De Winton (1902) [3], Osborn and Helmy (1980) [1] and Saleh *et al.* (2018) [4]. The taxonomic status of this species, however, has been recently a matter of controversy (Rueness *et al.*, 2011; Gaubert *et al.*, 2012 and Koepfli *et al.*, 2015) [5-7]. It was formerly treated as a subspecies of the Asiatic Golden Jackal *C. aureus*. Recent morphological and molecular investigations, however, strongly suggested a status as a distinct species (Saleh and Basuony, 2014; Koepfli *et al.*, 2015; Urios *et al.*, 2015 and Viranta *et al.*, 2017) [2, 7, 8 and 9]. Based on morphological comparison, Saleh and Basuony (2014) [2] resurrected the name *C. lupaster*, for its populations in Eastern Sahara, with two morphologically distinct subspecies identified in Nile Valley in Egypt. Abdel-Hamid (2016) [10] added a third subspecies; *C. lupaster. qattarensis*, which was formerly named *C. aureus qattarensis* from northwestern Egypt (Saleh and Basuony, 2014) [2]. Mitochondrial DNA sequence analysis recently showed that all North African *Canis* forms were very similar and were thus placed under the species *C. anthus* Cuvier, 1820. The larger Nile Valley canid was given the subspecific name *C. anthus lupaster* (Koepfli *et al.*, 2015 and Urios *et al.*, 2015) [7, 8]. The name *C. anthus* was subsequently considered a *nomen dubium* and the name *C. lupaster* was applied for all the wolf-like canids of the entire northern region of the African Continent (Viranta *et al.*, 2017) [9].

In this paper, we investigate the validity of the current, morphologically based taxonomic relations of *Canis* populations in Egypt and neighboring regions based on *Cyt b* mitochondrial DNA sequence analysis. The comparison includes all known *Canis* populations of Egypt, including those of the inland oases of Egypt, which were never examined by previous investigators.

MATERIALS AND METHODS

Sampling:

Tissue samples were obtained from a total of 18 *Canis* specimens collected from all known populations/taxa in Egypt. Samples were obtained from pectoral muscles of live trapped or animals freshly killed by farmers and 2 domestic dogs. All samples were obtained during the course of this study. Table

1 shows the list of samples used in this study. Figure 1 shows sampling localities and known collection localities of *Canis* species in Egypt based on all published records and material in Al-Azhar University Zoological Collection (AUZC).

DNA Extraction:

All tissue samples for DNA analysis was preserved in 100% ethyl alcohol and kept at -20 °C until used for the analysis. Total genomic DNA was extracted from pectoral muscles using the DNeasy Tissue Kit (QIAGEN), following the manufacturer's instructions, DNA concentration and purity were measured by Nanodrop.

Amplification segment of the mitochondrial *Cyt b* gene:

A segment of the mitochondrial *Cyt b* 402 base pairs was amplified from each sample by polymerase chain reaction (PCR) using the primer pair L14724 (5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′) (Irwin *et al.,* 1991) [11] and H15149 (5′-AAACTGCAGCCCCTCAGAATGATATT TGTCCTCA-3′) (Kocher *et al.,* 1989) [12]. Each PCR mixture consisted of 12.5 µl PCR master mix, 1 µl of each primer (10 pmol/µl) and 5 µl DNA template and the volume was brought to 25 µl by deionized sterile water, all in Thermowell® GOLD 0.2 ml PCR Tubes with Flat Cap.

PCR conditions were as follows: initial denaturation on 94°C for 7 min followed by 35 cycles, each one consisting of a denaturation step (45 sec at 94°C), annealing step (30 sec at 50°C) and an extension step (45 sec at 72°C), and an extra final extension step was performed for 7 min at 72°C. PCR products were kept in 4°C or -20°C until being electrophoresed and analyzed. DNA sequencing was applied by 3500 genetic analyzer (Applied Biosystems). At least two independent PCR products were used for sequencing per species.

Phylogenetic Analyses:

DNA sequences were checked and edited using the program BioEdit v.7.0.9.0 (Hall, 2007) [13]. The number of segregating sites (S), haplotype diversity (h), nucleotide diversity (π) and frequency of each haplotype were calculated using DNASP v. 5.10.01 (Librado and Rozas, 2009) [14]. Genetic distances were estimated under the Kimura 2-parameter (K2P) nucleotide substitution model (Kimura, 1980) [15] in MEGA6 v. 6.01 (Tamura *et al.,* 2013) [16].

To place these new mitochondrial DNA data in a wider context, we included nearly all previously published *Cyt b* gene sequences of the genus *Canis* in the phylogenetic analyses. Table 2 shows GenBank mt *Cyt b* gene sequences used in this study.

Phylogenetic analysis was performed based on 402 bp of *Cyt b* gene. Multiple alignments were performed by ClustalX 1.83 (Thompson *et al.,* 1997) [17], with the default parameters. The results of alignments were also carefully checked and edited by eye. Maximum-parsimony and Neighbor joining analyses were performed with Paup v4 (Swofford, 2001) [18], with heuristic searches using stepwise addition and performing tree bisection reconnection (TBR) branch swapping (Swofford *et al.,* 1996) [19]. Confidence in the nodes was evaluated by 1000 bootstrap replicates (Felsenstein, 1985) [20] with random addition of taxa.

Geographical structuring of *Canis* populations/taxa was inferred using Bayesian inference implemented with MrBayes 3.1.2 (Ronquist *et al.,* 2012) [21]. MrModeltest 2.3 (Langmead and Salzberg, 2012) [22] was used to select best fit models of nucleotide evolution, based on the Akaike information criterion (AIC) (Akaike, 1987) [23]. The analysis was conducted with three heated and one cold Markov chain (MCMC), sampling trees every 5000 generations for 10 million generations. Output parameters were visualized using Tracer 1.5 (Rambaut and Drummond, 2007) [24] to ascertain stationary and convergence. All samples obtained during the first million (25%) generations were discarded as burn-in. The default parameters were used for the Metropolis-coupled Markov Chain Monte Carlo (three hot chains and one cold chain). To keep state swap frequencies between 10% and 70%, the heating parameter was changed to 0.01. Each partitioning scheme was running for 10 million generations, 1000 generations and discarding the first 25% as burn-in. An ultrametric tree was generated with BEAST v. 1.8 **(**Nylander, 2004 and Drummond *et al.,* 2012) [25, 26].

