

Original Research Paper

Phylogeographic study for the conservation of the genus *Gazelle*

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Abstract

The phylogeographic analyses obtained in this work were carried out using various software programs such as MEGA (Molecular Evolutionary Genetics Analysis) for the reconstruction of the phylogenetic tree, DnaSP (DNA Sequence Polymorphism Analysis of Large Data Sets) for the identification of haplotypes, Network for the evolution of species, with the use of various methods such as UPGMA, Kimura- 2 parameters; These analyses, applied to 66 cytochrome b sequences and 16 D-Loop sequences of mitochondrial DNA from 19 species belonging to the *Gazella* genus taken from different geographical regions, demonstrate the monophyly of the *Gazella* genus. Phylogeographic analysis methods, whether by bifurcation, haplotypes or network using MEGA, DnaSP and Network software, have shown the existence of two distinct clades in the genus *Gazella*.

Keywords: *Gazella*, cytochrome b, D-Loop, phylogeography, software.

الخلاصة

تم إجراء التحليلات الجغرافية التطورية التي تم الحصول عليها في هذا العمل باستخدام برامج مختلفة، واحد لإعادة بناء الشجرة التطورية، وآخر لتحديد النمط الوراثي، وواحد لتطور الأنواع، مع استخدام أساليب مختلفة؛ أظهرت هذه التحليلات، المطبقة على 66 تسلسلاً من السيتوكروم ب و 16 تسلسلاً من منطقة التحكم في الحمض النووي للميتوكوندريا لـ 19 نوعاً تنتمي إلى جنس غزير والتي تم جمعها في مناطق جغرافية مختلفة، أن جنس الغزال أحادي الفصيلة. كما أظهرت طرق التحليل الجغرافي، سواء عن طريق التشعب أو النمط الوراثي أو الشبكة، وجود مجموعتين متميزتين في جنس الغزال

الكلمات الرئيسية: غزير، سيتوكروم ب، ميتوكوندريا، التحليل الجغرافي، برامج.

Introduction

Bioinformatics is a multidisciplinary field that combines biology, computer science and mathematics. The recent rapid development of genomics and proteomics has led to increasingly close collaboration between specialists in the life sciences and computer science, in order to develop new approaches and new analytical methods for examining the massive amount of biological data available. For phylogenetic tree reconstruction (Darlu et al., 1993; Lecointe and Le Guyader, 2001), there are a number of limits to the use of bioinformatics tools. This reconstruction is based on the fact that some characters are inherited from one species to another, while others have been transformed over time, leaving traces of evolution. The evolution of species is understood as a divergent process, most often modelled in the form of a tree whose leaves represent contemporary species and whose nodes represent ancestral species. This is known as an evolutionary tree or, more commonly, phylogeny, and phylogeography governs the distribution of genealogical lineages (Avise, 1998; Kidd and Ritchie, 2006).

In order to conserve them, a number of studies have been carried out on gazelles over the last decade (Bärmann et al., 2013). Gazelles are mammals in the bovid family and comprise four genera: cattle, goats, sheep and antelopes (Lerp et al., 2013). The Antilopinea tribe currently comprises 13 genera: *Raphiceros*, *Ourebia*, *Madoqua*, *Dorcatragus*, *Saiga*, *Litocranius*, *Ammodorcas*, *Antidorcas*, *Procapra*, *Eudorcas*, *Nanger*, *Antelope* and *Gazella* (Effron et al., 1976; Gentry, 1992; Rebholz et al., 1999; Groves, 2000; Grubb, 2005; Groves et al., 2011; Hassanin et al., 2012). The genus *Gazella* (Bovidae, Artiodactyla) is one of the most common taxa in the fauna, widely distributed in Eurasia and Africa (Arambourg, 1959; Gentry, 1970; 2010); and one of the most species-rich genera in horned ruminants,

despite the overall similarity in body size and morphology (Bärmann et al., 2013). Many gazelle species are endangered or threatened due to hunting, habitat loss and competition with domestic livestock (Cloudsley-Thompson, 1992; East, 1988; 1990; 1992; IUCN, 1988; Loggers et al., 1992; Ryder, 1987; Saleh, 1987). The IUCN has classified several species of the genus *Gazella* as extinct in the wild and their hunting is prohibited. With the aim of conserving this genus, we carried out this work based on phylogeographic analyses using several bioinformatics software packages to find the representative tree and the position of different species of the genus *Gazella*.

Material and methods

Our study utilized already published sequences obtained through Polymerase Chain Reaction (PCR) (Kleppe et al., 1971) and sanger sequencing. The researchers who published the results collected the samples, extracted the DNA, amplified a targeted DNA fragment, and sequenced that fragment. These sequences, generated by various research laboratories globally, were then submitted to biological data repositories such as GenBank, ensuring the data is freely accessible to researchers worldwide (Sayers et al., 2020).

We extracted all available DNA sequences of the *Gazella* genus from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). Subsequent analysis and classification of these sequences were performed using multiple molecular data processing software tools (Derouiche et al., 2024).

FaBox (Toolbox for Fasta Sequences): A collection of simple, intuitive web services designed for quick tasks involving sequence data, enabling biologists and medical researchers to efficiently process and analyse sequences (Villesen, 2007).

MEGA (Molecular Evolutionary Genetics Analysis): This software provides integrated tools for statistical analysis of DNA and protein sequence data from an evolutionary perspective. It constructs sequence alignments and phylogenetic trees, allowing the application of evolutionary bioinformatics methods in biology, biomedicine, and evolution (Tamura et al., 2011; Yoann, 2012).

