Journal of Molecular and Genetic Medicine

Salih et al., J Mol Genet Med 2018, 12:4
DOI: 10.4172/1747-0862.1000379

Research Article Open Access

Phylogenetic Relationships of Sudanese *Gazella dorcas* Based on Mitochondrial Cytochrome-B Gene Sequences

Salih RRM*, Imad-Eldain ETA and Abd-Elrahim EK

Department of Clinical Medicine, Faculty of Veterinary Medicine, University of Khartoum, Shambat, Bahry, Khartoum, Sudan

Abstract

Dorcas gazelles are critically endangered mammals on the Arabian World. Past conservation efforts have been plagued by confusion about the phylogenetic relationship among various 'phenotypically discernable' populations, and even the question of species boundaries was far from being certain. This lack of knowledge has had a direct impact on conservation measures. Here, we provide a phylogenetic framework, based on the analysis of mtDNA sequences. We applied molecular methods to document for the first-time patterns of genetic diversity and population structure of *Gazella dorcas* in Bahry and East of the Nile (Sudan) using 421 bp fragment of mitochondrial DNA in seventeen populations.

Keywords: Mitochondrial DNA; Cytochrome-b gene; DNA extraction

Introduction

The *Dorcas gazelle* (*Gazella dorcas*), which was once common throughout peri-Saharan North Africa, is no exception in this regard. *Dorcas gazelles* are thought to exist in a wide variety of habitats, from Sahelan savannahs to semi-arid gravel and sand deserts, while avoiding hyper arid areas and the upper elevations of the central-Saharan massifs [1-11]. Conservation efforts for *Dorcas gazelles* in different countries include the prohibition of hunting and establishment of protected areas. Most habitats across the species' natural range have not been degraded through over exploitation, so reintroduction programs remain a feasible option given that poaching is prevented. Various breeding centers have started actions to preserve *Dorcas gazelles*. Several subspecies of *Dorcas gazelles* were described on the basis of phenotypic variation, such as fur coloration, horn shape and length, and other morph metric measures, but genetic differentiation is generally not well documented, and the presumed taxa seem to show no obvious ecological differences congruent with the proposed taxonomic classification [12-16].

Mitochondrial DNA (mtDNA) is a good target for phylogenic reconstruction at several taxonomic levels. Phylogenic approaches normally use sequences from a single gene such as mitochondrial cytochrome- b gene, which is utilized for species and family level analysis as well as for resolution of taxonomic controversies. The increase in mutation rate of mtDNA is 5-10 times relative to a single copy nuclear gene which resulted in an accumulation of base substitutions over a long period of time. The ordering of mitochondrial genes often remains unchanged over long periods of evolutionary time [17-21].

No thorough phylogenetic or phylogeographic analyses focusing on *Gazella dorcas* have been conducted until now [19]. Mitochondrial DNA is suitable for phylogenic applications due to its very low recombination level and its abundance in small size compared with genomic DNA make [8]. In authenticating food products, a number of mtDNA genes are used as target for detecting or isolating different animal species. Cytochrome- b (Cyt-b) gene region is one of the conserved regions used as a molecular marker for this purpose [18]. Our present study was designed to fill this gap of knowledge.

The objective of this study to detect the phylogeny and phylography of *Gazella dorcas* by use of mcyt- b and increase the knowledge for wildlife field as one of the most important issues needs to be further research on it.

Materials and Methods

Whole blood in EDTA were collected from 17 *Gazella dorcas* from Hilat Kuku Zoo, Elkadaru's farm and Mozamel Elkurdi's farm in East of the Nile, Khartoum State. The origins of these animals are Dongola, River Nile- Northern Sudan and Butana- Central Sudan. Other blood samples were collected in EDTA from Shambat- Bahry from sheep, goats, cows, camels, donkeys, horses and pigs. The blood samples were used for DNA extraction by using commercial kit (QIAamp blood kit- QIAGEN Inc Chatsworth, CA, USA). Other samples were also taken from a slaughtered gazelle from heart, lung, liver, kidney, spleen, tongue, muscles of scapula, intestine, rumen, reticulum, omasum and lymph nodes. The size of PCR product was 421 bp. For specificity of PCR, DNA extracted from (dorcas gazelles, cows, sheep, goats, camels, pigs, horses and donkeys) was used with GZ1 and GZ2 primers.