Table 1: List of *Canis* samples from Egypt used in this study.

Sample	AUZC Number	Locality
C. lupaster	M00155	Al Qalyubiya, Nile Delta
C. lupaster	M00156	Dmuh, Faiyum
C. lupaster	M00409	Mansheyt Otefa, Senorus, Faiyum
C. lupaster	M00159	Mansheyt Otefa, Faiyum
C. lupaster	M00160	Mansheyt Otefa, Faiyum
C. lupaster	M00403	Luxor, Nile Valley
C. lupaster	M00404	Luxor, Nile Valley
C. lupaster	M00407	Sohag, Nile Valley
C. lupaster	M00408	Sohag, Nile Valley
C. lupaster	M00163	Qara Oasis, Qattara Depression, Matruh
C. lupaster	M00164	Qara Oasis, Qattara Depression, Matruh
C. lupaster	M00166	Bawiti, Bahariya Oasis, Giza
C. lupaster	M00197	Bawiti, Bahariya Oasis, Giza
C. lupaster	M00167	Baris Oasis, El Wadi El Gedeed
C. lupaster	M00169	Baris Oasis, El Wadi El Gedeed
C. lupaster	M00168	Farafrah Oasis, El Wadi El Gedeed
C. lupus familiaris	Egy 1	Beheira, Nile Delta
C. lupus familiaris	Egy 2	Beheira, Nile Delta

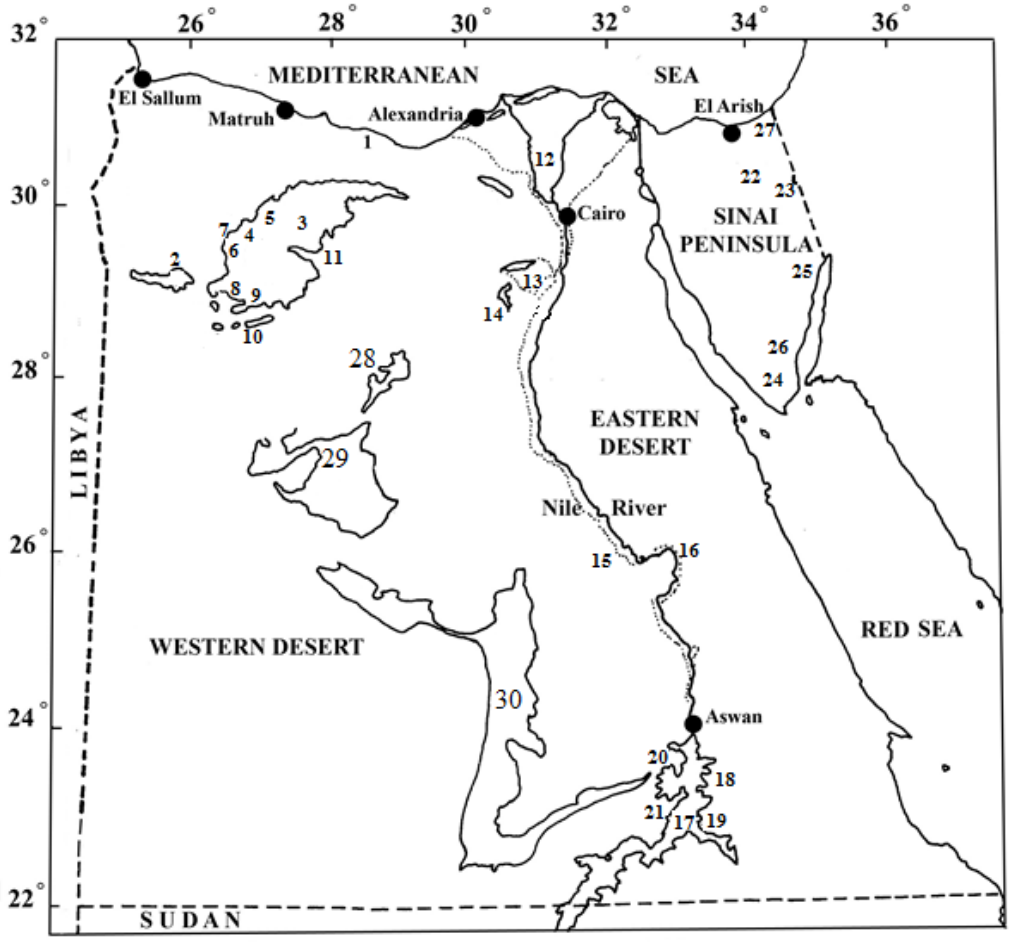

Figure 1: Collection localities of genus *Canis* samples from Egypt.

Table 2: Gene Bank sample, accession numbers of *Cyt b* previously used in phylogenetic analysis of *Canis sp*.

Taxon	Locality	Accession numbers of *Cyt b*	Authors
C. lupaster	Algeria	JQ088659	Gaubert *et al.* (2012) [6]
C. lupaster	Algeria	JQ088660	Gaubert *et al.* (2012) [6]
C. lupaster	Algeria	JQ088661	Gaubert *et al.* (2012) [6]
C. lupaster	Algeria	JQ088662	Gaubert *et al.* (2012) [6]
C. lupaster	Algeria	JQ088663	Gaubert *et al.* (2012) [6]
C. lupaster	Senegal	JQ088664	Gaubert *et al.* (2012) [6]
C. lupaster	Mali	JQ088665	Gaubert *et al.* (2012) [6]
C. anthus	Morocco	KT378607	Urios *et al.* (2015) [9]
C. anthus	Morocco	KT447762	Urios *et al.* (2015) [9]
C. anthus	Morocco	KT378605	Urios *et al.* (2015) [9]
C. anthus	Morocco	KT378606	Urios *et al.* (2015) [9]
C. anthus	Mauritania	KT447761	Urios *et al.* (2015) [9]
C. anthus	Mauritania	KT447760	Urios *et al.* (2015) [9]
C. anthus	Mauritania	KT447759	Urios *et al.* (2015) [9]
C. lupus	Spain	NC008092	Bjornerfeldt *et al.* (2006) [27]
C. lupus	Saudi Arabia	EU789787	Pang *et al.* (2009) [28]
C. simensis	Ethiopia	KT448281	Koepfli *et al.* (2015) [7]
C. aureus	India	AY291433	Aggarwal *et al.* (2007) [29]
C. latrans	USA	EU789789	Bjornerfeldt *et al.* (2006) [27]
C. adustus	Guinea	JQ088650	Gaubert *et al.* (2012) [6]
C. adustus	Guinea	JQ088651	Gaubert *et al.* (2012) [6]
Lycaon pictus	Africa	AF028147	Wayne *et al.* (1997) [30]