DnaSP (DNA Sequence Polymorphism Analysis of Large Data Sets): A bioinformatics tool designed for comprehensive analysis of DNA sequence variation, facilitating detailed polymorphism studies (Rozas et al., 2017).

Network Software: Utilized for reconstructing phylogenetic networks, this software infers ancestral types, potential evolutionary paths, branching patterns, and estimates datings. It generates evolutionary trees and networks from genetic, linguistic, and other data (Huson and Bryant, 2012; Forster and Forster, 2020).

Results and discussion

I. GenBank results

19 species belonging to the genus *Gazella*, retrieved from GenBank. The species used in these phylogeographic analyses are: *G. arabica*, *G. bennetti*, *G. cuvieri*, *G. dorcas*, *G. leptoceros*, *G. marica*, *G. saudiya*, *G. spekie*, *G. subguttorsa*, *G. gazella*, *G. dorcas pelzelinii*, *G. dorcas x leptoceros*, *G. erlangeri*, *G. gazella muscatensis*, *G. subgutturosa marica*, *G. subgutturosa sairenses*, *G. subgutturosa subgutturosa*, *G. subgutturosa yarkandensis*, *G. sp.* located in different geographical regions. There are several sequences that are 100% identical, so we will only consider one representative sequence (Table I).

From the table above, we can see that there are 214 genomic DNA sequences and 1026 mitochondrial DNA sequences, mainly cytochrome b and the D-Loop control region. Our study is therefore based on mitochondrial DNA (cytochrome b, D-Loop (Rustin and Rötig, 1995) in order to establish links of relationship between species of the genus *Gazella*; bearing in mind that, in general, mutation rates in mitochondrial DNA are greater than those in nuclear DNA, mitochondrial DNA has been widely used as a tool for deciphering the evolutionary and demographic history of populations and species (Ballard et al., 2004).

II. Phylogeographic analyses

1. Construction of phylogeographic trees using MEGA

The phylogeographic tree was constructed using the MEGA program. Firstly, we selected representative sequences of cytochrome b and D-Loop based on their geographical origins.

Table I: Nuclear and mitochondrial DNA sequences of the genus *Gazella* extracted from GenBank.

Species	Total number of sequences	Nuclear DNA	Mitochondrial DNA	Cytochrome b sequences	D-Loop sequences	sequences
<i>G. arabica</i>	77	34	43	26		/
<i>G. bennetti</i>	73	28	45	34	0	/
<i>G. cuvieri</i>	24	12	12	7	2	/
<i>G. Dorcas</i>	299	49	249	155	3	1
<i>G. dorcas pelzelni</i>	1	0	1	1	0	/
<i>G. dorcas x leptoceros</i>	21	1	20	20	0	/
<i>G. erlangeri</i>	2	0	2	0	0	/
<i>G. gazelle</i>	336	19	317	162	91	/
<i>G. gazella gazelle</i>	3	0	3	1	1	/
<i>G. gazella muscatensis</i>	1	0	1	1	0	/
<i>G. leptoceros</i>	30	13	13	10	0	4
<i>G. saudiya</i>	10	0	4	9	0	/
<i>G. spekei</i>	29	21	8	5	0	/
<i>G. subgutturosa</i>	143	19	116	69	17	8
<i>G. subgutturosa marica</i>	94	18	76	33	21	/
<i>G. subgutturosa sairenses</i>	4	0	4	0	4	/
<i>G. subgutturosa subgutturosa</i>	76	0	76	0	76	/
<i>G. subgutturosa yarkandensis</i>	20	0	20	0	20	/
<i>G. sp.</i>	18	0	10	10	0	8
Total	1261	214	1026	543	634	21

Cytochrome b

- *G. arabica*: In total, there are 6 sequences, 3 sequences from Saudi Arabia, 2 sequences from Palestine and one sequence from Jordan. We chose 3 sequences from Saudi Arabia of different sizes (between 336 bp and 1149 bp), one sequence from Palestine (1132 bp) and one from Jordan (1195 bp).
- *G. bennettii*: 12 sequences in total, 5 sequences from India in size (204 bp to 751 bp), 2 sequences from Pakistan (300 bp and 1149 bp), 2 sequences from the Arabian Peninsula (333 bp and 375 bp), 2 sequences from Saudi Arabia (1149 bp and 1018 bp) and a single sequence from Iran (410 bp). We chose a single sequence from India and one from Pakistan of size (415 bp) and (1149 bp) respectively, the 2 sequences from the Arabian Peninsula (333 bp and 375 bp), 2 sequences from Saudi Arabia (1149 bp and 1018 bp) and the sequence from Iran of (410 bp).
- *G. cuvieri*: We will take all the sequences in this genus, a single sequence from the Arabian Peninsula (333 bp), one from North Africa (300 bp) and 2 sequences from Spain (1095 bp and 1149 bp).
- *G. dorcas*: 17 sequences were found, including 2 sequences from Morocco (375 bp and 716 bp), 2 sequences from North Africa (300 bp and 450 bp), 2 sequences from Algeria (1152 bp) and a single sequence from: Mali (1152 bp), Sudan (1152 bp), Qatar (1152 bp), Saudi Arabia (336 bp), Arabian Peninsula (336 bp), Germany (336 bp), Chad (1152 bp), Tunisia (1152 bp) and 3 sequences from Palestine (790 bp to 1152 bp), only 2 sequences were eliminated, one from Morocco of 375 bp and the other from Palestine of 790 bp.
 - *G. dorcas pelzelni*: A single Qatar sequence with a size of 1140 bp.
 - *G. dorcas x leptoceros*: A single sequence from North Africa with a size of 450 bp.
 - *G. gazella*: We found 12 sequences including 3 sequences from Palestine of different sizes (790 bp to 1149 bp), we chose a single sequence of 1149 bp, 2 sequences from Saudi Arabia (336 bp and 1152 bp), we chose the sequence of 1152 bp, a single sequence for the following countries: Turkey of 400 bp, North Africa (300 bp), Arabian Peninsula (375 bp), Dubai (1011 bp), Germany (360 bp), Qatar (981 bp) and Eurasia (1134 bp).
 - *G. gazella gazella*: A single sequence from Germany, size 336 bp.
 - *G. gazella muscatensis*: A single sequence from the Arabian Peninsula of size 375 bp.