Selection of the primers for PCR

For detecting the mitochondrial cytochrome-b gene for gazelle we designed the primers by use bioedit software with [accession number JN410257.1 of *Gazella dorcas* isolate from west 7 cytochrome- b (cytb) gene, complete cds; mitochondrial Tunisia from GenBank]. The left primers included bases of the positive sense strand of the positive sense strand 5: CCT AGT TCT CAC ACT CCT AGT T. The right primers include bases of the complementary strand 5: GAG ACT ATT TTC AAT GGT ACT. The PCR product 421 bp and the primers were synthesized by World Meridian 10F, Gasan-dong, Geumcheon-gu, Seoul, 153-781 Korea.

Extraction of DNA from blood samples

For extraction of the DNA from blood samples we used commercial

***Corresponding author:** Dr. Reem Rabie Mohammed Salih, Head of the Department of Clinical Medicine, Faculty of Veterinary Medicine, University of Khartoum, Shambat, Bahry, Khartoum, Sudan, Tel: 00249911388234; E-mail: reemat7@yahoo.com

Received September 14, 2018; **Accepted** November 10, 2018; **Published** November 14, 2018

Citation: Salih RRM, Imad-Eldain ETA, Abd-Elrahim EK (2018) Phylogenetic Relationships of Sudanese *Gazella dorcas* Based on Mitochondrial Cytochrome-B Gene Sequences. J Mol Genet Med 12: 379 doi:10.4172/1747-0862.1000379

kit (QIAamp blood kit- QIAGEN Inc Chatsworth, CA, USA) according to the manufacturer´s instructions. Briefly, 200 µl from the Lysing buffer (L.A) put in sterile epindorf tube, then added 200 µl from the blood sample and added 10 µl from lyses enhancer, vortexed the tubes then incubated in water bath at 70°C for 10 minutes. Add 200 µl absolute alcohol, vortexed and incubated in water bath at 70°C for 2 minutes. Transferred all contents from the epindorf tubes and put to QIA spin column. Spin at 8000 rpm for 2 minutes and discarded the deposite. Washed with 500 µl buffer one, vortexed and spin at 8000 rpm for 2 minutes. Changed the QIAamp column and washed with 500 µl buffer two, vortexed and spin at 12000 rpm for 3 minutes. Discarded the deposit and put the QIAamp column in epindorf tube and add 200 µl from elution buffer and left for 1 minute. Put in the spin for 8000 rpm for 2 minutes then discard the column and saved the epindorf tube at freezing.

Tissues preparation

One dorcas gazelle slaughtered and took small part from different organs like: Tongue, lung, heart, liver, kidney, spleen, muscles, lymph node, rumen, reticulum, omasum, intestine). The tissues were prepared from different organs by chopping to small pieces finally to form homogenous extract by scalpel, put every tissue in separate epindorf tube, then add 300 µl of distilled water (D.W) and voertexed hardly, put the tubes in deep freezing for 10 minutes, thawing and vortexed. Repeated these steps 3 times, then centrifuged all samples at 300 rpm for 1 minute. Aspirated 200 µl from supernatant for DNA extraction.

Cooked meat preparation

About 2 gm of scapular muscles was poiled for 5 minutes, chopped finely to form homogenous extract, the whole volume was moved to an epindorf tube and diluted with added 300 µl distilled water, vortexed hardly. The tube was kept into -20°C in deep freezer for 10 minutes, then thawed and vortxed, three times successively. All the samples were centrifuged at 300 rpm for 1 minute. A volume of 200 µl of supernatant was aspirated to be used for DNA extraction [22-30].

DNA extraction from tissues and cooked meat

For extraction of DNA used the different organs of slaughtered gazelle and cooked meat. Commercial kit (QIAamp blood kit- QIAGEN Inc Chatsworth, CA, USA) was used according to the manufacturer´s instructions. Briefly, 200 µl from the Lysing buffer (L.A) were put in sterile epindorf tube, then 200 µl of tissue lyses buffer were added, then, 200 µl from the supernatant sample were added, vortexed the tubes then incubated in water bath at 70°C for 10 minutes. A volume of 200 µl absolute alcohol were added, vortexed and incubated in water bath at 70°C for 2 minutes. The contents were transferred from the epindorf tubes and put to QIA spin column. Spinning was performed at 8000 rplm for 2 minutes and the deposit was discarded. Washing was carried out with 500 µl buffer one, vortexed and centrifuged at 8000 rpm for 2 minutes. The QIAamp column was changed and washed with 500 µl buffer two, vortexed and centrifuged at 12000 rpm for 3 minutes. The deposit was discarded and put the QIAamp column in epindorf tube and 200 µl from elution buffer were added and left for 1 minute. Centrifugation was carried out at 8000 rpm for 2 minutes, then, the column was discarded and the epindorf tube was kept at freezing degree.