RESULTS AND DISCUSSION

Our *Cyt b* mt DNA data set consisted of 368 aligned nucleotides. In total, 284 (77.17%) bases were constant, 30 (8.15%) bases were variable and 54 (14.67%) were parsimony-informative. Nucleotide composition was clearly biased towards A–T. The mean values of T, C, A and G within the sequence data are 29.8, 25.1, 29.8 and 15.3%, respectively. Within the 368 bp length sequences, 84 polymorphic segregating sites were detected. Haplotype diversity (h) and nucleotide diversity (π) were 0.435 and 0.02677, respectively. The sequence divergences among the *Canis* haplotypes ranged from 0.00 to 0.12, with an average of 0.06.

Maximum Parsimony

We performed the maximum parsimony analysis within 368 bp in the length of the sequences, the gaps treated as missing produced two most-parsimonious trees with a length of 105 steps (homoplasy index = 0.681; consistency index = 0.810; retention index = 0.841).

The resulting tree Figure 2 shows seven distinct groups representing *Canis* taxa in the regions and *Lycaon pictus* as an out group. The first clade is strongly supported with a Bootsrap value 84. This clade, which represents one haplotype shared by 30 individuals belonging to the taxa recognized as *C. lupaster* (Saleh and Basuony, 2014) [2], *C. lupus lupaster* (Gaubert *et al.*, 2012) [6] and *C. anthus* (Koepfli *et al.*, 2015; Urios *et al.*, 2015) [7, 8] which are assumed to represent *Canis* species in part or all of a vast geographical range spread across North Africa from Mauritania to Egypt.

Our analysis also clearly shows that the second clade is shared by the gray wolf *C. lupus lupus* from Spain, the Arabian wolf *C. lupus. arabs* from Saudi Arabia and the domestic dog *C. lupus. familiaris* from Egypt, and is supported by a Bootsrap value of 100. The third clade includes *C. simensis* from Ethiopia and is supported by a Bootsrap value

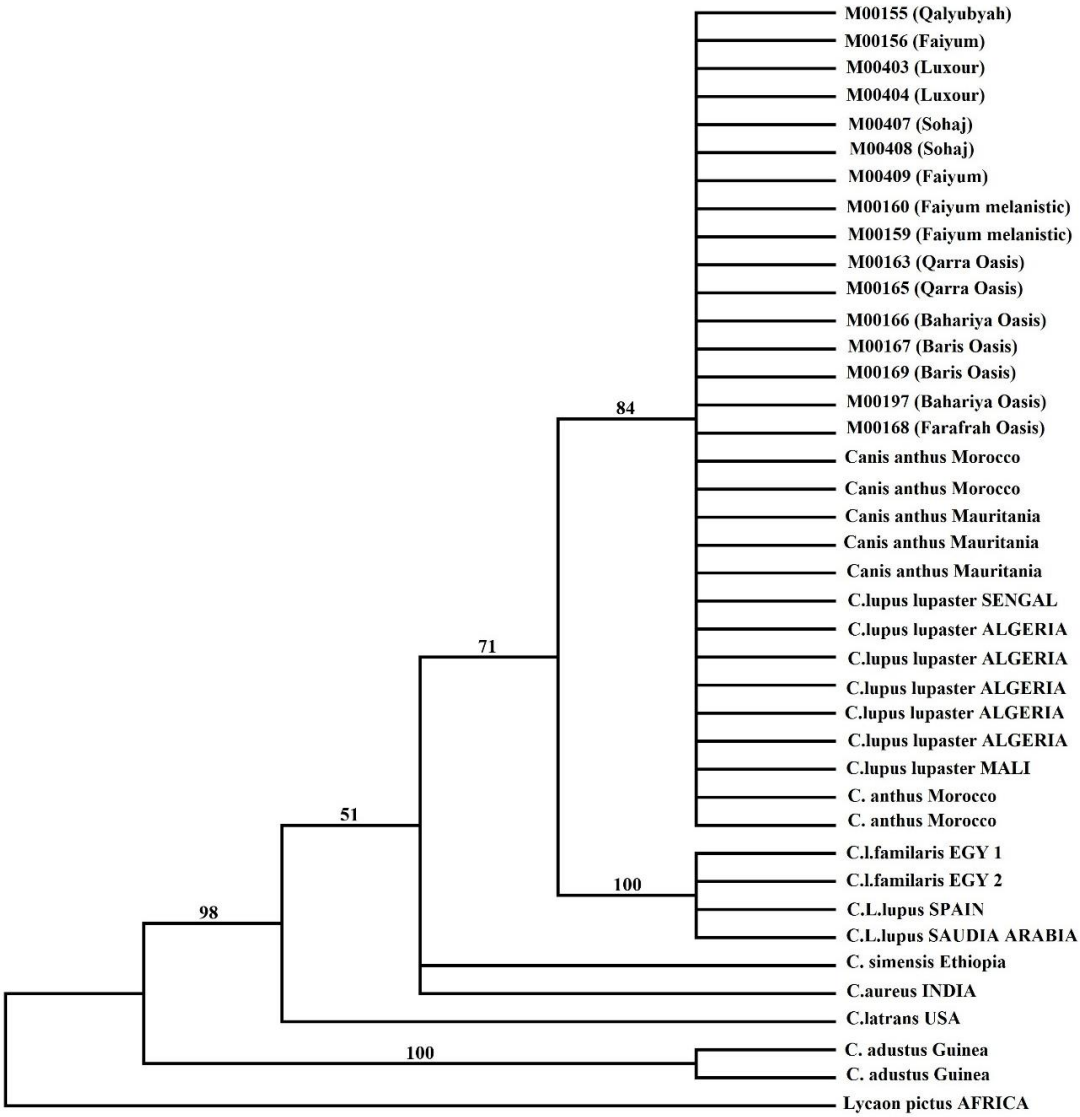

Figure 2: Maximum parsimony phylogenies of genus *Canis* mt DNA sequences fragment of the *Cyt b* region 368 bp. Number above branches indicate bootstrap values calculated with 1000 replicate. The tree was performed with Paup v4.