- *G. leptoceros*: We will take all the sequences from this species. In total we have 7 sequences including 2 sequences from the Arabian Peninsula (333 bp and 375 bp), a single sequence from each of the following countries: North Africa (300 bp), Tunisia (1025 bp), Egypt (1104 bp), Algeria (1152 bp), and the Czech Republic (1140 bp).
- *G. saudiya*: We have a total of 3 sequences, including one from North Africa at 300 bp, and 2 sequences from Saudi Arabia at 375 bp and 404 bp. We took the North African sequence and a single Saudi Arabian sequence of 404 bp.
- *G. spekei*: We have 2 sequences, one from North Africa (300 bp) and the other from Dubai (1149 bp).
- *G. subgutturosa*: In total we have 14 sequences including: 2 sequences from Mongolia (375 bp and 1083 bp), a single sequence from the following countries: Eurasia (1143 bp), Azerbaijan (333 bp), Turkestan (332 bp), Arabian Peninsula (375 bp), North Africa (300 bp), Qatar (1128 bp), China (1143 bp). 4 sequences from Iran of different sizes (258 bp to 470 bp). Mongolia (375 bp) and Iran (333 bp) were eliminated.
- *G. subgutturosa marica*: We have a total of 6 sequences, one from Saudi Arabia (1116 bp), one from Oman (333 bp), one from the UAE (333bp) and one from the Arabian Peninsula (375 bp).
- *G. sp.*: There are 2 sequences, one from the Arabian Peninsula (375 bp) and the other from Iran (383 bp).

Based on the size and origin of the DNA sequences, we studied 70 selected cytochrome b sequences for phylogeographic analysis.

We used Fabox to convert our sequence file into Fasta format in order to organise the azotic bases that form a single-line sequence.

The phylogeographic tree was constructed using the UPGMA (Unweighted pair group method with arithmetic mean) method. The distance used is that of Kimura-2p (= 2 parameters) which takes into account the fact that transitions and transversions are not equiprobable during the evolution of sequences, particularly for mitochondrial DNA where transitions can represent 90% of mutations (Kimura, 1980). In biological reality, transitions account for the majority of nucleotide substitutions. So, when the genetic distance between two sequences increases, the number of transitions and transversions increases proportionally, with the number of transitions always being greater. However, in the case of increasingly distant sequences, saturation of substitutions can be reached and transversions become more numerous than transitions. 1000 bootstrap replications are required for this method to be statistically valid. The relationships between *Gazella* cytochrome b sequences were represented using a tree created with the MEGA programme (Figure 1). The names of the sequences represented in our tree are the names given by GenBank.

Correction of the raw tree

We noticed in the tree obtained that there are certain species that are not in their correct clades (*G. gazella*, *G. saudiya*, *G. leptoceros*, *G. subgutturosa*). These four sequences are published by Rebholz and Harley (1999) who base the identification and naming of the species on the morphology of the animals, so we completely eliminated the 4 sequences. Thanks to our tree (Figure 1) we were able to identify the two *Gazella sp* sequences, according to their position in the tree, *G. sp.* from the Arabian Peninsula (AF187721) is part of the species *G. subgutturosa* and *G. sp.* from Iran (KR873132) is part of the species *G. bennettii*. In the end, we obtained a corrected tree with 66 cytochrome b sequences (Figure 2).