Phylogeny and sequencing

The PCR product of 17 blood samples from *Gazella dorcas* sent to Macrogen Company for sequencing using forward primer (GZ1) and reverse primer (GZ2) of mitochondrial cytochrome- b gene. Used the sequences for phylogenetic analysis of *Gazella dorcas* in Sudan compared with other *Gazella dorcas* in GenBank in (Sudan, Saudi Arabia, King Khalid Wildlife in Saudi Arabia, Tunisia, Algeria, Chad, captive gazelle in Alwabra Wildlife Preservation in Qatar, Mali and Israel). For phylogeny used CLC DNA workbench and Mega 5 softwares. In this study the isolated species from Sudan were as follows: (1R, 2R, Q3, 4R, 5R, 6R, 7R, 8R, 9R, 10R, Q11, 12R, Q13, Q14, Q15, Q1 and Q2) by forward primer (GZ1). The same samples read by using reverse primer (GZ2) for the same species (1, 2, Q3, 4, 5, 6, 7, 8, 9, 10, Q11, Q12, Q13, Q14, Q15, Q1 and Q2).

Results

Sequencing and phylogeny of *Gazella dorcas*

Table 1 shows the comparison between *Gazella dorcas* isolated from Sudan with reference from GenBank. Figure 1 shows the sequence of mitochondrial cytochrome- b gene of *Gazella dorcas* in Sudan. Figure 2 shows JN410247.1: Accession number of *Gazella dorcas* isolated from east of the Nile- Sudan from the GenBank, (GZ) Sudan: Isolated *Gazella dorcas* from different places in Sudan in this study. The PCR product of 17 blood samples from *Gazella dorcas* sent to Macrogen Company for sequencing using forward primer (GZ1) and reverse primer (GZ2) of mitochondrial cytochrome- b gene. Used the sequences for phylogenetic analysis of *Gazella dorcas* in Sudan compared with other *Gazella dorcas* in GenBank in (Sudan, Saudi Arabia, King Khalid Wildlife, Tunisia, Algeria, Chad, captive gazelle in Alwabra Wildlife

TCCCACTCCTAGTTCTCACACTCCTAGTTTCTCACACTCCTAGTTCTCACACTCCTAGTTCTCACACTCCTAGTTCTCAC
ACTCCTAGTTCTCACACTCCTAGTTCTCACACTCCTAGTTCTCACACTCCTAGTTCTCACACTCCTAGTTTTCACACACC
TAGTAGTTCTCACACTCCTAGTTCTCACACTCCTAGTTCTCACACTCCTAGTTCTATTCTCACCAGACCTACTCGGAGACCCAGACAACTATAC
ACCAGCAAATCCACTCAACACACCCCCACACATCAAACCTGAATGATACTTCTTATTCGCATATGCAATTCTCCGATCAATTCCCAATAAACTA
GGAGGAGTCCTAGCCTTAGTCCTATCAATTCTAATCTTAATCCTTATACCCCTACTTCATACATCTAAACAACGAAGCATGATATTTCGGCCAAT
CAGCCAATGCCTATTCTGAATTCTAGTAGCAGACCTGCTAACACTTACATGAATCGGAGGACAACCAGTCGAACACCCATACATTATTATCGG
ACAACTAGCATCAATTATATATTTCCTACTCATTCTAGTACTGATACCAGCAGCCAGTACCATTGAAAATAGTCTCGGTGTGCCGTGGGACAAC
GAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACT
AGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTA
GGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAG
GAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGA

Figure 1: The sequence of mitochondrial cytochrome- b gene of *Gazella dorcas* in Sudan.