of 71. The fourth recognizable clade includes *C. aureus* from India and is supported by a Bootsrap value of 71. *C. latrans* from North America forms the fifth clade and is supported by a Bootsrap value of 51. The Sixth clade contains *C. adustus* from Guinea and is supported by a Bootsrap value of 98. The Seventh clade contains the African hunting dog *Lycaon pictus* as outgroup.

Similarly, the neighbor joining analysis generated a tree Figure 3 shows the results of the neighbor joining analysis. The resulting tree has a general topology nearly identical to the maximum parsimony tree shown in Figure 2. The phylogenetic analysis reveals seven strongly supported clades identical with those revealed by the maximum parsimony analysis.

The general topology of the Bayesian inference tree shown in Figure 4 is very similar to both the maximum parsimony (Figure 2) and neighbor joining trees (Figure 3). This analysis clearly shows that the second clade encompasses the gray wolf *C. l. lupus*, *C. l. arabs* and *C. l. familiaris*. This clade is supported by a posterior probability value 1. The third clade includes *C. simensis* from Ethiopia and supported by posterior probability value 0.92. *C. aureus* from India represents the fourth recognizable clade and is weakly supported by a posterior probability value of

0.36. *C. latrans* from USA forms the fifth clade and is supported by a posterior probability value of 0.68. The Sixth clade contains *C. adustus* from Guinea and is supported by a posterior probability value 1. The Seventh clade contains the African hunting dog *Lycaon pictus* as outgroup.

Genetic Distance and Taxonomic Implications

Based on the 368 bp mt DNA *Cyt b* data set, genetic distances were estimated, using mega 6 (Tamura and Nei, 1993) [31]. Table 3 shows the genetic distance based on 368 bp mitochondrial *Cyt b* sequences. The table shows that the *C. lupaster* phylogroup, which consists of *C. lupaster* and *C. anthus* is separated from the nearest phylogroup containing the gray wolf *C. lupus* and the domestic dog *C. l. familiaris* by a genetic distance of 3.1%. Within the first phylogroup, however, all *Canis* taxa seem to be nearly identical with inter-population difference in the 368 bp mitochondrial *Cyt b* sequences being undetectable.

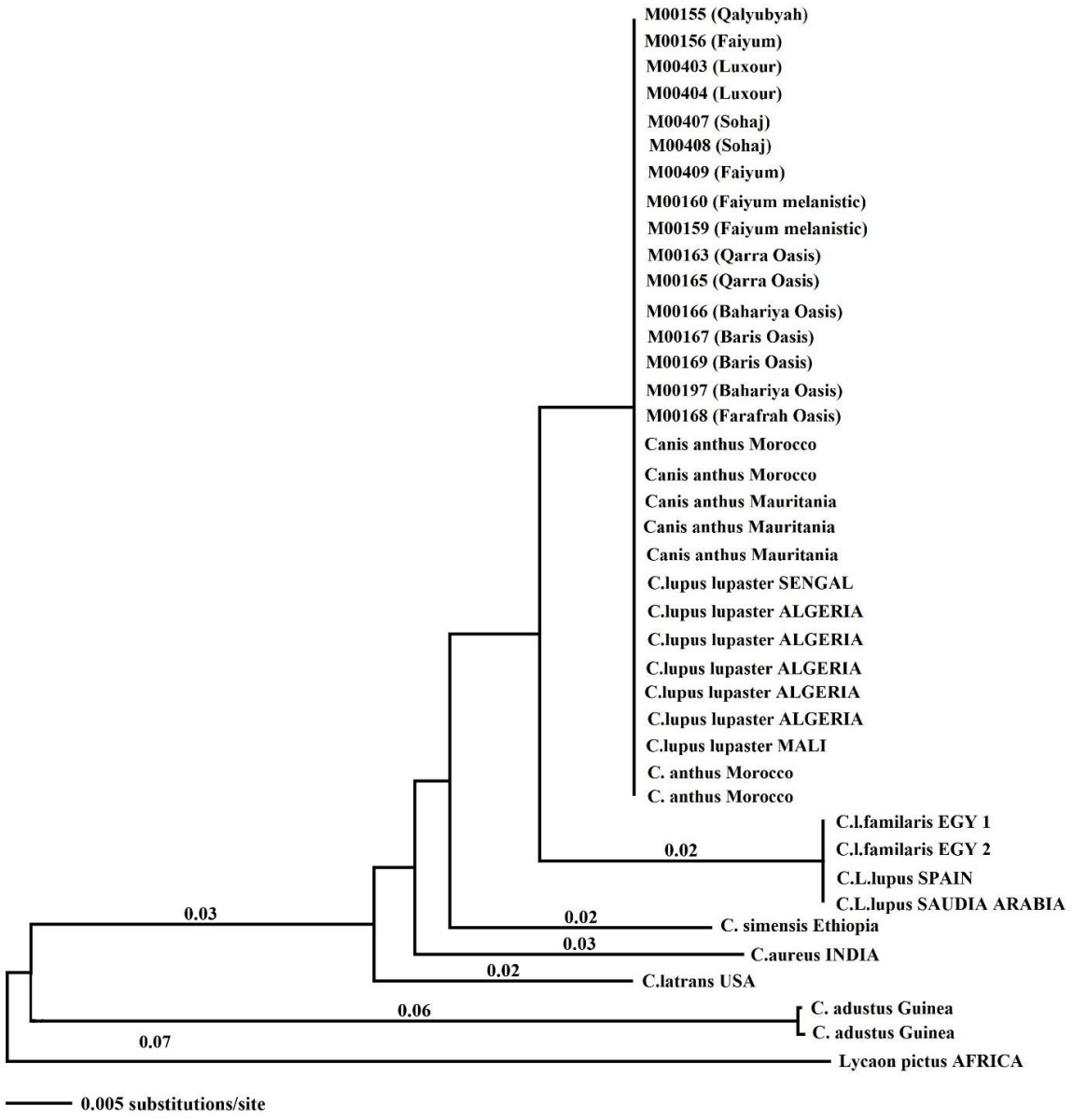

Figure 3: Neighbor joining phylogenies of genus *Canis* mt DNA sequences fragment of the *Cyt b* region 368 bp. Number above branches indicate distance values between genus *Canis* populations. The tree was performed with Paup v4.