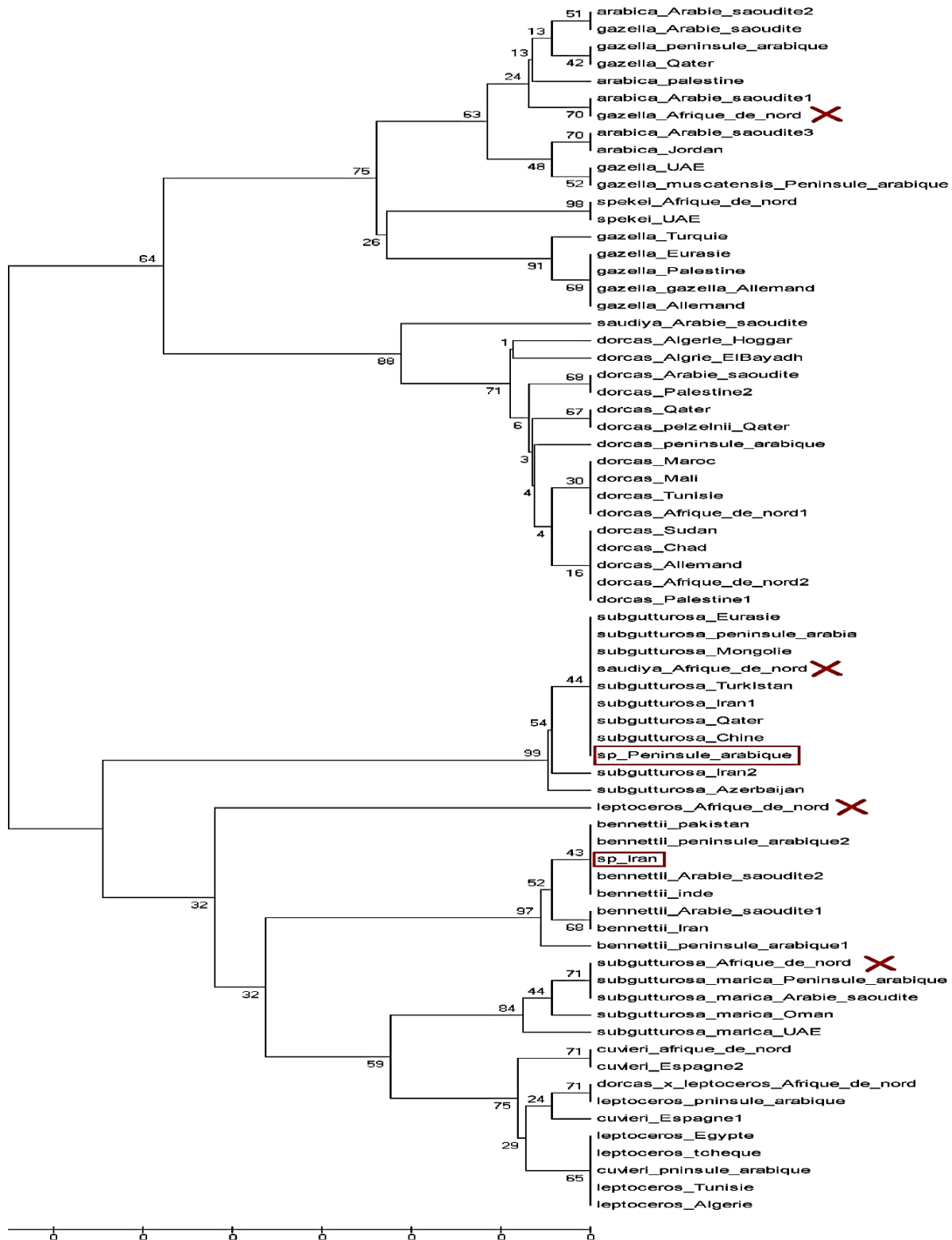


Figure 1: Phylogeographic tree reconstructed using the UPGMA method based on 70 cytochrome b sequences from the *Gazella* genus using MEGA.

A phylogeographic reconstruction is then made from each chimeric alignment generated, and the value associated with each node in the tree corresponds to the number of times out of 1000 replicates that the same topology was found.