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(GZ)Sudan    ------------------------------------------------------------TC
JN410247.1   ATATCTTTCTGAGGAGCAACAGTTATCACGAACCTCCTCTCAGCGATCCCATACATCGGC
                                         *

(GZ)Sudan     CCACTCCTAGTTCTCACACTCCTAGT----TTCTCACACTCCTAGTTCTCACACTCCTAG
JN410247.1    ACAAGCCTAGTAGAATGAATCTGAGGGGGATTCTCAGTAGATAAAGCAACACTTACCCGA
          ** ******    * ** **   ******    *   ***  **

(GZ)Sudan     TTCTCACACTCCTAGTTCTCACACTC-CTAGTTCTCACACTCCTAGTTCTCACACTCCTA
JN410247.1    TTCTTTGCTTTTCACTTTATTCTCCCATTCATCATCGCAGCCCTCGCTATAGTCCACTTA
          ****   *  * **  * * * * * ** ** *** * * *  * * **
(GZ)Sudan     GT----TCTCACACTCCTAGTTCTCACACTCCTA--GTTCTCACACTCCTAGTTCTCACA
JN410247.1    TTATTTCTTCACGAAACAGGATCTAATAACCCCACAGGAATTTCATCAGACGCAGACAAA
           *    ****   * * *** * *  ** * *  * *  **    *   ** *
(GZ)Sudan     CTCCTAGTTTTCACACACC-TAGTAGTTCTCACACTCCTAG-----TTCTCACACTCCTA
JN410247.1    ATTCCATTTCACCCCTACTACACCATCAAGGACATTCTAGGAGCGCTACTACTAATCCTA
           * * * ** * * **  * *    *** **  *   * **  * *****

(GZ)Sudan     GTTCTCACACTCCTAGTTCTATTCTCACCAGACCTACTCGGAGACCCAGACAACTATACA
JN410247.1    GTTCTCACACTCCTAGTTCTATTCTCACCAGACCTACTCGGAGACCCAGACAACTATACA
          ************************************************************

(GZ)Sudan     CCAGCAAATCCACTCAACACACCCCCACACATCAAACCTGAATGATACTTCTTATTCGCA
JN410247.1    CCAGCAAATTCACTCAACACACCCCCACACATCAAACCTGAATGATACTTCTTATTTGCA
          ********* ********************************************** ***
(GZ)Sudan     TATGCAATTCTCCGATCAATTCCCAATAAACTAGGAGGAGTCCTAGCCTTAGTCCTATCA
JN410247.1    TATGCAATTCTCCGATCAATTCCCAATAAACTAGGAGGAGTCCTAGCCTTAGTCCTATCA
          ************************************************************

(GZ)Sudan     ATTCTAATCTTAATCCTTATACCCCTACTTCATACATCTAAACAACGAAGCATGATATTT
JN410247.1    ATTCTAATCTTAATCCTTATACCCCTACTTCATACATCTAAACAACGAAGCATGATATTT
          ************************************************************

(GZ)Sudan     CGGCCAATCAGCCAATGCCTATTCTGAATTCTAGTAGCAGACCTGCTAACACTTACATGA
JN410247.1    CGGCCAATCAGCCAATGCCTATTCTGAATTCTAGTAGCAGACCTGCTAACACTTACATGA
          ************************************************************

(GZ)Sudan     ATCGGAGGACAACCAGTCGAACACCCATACATTATTATCGGACAACTAGCATCAATTATA
JN410247.1    ATCGGAGGACAACCAGTCGAACACCCATACATTATTATCGGACAACTAGCATCAATTATA
          ************************************************************

(GZ)Sudan     TATTTCCTACTCATTCTAGTACTGATACCAGCAGCCAGTACCATTGAAAATAGTCTCGGT
JN410247.1    TATTTCCTACTCATTCTAGTACTGATACCAGCAGCCAGTACCATTGAAAATAGTCTCC-T
          ************************************************************ *
(GZ)Sudan     GTGCCGTGGGACAACGAGGAGTGTGAGAACTAGGAGTGTGAGAACTAGGAGTGTGAGAAC
JN410247.1    AAAATG-AAGATAA----------------------------------------------
            *  ** **
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Figure 2: JN410247.1: Accession number of *Gazella dorcas* isolated from east of the Nile- Sudan from the GenBank. (GZ) Sudan: Isolated *Gazella dorcas* from different places in Sudan in this study.