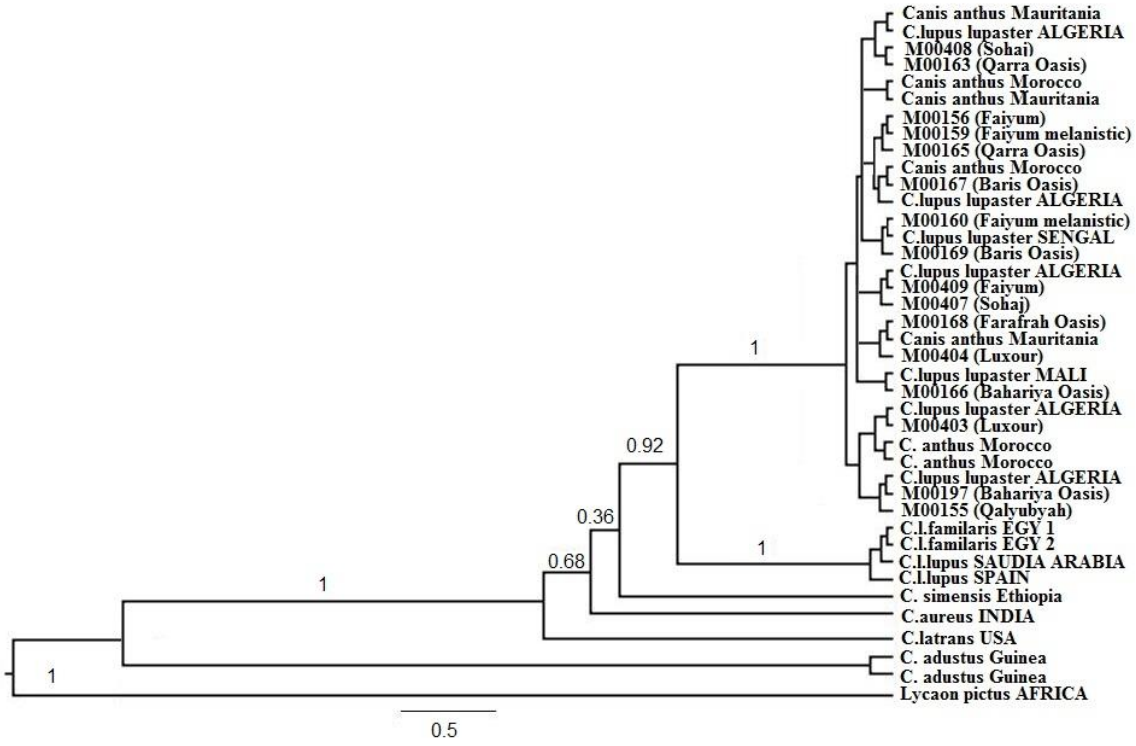

Figure 4: Bayesian inference tree phylogenies of genus *Canis* mt DNA sequences fragment of the *Cyt b* region 368 bp (50% majority rule consensus tree). Numbers above nodes indicate the posterior probabilities.

Current taxonomic literature refers North African *Canis* to either *C. lupaster or C. anthus* (Saleh and Basuony 2014; Koepfli *et al.*, 2015; Urios *et al*., 2015) [2, 7, 8]. Previously recognized systematics and nomenclature treating these canids as either a large subspecies of the Golden Jackal, *C. aureus*, (Setzer, 1961; Clutton-Brock *et al.*, 1976; Osborn and Helmy, 1980; Wassif, 1995) [32, 33, 1, 34], or small subspecies of the gray wolf *C. lupus* (Ferguson, 1981) [35] were proven to be erroneous.

Rueness *et al.* (2011) [5], based on mitochondrial DNA analysis, concluded that the Egyptian Wolf is not a golden jackal and should be placed within the Gray Wolf species complex. Gaubert *et al.* (2012) [6] adopted the combination *C. lupus lupaster* stating that the species clearly appears as a distinct, relatively ancient gray wolf lineage occupying a range 6000 km wide, stretching from Senegal to Egypt. Saleh and Basuony (2104) [2] showed that the Egyptian wolf is morphologically very different from both *C. lupus* and *C. aureus* and should be treated as a distinct species under its original name *C. lupaster*. These authors (Saleh and Basuony, 2104) [2], however, demonstrated significant morphological differences between discrete populations northeast Africa that they considered sufficient to treat these populations as distinct subspecies.

Koepfli *et al.* (2015) [7], Rueness *et al.* (2015) [36] and Urios *et al*. (2015) [8] using molecular data demonstrated that the North African canid is a unique taxon and not a hybrid between other canids. Koepfli *et al.* (2015) [7] concluded that populations of the golden jackals from Africa and Eurasia represent distinct monophyletic lineages separated from each other for more than one million years, which is sufficient to merit formal recognition as distinct species. These authors resurrected the name *C. anthus* (African Wolf) as the name that first was classified as species by Cuvier (1820).

Our molecular data clearly show that the canids of North and northeast Africa are nearly genetically identical with those of northwest

Africa but decidedly distinct at the specific level from both *C. aureus* and *C. lupus*; a conclusion already reached by Saleh and Basuony (2014) [2] based on morphological data and Koepfli *et al.* (2015) [7] and Urios *et al*. (2015) [8] based on molecular phylogenetic analysis of mitochondrial DNA *Cyt b* sequences. This may suggest an un-interrupted gene flow between *Canis* populations from the Nile Valley, across the Sahara to western North Africa and the Sahel. As the Nile Valley population is currently isolated from populations in the rest of North Africa and the Sahel, it may be assumed that the observed population fragmentation is very recent.