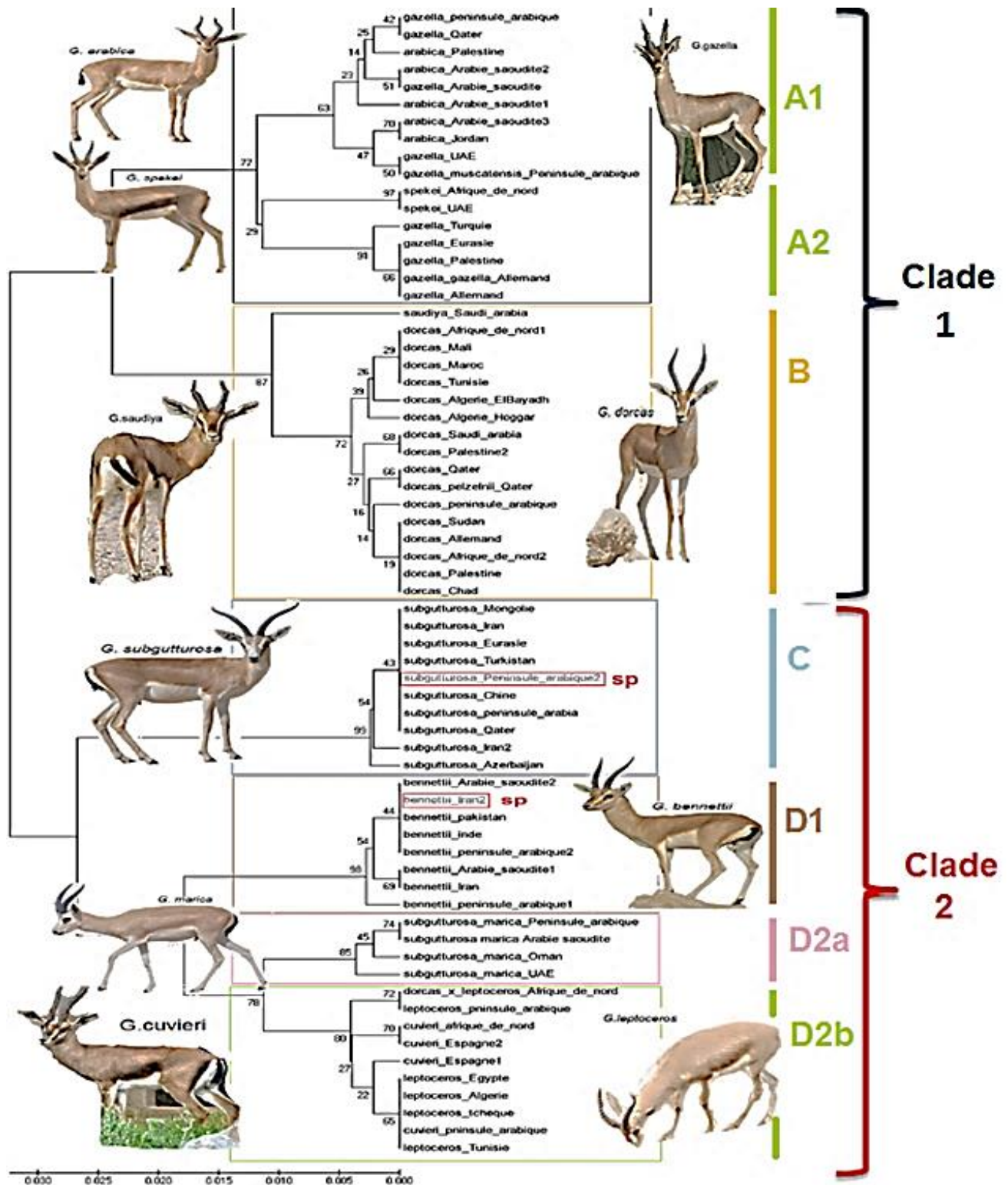


Figure 2: Final *Gazella* genus tree based on 66 cytochrome b sequences using the UPGMA method in the MEGA computer suite.

According to the tree, cytochrome b sequences fall into 2 main clades:

Clade 1: This includes *G. arabica*, *G. spekei*, *G. gazella*, *G. dorcas* and *G. saudiya*. This clade is subdivided into two sub-clades :

- ✓ Subclade A (A1 and A2): Includes the species *G. arabica*, *G. spekei*, *G. saudiya* and the subspecies *G. gazella gazella* and *G. gazella muscatensis*.
- ✓ Subclade B: Includes *G. dorcas*, *G. saudiya* and the subspecies *G. dorcas pelzelinii*.

Clade 2: This includes *G. subgutturosa*, *G. bennettii*, *G. leptoceros*, *G. cuvieri* and *G. subgutturosa marica*. This clade is subdivided into 2 sub-clades :

- ✓ Subclade C: This is represented by the only species corresponding to *G. subgutturosa*.
- ✓ Sub-Clade D: Also subdivided into:
 - D1: It is represented only by the species corresponding to *G. bennettii*.
 - D2a: Corresponds to *G. subgutturosa marica*.
 - D2b: Contains the species *G. leptoceros*, *G. cuvieri* and *G. dorcas x leptoceros*.

In general, the tree obtained in this section shows that *Gazella* is monophyletic (Wronski et al., 2010). The absence of nodes means that there is no clear separation between species, so they are very closely related.

We used the Blast programme to compare and vitrify our results. Blast identifies regions of local similarity between sequences, compares nucleotide or protein sequences and calculates the significance of the results. The sequences are ranked according to a 'score' that depends on their homology with the query sequence, the size of the library and the value of the 'E-Value'. The smaller the E-Value, the greater the homology between the query sequence and the sequence in the library. Using Blast, we checked whether the sequences in the clades are really close; we compared the sequences of *G. arabica* and *G. gazella* from A1 in clade 1. The homology between these sequences is 99%. We also compared the two sequences of *G. sepkie* with the sequences of *G. gazella* and A2 from clade 1. These have a similarity of 97%. This result shows that these sequences are homologous. We also compared the Saudi Arabian sequence of *G. saudiya* with the other sequences of *G. dorcas*. The latter have a similarity of 98% with the *G. dorcas* sequence, and the results show that these sequences are also homologous.

D-Loop

- *G. arabica*: We found 2 sequences, one from Saudi Arabia with a size of 337 bp, and one from Germany with a size of 307 bp.
- *G. cuvieri*: A single sequence from Spain with a size of 784 bp.
- *G. dorcas*: We have 2 sequences, one from Saudi Arabia (250 bp) and the other from Germany (268 bp).
- *G. gazella*: We found 3 sequences, 2 from Germany (234 bp and 211 bp) and a single sequence from Saudi Arabia (366 bp).
- *G. gazella gazella*: A single sequence from Germany, size 362 bp.
- *G. subgutturosa*: A single sequence from China, size 984 bp.
- *G. subgutturosa marica*: We have 2 sequences, one from Saudi Arabia with a size of 358 bp and the other from Saudi Arabia with a size of 1106 bp.
- *G. subgutturosa sairenses*: A single sequence from China with a size of 785 bp.
- *G. subgutturosa subgutturosa*: We found 2 sequences, one from Azerbaijan with a size of 980 bp and the other from Uzbekistan with a size of 973 bp.
- *G. subgutturosa yarkandensis*: A single sequence from China, size 785 bp.

The *Gazella* genus phylogenetic tree was created using 16 sequences from the control region (D-Loop) of mitochondrial DNA (Figure 3).