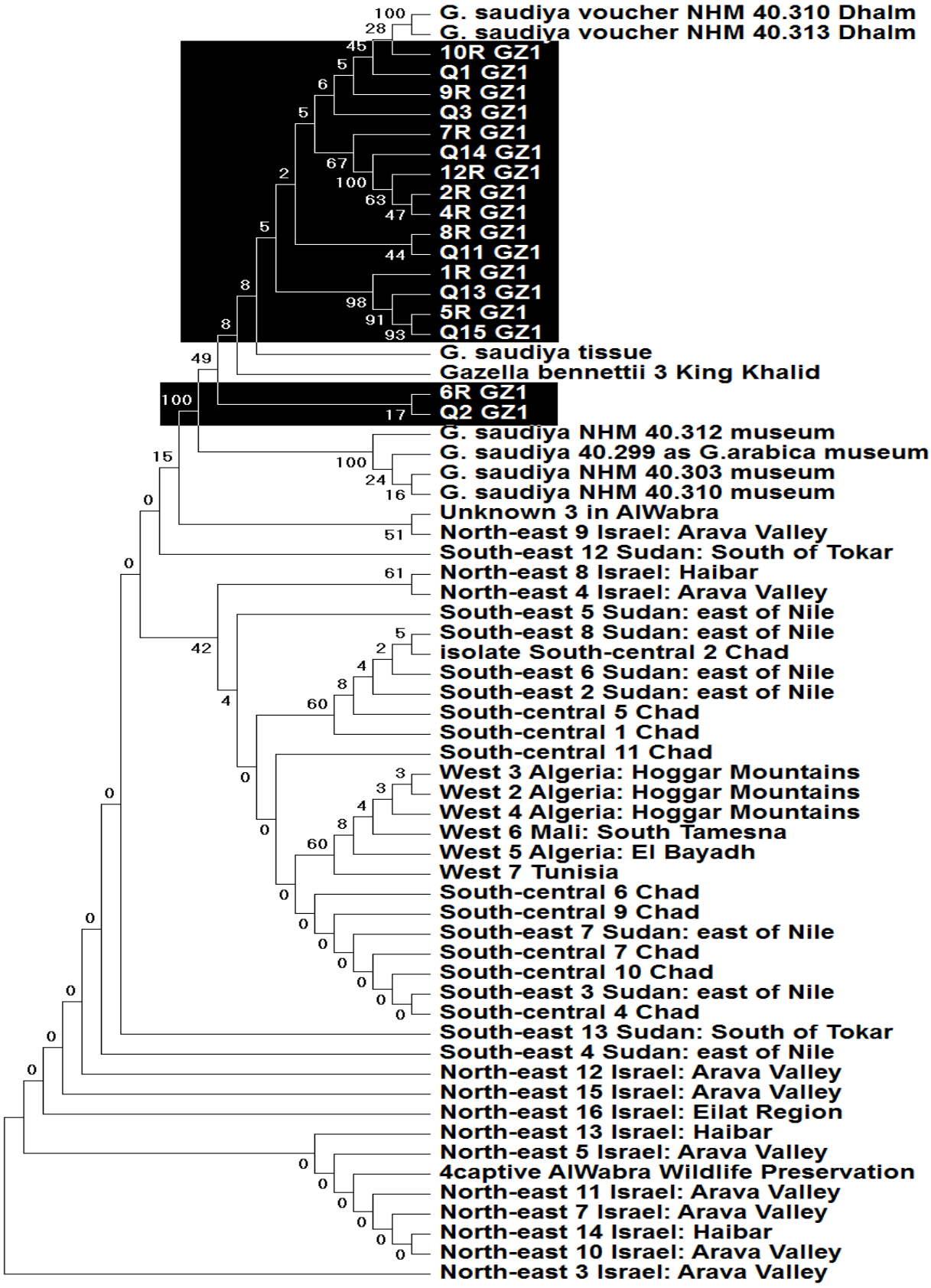

Figure 3: Phylogenetic tree for *Gazella dorcas* using GZ1.