Molecular Phylogeography

To estimate divergence time between different phylogroups of the genus *Canis*, we constrained the root of our tree (the split between *Lycaon pictus* and *C. lupus*) based on the estimate of 4.3 Mya (credibility region between 3.4 and 5.5 Mya) as provided by Perini *et al.* (2010) [37]. This estimate was used as a calibration point for the time of the most recent common ancestor of all taxa of the genus *Canis* included in the tree. The species tree was then reconstructed and divergence times was estimated using BEAST v. 1.6.2 (Drummond and Rambaut, 2007) [38]. Best-fit models of evolution were selected using Mr Modeltest 2.

The tree in Figure 5 shows the estimated divergence times for *Canis* species of North Africa and the Middle East w
lupus and the domestic dog *C. lupus familiaris* during late Pleistocene 0.45 to 1.39 Mya, with a mean, estimated node age of 0.84 Mya.

The phylogeographic history of the genus *Canis* in Egypt and the rest of North Africa as inferred from the molecular results, can be best understood within the framework of the ecological history of the region. Key to this historical framework are the dramatic changes in the geology, geomorphology and geography of the region and the development of desert conditions in North Africa and the Middle East.

Since the first onset of arid conditions in the Sahara during the Late Miocene-Pliocene, around 7 Mya (Schuster *et al.*, 2006; Swezey, 2009) [39, 40], the Sahara has experienced periodic shifts between humid and arid conditions (Foley *et al.*, 2003; Kropelin *et al.*, 2008; Geraads, 2010; Drake *et al.*, 2011) [41-44]. These climatic shifts led to periodic constrictions and expansions of arid and green regions of the Sahara. These dramatic changes in climate, particularly those occurring during the last 3 million years, had profound influences on the evolution of vertebrates in Africa (Almogi-Labin, 2011) [45] and seem to have driven the evolution of *Canis* in North Africa.

Three major climatic episodes, leading to important changes in the paleoenvironment are recognized, and have been dated at 2.6–2.4 Mya, 1.8–1.6 Mya, and 1.2–0.9 Mya (De Menocal, 2004) [46]. These major climatic episodes, are characterized by step-like increases in aridity, had very pronounced consequences on the evolution of African faunal assemblages and, possibly accelerated speciation. Changes toward desert-adapted African faunal assemblages are clearly associated with the onset of each of these periods (De Menocal, 2004) [46]. Paleoclimatic oscillations following the initial formation of the Sahara are suggested to have occurred, with episodes of intensified rainfall, turning the desert into a green savannah-like environment (Drake *et al.*, 2011) [44]. These Saharan climatic changes greatly shaped the range of desert and savannah environments and constrained species distribution and genetic structure (Szabo *et al.*, 1995; Drake *et al.*, 2011) [47, 44].

Spatial and temporal expansion and contraction of desert conditions in the Sahara appear to have acted as an important driver of faunal diversification and speciation events. Paleoclimatic cycles continually adjusted the boundaries between the desert and savannah environments and their associated biodiversity (Dumont, 1982; Le Houerou, 1992; 1997; Drake *et al.*, 2011) [48, 49, 50, and 44]. Vicariance events associated with Saharan aridity episodes become the main diversification force for post Pleistocene allopatry (Douady *et al.*, 2003; Nyari *et al.*, 2010) [51-52]. Such events are believed to have resulted in allopatric isolation, which in turn induced the interruption of gene flow and the evolution of independent lineages or new species.

The response of a give animal taxon to Saharan vicariant events varies according to the taxon's habitat requirements. During humid periods, desert-adapted animals become restricted to remaining desert habitat fragments, or the remaining arid core of the Sahara. In their isolation, they are likely to undergo morphologic and genetic allopatric diversification (Boratynski *et al.*, 2012) [53]. Animals of mesic habitats would disperse across the newly created mesic habitats expanding their geographical range and possibly undergoing sympatric speciation. If geographical distance and topography allow, free genetic flow will largely remain across a relatively wide geographical area.

During a subsequent arid episode, isolated populations of desert-adapted species will expand their ranges, possibly merging the different meta-populations into larger populations. If the previous allopatric divergence was not sufficient to result in reproductive isolation, genetic mixing will take place and a uniform population with a free gene flow will result. For animals of mesic habitats, populations will become fragmented with isolated populations becoming restricted to oases and mountain refuges where allopatric speciation may occur.

For species of mesic habitat requirements, overland route to across the Sahara during dry episodes were available via the green flood plains of the Nile and other river systems, which acted as a sustained migration conduit during Pleistocene arid periods (Said, 1993; Derricourt, 2005; Vermeersch, 2006) [54, 55, 56]. Through these drainage systems, or what remains of them during extremely dry periods, African fauna could reach the less arid Mediterranean coast to spread east across northern Sinai Peninsula to the Levant and hence the rest of the world, or west to African Atlantic coast and possibly across the Strait of Gibraltar to Europe. The same routes can allow Palearctic species of mesic habitat requirement to reach sub-Saharan Africa. The modern Nile Valley, Wadi Qena, Wadi Araba and Wadi El Arish route seems to have provided a near direct dispersion route between Africa and the Levant.

A period of increased aridity in North Africa extended for some 500,000 years during the Late Pliocene some 2.7 Mya. According to Almogi-Labin (2011) [45], climate change during that period is recognized as having profound influences on the evolution of vertebrates in Africa. Our molecular phylogenetic analysis suggests that this period

Table 3: Genetic distance based on 368 bp mitochondrial *cyt b* sequences between different *Canis* taxa.