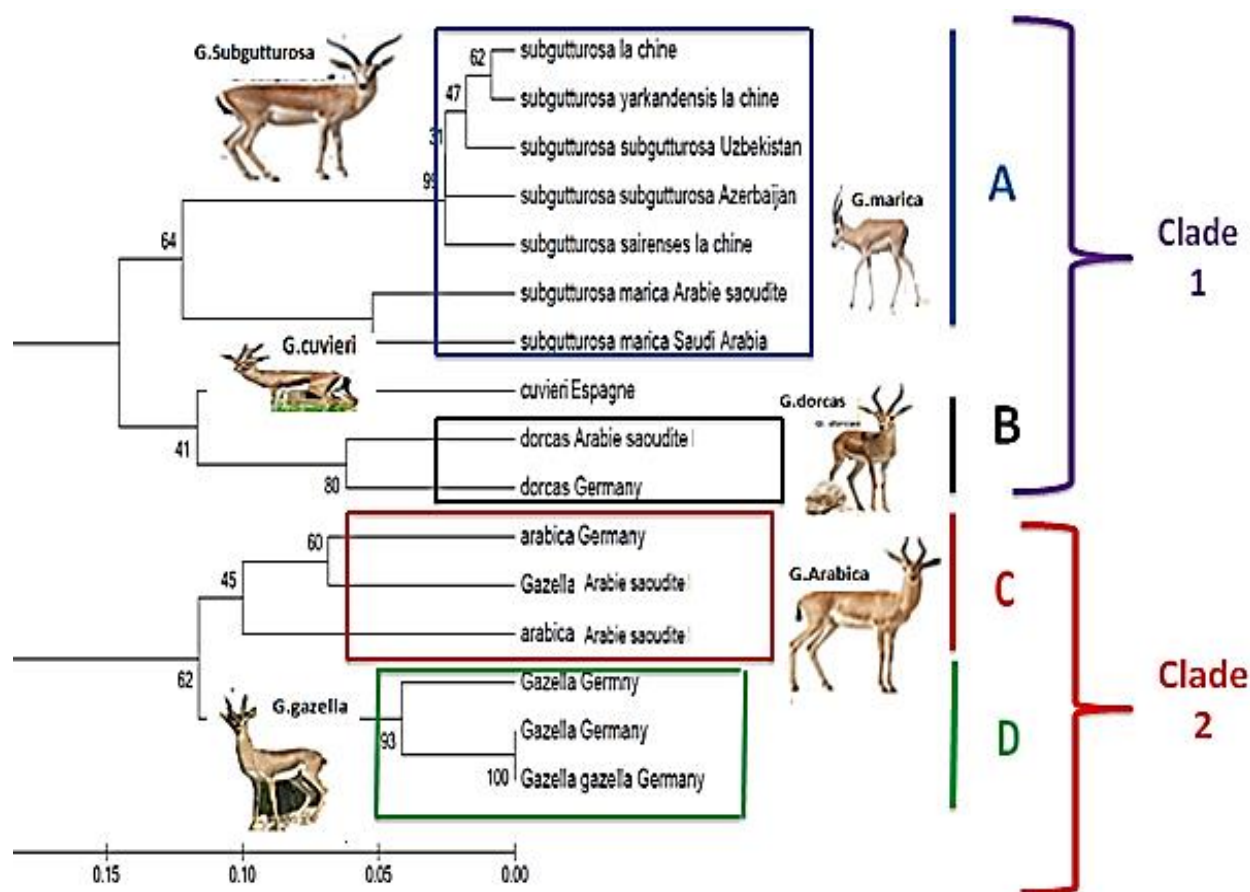


Figure 3: Phylogeographic tree of the *Gazella* genus based on 16 D-Loop sequences using the UPGMA method with MEGA software.

According to the tree, D-Loop sequences fall into 2 main clades:

Clade 1: Includes the majority of species: *G. subgutturosa*, *G. subgutturosa yarkandensis*, *G. subgutturosa subgutturosa*, *G. subgutturosa sairenses*, *G. subgutturosa marica*, *G. cuvieri*, *G. dorcas*. This clade is subdivided into 2 sub-clades:

- ✓ Subclade A : Includes *G. subgutturosa marica*, *G. subgutturosa sairenses*, *G. subgutturosa subgutturosa* and *G. subgutturosa yarkandensis*.

We used the Blast server to compare the sequences of clade A1 with those of the GenBank database. This method enabled us to obtain the percentage of similarity between the sequences. The Blast results (Table II) show that there is similarity between the subspecies.

Table II: Tables showing the percentage of similarity between sequences using Blast.

	<i>G. subgutturosa yarkandensis</i>	<i>G. subgutturosa subgutturosa</i>	<i>G. subgutturosa sairenses</i>	<i>G. subgutturosa marica</i>
<i>G. subgutturosa yarkandensis</i>	100 %	99 %	100 %	100 %
<i>G. subgutturosa subgutturosa</i>	99 %	100 %	100 %	100 %
<i>G. subgutturosa sairenses</i>	100 %	100 %	100 %	70 %
<i>G. subgutturosa marica</i>	100 %	100 %	70 %	100 %

- ✓ Subclade B: In total we have 2 sequences of the *dorcas* species from Saudi Arabia and Germany.
- Clade 2: Comprising the species *G. arabica* and *G. gazella*, this clade is subdivided into 2 sub-clades:
- ✓ Subclade C: In total, there are 2 sequences of the *G. arabica* species and one sequence of *G. gazella*.

✓ Sub-clade D: We have 2 sequences of the species *G. gazella* and one sequence of *G. gazella gazella*.

We used Blast to compare the sequences of *G. gazella* with a sequence of *G. arabica*, the latter having a similarity of 90% with the sequence of *G. gazella*.

Interpretation of the phylogenetic relationships between the different species of the genus *Gazella*

Gazelles are identified as the most taxonomically complex group of bovids (Groves, 1967; 1997). This problem is largely caused by considerable intraspecific variation being confused with interspecific similarity, particularly in characters such as horn shape, body size and coat colouration (Groves, 1996).

According to the global cytochrome b and D-Loop trees of the *Gazella* genus, we have two major clades: (1) an Asian clade (*G. bennettii*, *G. subgutturosa*, *G. marica*, *G. leptoceros* and *G. cuvieri*) and (2) an African clade (*G. saudiya*, *G. dorcas*, *G. spekei*, *G. gazella*, and *G. arabica*).

- *G. marica* (Thomas, 1897), was included in *G. leptoceros* by Ellerman and Morrison-Scott (1951). Later, *G. marica* was considered to be a subspecies of *G. subgutturosa* on the basis of morphological similarities (Groves, 1967; Kingswood et al., 1996 a, b; 1997).
- Early classifications place *G. bennettii* as a subspecies of *G. gazella* (Haltenorth and Diller, 1977; Roberts, 1977) or as a subspecies of *G. dorcas* (Gentry, 1964; Groves, 1969; Lange, 1972).
- Karyology data have shown that *G. bennettii* is independent of *G. gazella* (Furley et al., 1988; Kumamoto et al., 1995).
- The taxonomic classification of *G. cuvieri* and *G. leptoceros* remains confusing. Lange (1972) classified *G. cuvieri* under *G. gazella* and *G. leptoceros* were considered a subspecies of *G. subgutturosa*.
- However, Hassanin et al (2012) suggest that *G. marica* and *G. leptoceros* are subspecies of *G. cuvieri* because of their mitochondrial divergence.
- Several subspecies of *G. dorcas* have been described on the basis of phenotypic variation, such as coat colouration and horn shape and length (Groves et al., 2011; Groves, 1981; 1969; Alados, 1987; Yom-Tov et al., 1995).
- A phylogeographical study based on mitochondrial sequence variation of cytochrome b and control region indicates that *G. dorcas* comprising *G. saudiya* and *G. pelzelni* represents a reciprocally monophyletic group with sister-group relationships to *G. gazella* and *G. arabica* (Lerp et al., 2011).

2. Haplotype determination by DnaSP

We used the DnaSP to determine the haplotypes (Table III); the term haplotype corresponds to a nucleotide sequence, which may be common to several individuals, but differs from other haplotypes by one or more nucleotide substitutions.

Cytochrome b: Analysis of cytochrome b reveals very high genetic diversity; the 66 individuals studied enabled 30 different haplotypes to be characterised. 13 private haplotypes were detected (a private haplotype is one that is only found in a single population). Haplotypic and nucleotide diversity were therefore very high within our limited sample ($H_d = 0.9622$).

D-Loop: We used the FaBox software online to align the sequences, which simply removes the border zones that are full of gaps in order to use alignment result in the DnaSP software. Of the 16 D-Loop sequences of the *Gazella* genus obtained, 15 haplotypes could be identified and 14 private haplotypes were detected. Each haplotype contains a single sequence, except for haplotype 7, which contains 2 sequences.

We have noted that the results obtained by the DnaSP confirm the MEGA results, and the analyses of mitochondrial polymorphism provide interesting clues as to the history of the geographical expansion of each species. Relationships between individuals, haplotypes or species have previously been visualised through the construction of trees, by agglomerating the nearest neighbours (Saitou et al., 1987). The higher the haplotypic diversity within the sample, the greater the chance of observing different haplotypes when 2 individuals are selected at random (Nei, 1987). Nucleotide diversity measures the average number of differences between 2 randomly selected sequences in the sample (Nei and Li, 1979).

Table III: Comparison of diversity indices calculated with DnaSP on the samples studied.

Population	Clade	Size of the sample	Number of haplotypes h	Diversity haplotipic Hd	Number of polymorphic sites S	Variable number of sites
Cytochrome b	1	66	17	0,9622	1186	41
	2		13			
D-Loop	1	16	10	0.9917	1123	65
	2		4			

3. Haplotype networks built by Network

Haplotypes, which can be used for traceability analyses, but also to study the evolution of the species. Relationships between haplotypes can also be understood by constructing haplotype networks. These haplotype networks are constructed using Network

Cytochrome b: The results are shown in Figure 4.

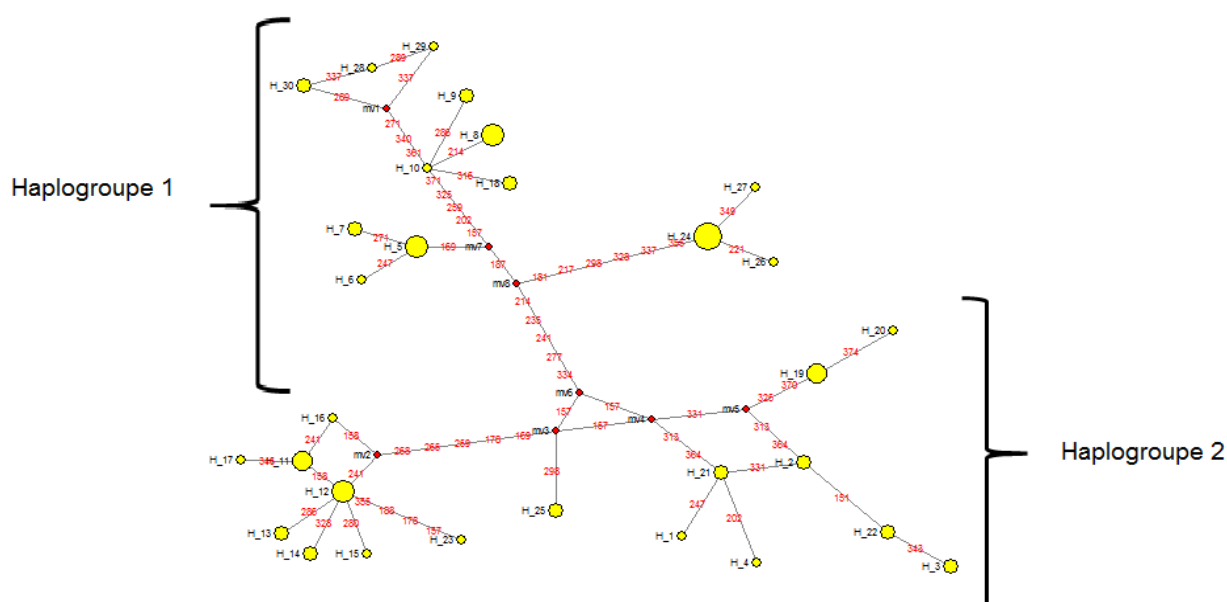


Figure 4: Haplotypic network produced by Network software from 66 *Gazella* cytochrome b sequences.