Taxon and location	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
M00155 (Qalyubyah)	0.0																			
M00403 (Luxour)	0.0																			
M00407 (Sohaj)	0.0	0.0																		
M00409 (Faiyum)	0.0	0.0	0.0																	
M00163 (Qarra Oasis)	0.0	0.0	0.0	0.0																
M00166(Bahariya Oasis)	0.0	0.0	0.0	0.0	0.0															
M00169 (Baris Oasis)	0.0	0.0	0.0	0.0	0.0	0.0														
M00168 (Farafrah Oasis)	0.0	0.0	0.0	0.0	0.0	0.0	0.0													
C. anthus Mauritania	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0												
C. l. lupaster Senegal	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0											
C. l. lupaster Algeria	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0										
C. anthus Morocco	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0									
C. l. familaris EGY 1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1								
C. l. lupus.Saudi Arabia	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	0.0							
C. l. lupus Spain	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	0.0	0.0						
C. simensis Ethiopia	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	5.1	5.1	5.1					
C. aureus India	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	6.3	6.3	6.3	5.1				
C. latrans USA	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	6.0	6.0	6.0	4.8	5.4			
C. adustus Guinea	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	14.9	14.9	14.9	12.8	11.1	13.2		
Lycaon pictus Africa	12.9	12.9	12.9	12.9	12.9	12.9	12.9	12.9	12.9	12.9	12.9	12.9	15.3	15.3	15.3	15.3	13.9	13.6	14.6	0.0

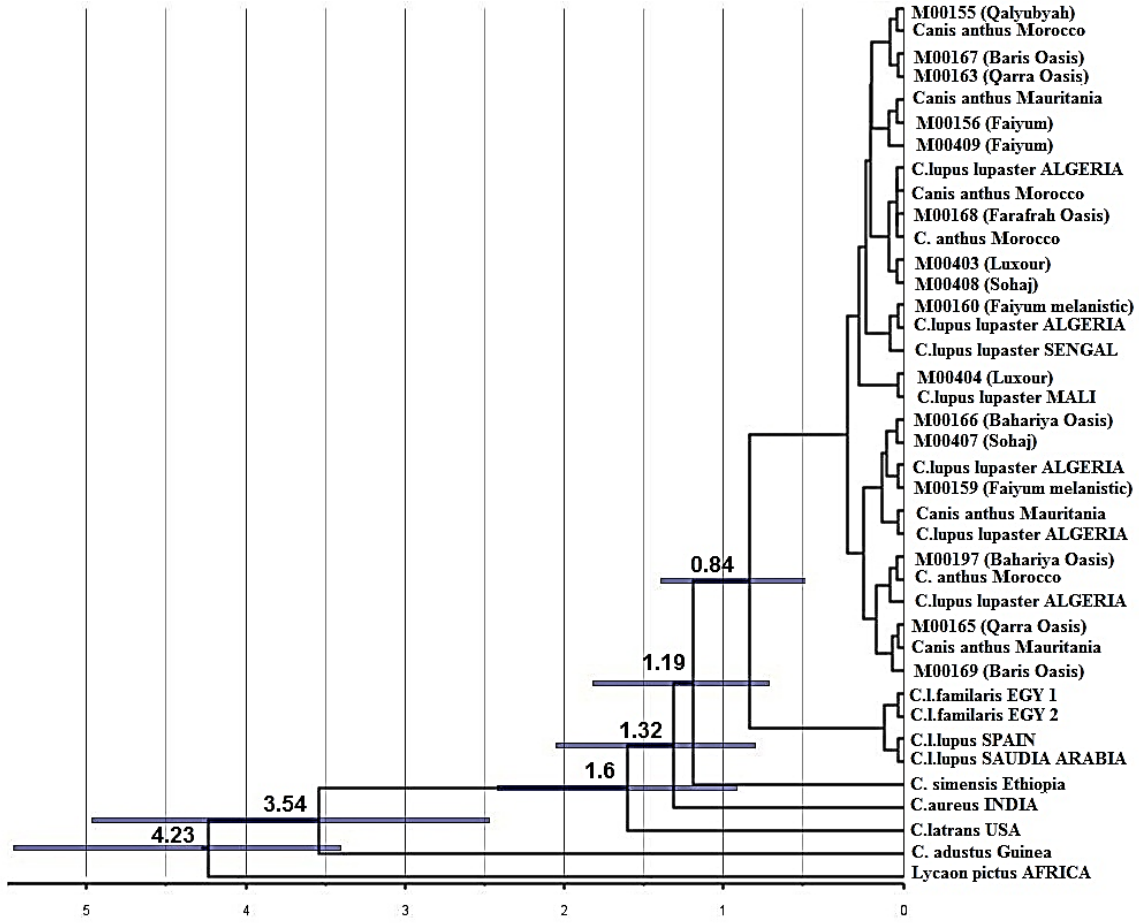

Figure 5: Phylogenetic tree based on 368 bp mitochondrial *Cyt b* sequence showing divergence time for *Canis* species of North Africa and the Middle East with *Lycaon pictus* as an out group. Bars on the nodes indicate the mean and range of divergence dates estimates in millions of years ago (Mya)

has witnessed the evolution of wolf-like canids in North Africa and Middle East, from which these species evolved to occupy diverse habitats in the Palearctic and the Sahel.

The period of increased aridity (Rea, 1994) [57], which followed between 2 and 1 Mya, was interrupted by two brief wet episodes. These pronounced climatic and consequently environmental changes led to faunal turnovers, the most significant of which in Africa occurred 1.8-1.6 Mya (Vrba, 1995; De Menocal, 2004) [58, 46].

Our data indicate that the clade consisting of *C. anthus/ lupaster* and *C. lupus* separated from its nearest relative clade of *C. aureus* about 1.32 Mya (0.8 to 2.05 Mya). This separation, which seems to have taken place in Asia or Europe, coincides with a major glaciation episode and the beginning of an arid spill in the Sahara and the Middle East. Ancestral *C. lupus/ C. lupaster* must have been able to disperse into newly formed, mesic habitats in the Green Sahara, presumably following favorable mesic habitat corridors along the Mediterranean coast, the southern margin of the Sahara or the river courses cutting across the Sahara. For the wolf-like ancestor with a high mobility, hopping from one favorable habitat patch to another across the semi-arid or even arid landscape would not have constituted a major problem. The divergence between these populations was further developed as the ancestral population dispersed over a very large geographical area.