The construction of these networks is based on the theory of coalescence (Kingman, 2000). This theory consists of a retrospective approach that mathematically describes the process of binary fusion of all the genealogical lineages of a sample of genes back to their closest common ancestor. In the haplotype network, each circle corresponds to a haplotype, with the size of the circle proportional to the frequency of the haplotype in the dataset. The length of the segments between each haplotype is proportional to the number of mutations separating them. Of the 66 *Gazella* cytochrome b sequences obtained; 30 haplotypes were identified. The structure of the network (Figure 4) appears to be more complex, with many divergent haplotypes.

Haplogroup 1 corresponds to the Asian group: *G. bennettii*, *G. subgutturosa*, *G. marica*, *G. leptoceros* and *G. cuvieri*, for the following haplotypes: H5, H6, H7, H8, H9, H10, H18, H24, H26, H27, H28, H29, H30.

Haplogroup 2 corresponds to the African group: *G. saudiya*, *G. dorcas*, *G. spekei* and *G. gazella* for the following haplotypes: H1, H2, H3, H4, H11, H12, H13, H14, H15, H16, H17, H19, H20, H21, H22, H23, H25.

D-Loop: The results are shown in Figure 5.

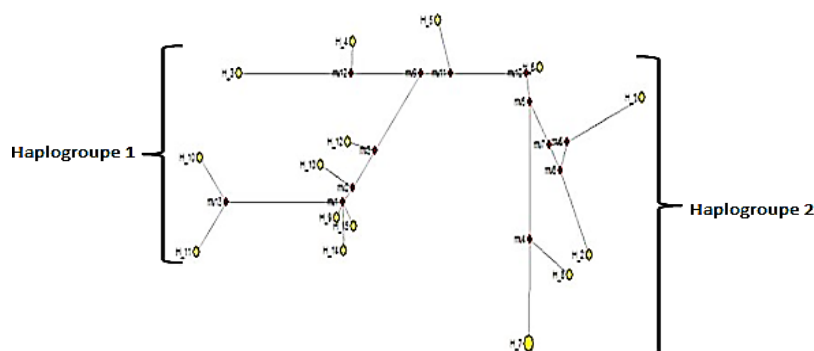


Figure 5. Growth of haplotypes of D-Loop sequences of the genus *Gazella* using Network software.

According to the network structure (Figure 5), the 16 D-Loop sequences fall into 2 main haplogroups:

Haplogroup 1 corresponds to the Asian group: *G. subgutturosa yarkandensis*, *G. subgutturosa subgutturosa*, *G. subgutturosa sairenses*, *G. subgutturosa marica*, *G. cuvieri*, *G. dorcas*, for the following haplotypes: H3, H4, H5, H9, H10, H11, H12, H13, H14, H15.

Haplogroup 2 corresponds to the African group: *G. arabica*, *G. gazella*, *G. gazella gazella*, for the following haplotypes: H1, H2, H6, H7, H8

The results obtained from the haplotypic networks of the two genes, cytochrome b and D-Loop, showed the existence of two major haplogroups representing the African clade and the Asian clade. The more different two homologous sequences are, the greater the evolutionary distance separating them, and therefore the longer ago the sequences diverged. Conversely, the closer two homologous sequences are, the smaller the evolutionary distance between them (Saitou and Nei, 1987). We have observed a high genetic distance between species of the genus *Gazella*, and this growth has taken place over a relatively short period.

Conclusion

The decline in gazelle numbers is worldwide, so there is an urgent need for researchers to determine their conservation units and describe the phylogeographical relationships between species in this genus. The genus *Gazella* is a complex genus, with species occurring in Africa, the Middle East and Asia. In general, gazelles are characterised by pronounced phenotypic variability that is not always reflected by molecular divergence of DNA sequences; some of this variation may be due to phenotypic plasticity. Phylogenetic reconstruction is a tool used in fields as diverse as taxonomy, epidemiology and population genetics. It is an essential procedure for tracing the evolutionary history of organisms at all taxonomic levels. This analysis is also of great importance for clarifying the evolutionary patterns of species and for understanding adaptive evolution at the morphological and molecular levels. The phylogeographic analyses obtained in this work were carried out using the MEGA, DnaSP and Network software packages. We have noted that the results obtained by DnaSP confirm the results of MEGA, and the analyses of mitochondrial polymorphism provide interesting clues as to the history of the geographical expansion of each species. Relationships between individuals, haplotypes or species were previously visualised via the construction of trees, by agglomerating the nearest neighbours. We also noted that the results obtained by the Network confirm the MEGA results, showing the existence of two haplogroups for the cytochrome b and D-Loop genes, representing the African clade and the Asian clade. Phylogeographic analysis methods using MEGA, DnaSP and Network software have shown the existence of two distinct clades for the *Gazella* genus: the Asian clade and the African clade.

Author's Contributions

Author 1: Data analysis, drafting the article.

Author 2: Data analysis.

Author 3: Data analysis.

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