According to our data, the *C. lupaster* phylogroup became separated from the nearest

phylogroup containing the grey wolf *C. lupus* and the domestic dog *C. l. familiaris* during late Pleistocene, about 0.84 Mya (0.45 - 1.39 Mya). This separation closely follows the onset of an arid episode (and northern latitude glacial intensification period) that lasted for almost one million years (1.8 – 0.8 Ma). During that period, northeast Africa was an arid landscape with no major rivers flowing. The desert conditions acted as a dispersion barrier preventing genetic flow between the *Canis* ancestral population in North Africa and its Levant counterparts, resulting in allopatric speciation leading to the evolution of the *C. lupaster* group.

It may be assumed that, during this long arid spill, *C. lupaster* population in North Africa began to follow the retreating rain lines north and south. Populations trapped in the expanding arid core of the Sahara broke down into fragmented populations isolated in oasis and river valley refugia where mesic conditions continued to prevail. Within at least some of the Saharan core refugia, isolated *C. lupaster* populations were evolving adaptations to arid conditions leading the several North African subspecies known today (Saleh and Basuony, 2014) [2].

This vicariance event appears to have resulted, by the end of this long arid spill of around 800 ka, in the allopatric, morphologic differentiation of *C. lupaster* population in the Mediterranean coastal desert and the northern region of the Western Desert and the appearance of the desert adapted *C. aureus qattarensis*. This subspecies appears to be particularly adapted to arid conditions of the maritime coastal desert and northern region of the western Desert. The subspecies appears to be unable to survive in the hyper arid hinterland of the Western Desert. The Egyptian wolf *C. lupaster*, however, continued to survive in isolated populations in the Nile Valley riverine oasis and in the larger oases of the Western desert where locally mesic conditions still prevail despite the overall hyper arid conditions.

The end of the dry spill has also witnessed the appearance of the Prenile. This vigorous river, with its headwaters in the Ethiopian and Central African highlands began flowing in Egypt after about one million years of absence of any major river system in northeast Africa. The flood plain of this river probably offered continuous mesic habitats across northeast Africa, which seems to have driven the evolution *C. lupaster*. About 400 ka later, the Prenile was replaced by the less significant Neonile. For about 400 ka this river was only intermittently connected to the Ethiopian highland, sometimes reduced to a seasonal stream with headwaters in the Red Sea Mountains. It appears that *C. lupaster* continued to be confined to the bank habitats of this river and continued its differentiation. The isolation of *C. lupaster* of today, remains in the modern Nile which came into existence about 12 ka.

The *C. lupaster* populations isolated in the Saharan oases seem to have been at least periodically connected to the rest of the North African populations, with which genetic desistance is extremely small. Uninterrupted genetic flow between the populations during the last Green Sahara period (ca. 10 ka BP) may have prevented the formation of separated mitochondrial lineages and the structuring in these populations in their present refugia. The absence of genetic differentiations suggests frequent re-connection and gene flow between these populations. These minor genetic differentiations, as suggested by the mitochondrial *Cyt b* sequence analysis, however, do not reflect the clear morphologic differentiations between the Nile Valley and other populations in arid North Africa.

Geological evidence suggests a possible corridor between Nile Valley and Kharga Depression via a major tributary of the now extinct Qena River near Toshka (Said, 1990; Issawi and McCauley, 1992) [59, 60]. The corridor seems to have connected the Nile Valley and Kharga Depression during the Quaternary when the successive stages of Nile replaced that part of Qena River. That possible corridor is marked with a number of oases and small uninhabited vegetation patches that seem to connect the great longitudinal hollow in what is now the Egyptian Western Desert to the Nile.

With the mid-Holocene advancement of aridity, the connections between Nile Valley and Kharga Depression were eventually severed, and the continuous habitats of this great depression broke down into isolated habitat patches that are entirely dependent on groundwater from artesian springs, creating the present-day oases of the New Valley (Said, 1990) [58]. These oases continued to retain ecological conditions similar to those of the Nile Valley, supported by an abundance of water from numerous springs. Fauna of the present-day oases of the Egyptian Western Desert, includes many Nile Valley elements, including *C. lupaster*, despite hundreds of kilometers of totally barren desert that separate these oases from the Nile Valley.

CONCLUSION

Analyses of cytochrome *b* (*Cyt b*) mitochondrial DNA sequences of different wolf populations represented different eco-geographical regions of Egypt and different eco-geographical regions from west, northwest Africa and the neighboring regions, clearly shows that the canids of Egypt, north and northeast Africa are nearly genetically identical without any genetic distances. This suggests that isolation among the different wolf populations in the regions was relatively recent or this piece of the cytochrome *b* gene is insufficient to explain these differences. Wheal the results clearly shows that the canids of Egypt, north and northeast Africa are distinct at the specific level from both *C. aureus* and *C. lupus*. The phylogeographic history of these wolves in Egypt and the region is discussed on the basis of the Quaternary climatic cycles and geomorphological changes affecting the Egyptian landscape.

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الملخص العربي :

تتناول هذه الدراسة العلاقات التطوريه الجينيه و علاقتها بالتوزيع الجغرافي للعشائر والانواع و ذلك في ضوء علم الوراثه الجزيئيه وهذا ما يعرف بالجغرافيا النسبيه. حيث تم التحقق من التعريف التصنيفي للعشائر المنعزلة من جنس كانيس في مصر باستخدام التحليل التطوري الجزيئي القائم على تحليل تتابعات الحمض النووي لجين سيتوكروم بي. تم الحصول على وفحص 18 عينة من أنسجة الذئاب التي تم جمعها من جميع المجتمعات المعروفة في مصر. وتم مقارنة تتابعات الحمض النووي لجين سيتوكروم بي الناتجة من عشائر/أصناف كانيس الحالية الموصوفة من القياسات المورفولوجية بعضها مع بعض، ومع عشائر/أصناف أخرى في شمال إفريقيا والشرق الأوسط. وأظهرت النتائج عدم وجود تمايز لهذه التتابعات قابل للتفريق بين جميع العشائر، مما يشير إلى أن الإنعزال بين عشائر الذئاب المختلفة في مصر كان حديثًا نسبيًا أو أن تلك القطعة من جين سيتوكروم بي غير كافية لإظهار تلك الفروق. كما تم مناقشة تقدير زمن الإنفصال بين العشائر المختلفة وتاريخ جغرافيا الأنساب لهذا الجنس في سياق إقليمي وجغرافي قديم.