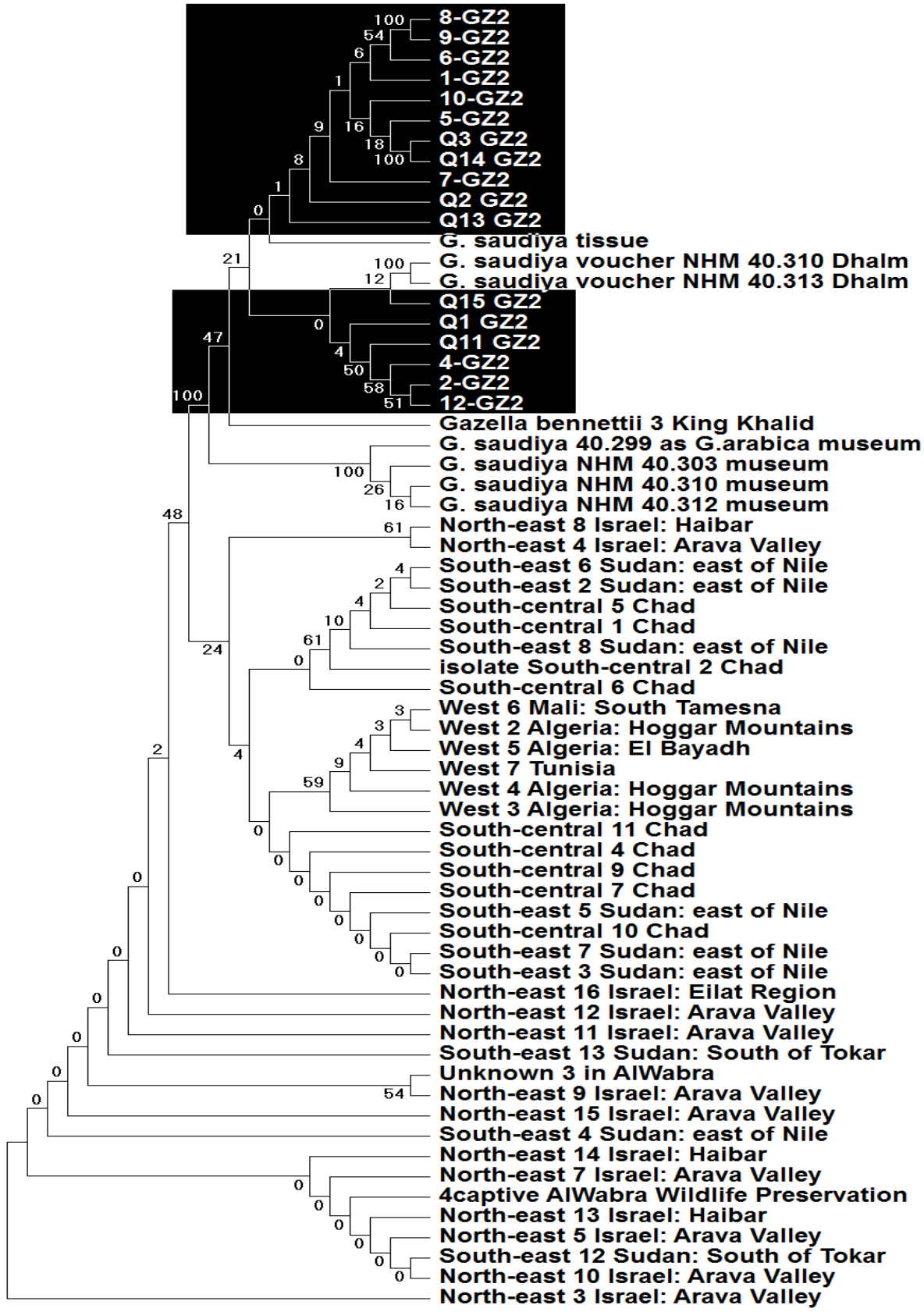

Figure 4: Phylogenetic tree for *Gazella dorcas* using GZ2.

Name of Sequence	The number of different ion between query and subject																							
1R- GZ1	76 T* A	79 T* A	82 -* C	86 -* T	92 C* -	101 -* A	145 G* T	381 G* A																
2R- GZ1	90 C* G	93 T* A	99 T* A	108 T* A	111 T* A	382 G* A																		
3Q- GZ1	73 T* A	76 T* A	88 C* T	97 T* A	109 T* A	380 G* A																		
4R- GZ1	86 A* T	90 C* T	93 T* A	99 T* A	111 T* A	382 G* A																		
5R- GZ1	74 T* A	89 C* T																						
6R- GZ1	103 A* T	121 C* A	125 T* A	350 C* T	356 G* A	384 G* A																		
7R- GZ1	75 T* A	81 -* C	83 G* T	86 G* T	91 A* G	93 G* A	98 -* C	99 G* A	108 G* A	109 A* T	110 -* C	126 T* A	143 G* T	144 T* C	145 G* C	161 G* T	176 G* T	194 -* A	195 C* A	241 A* T	244 G* T	246 C* G	248 T* A	272 G* C
8R- GZ1	294 T* A	377 A* G																						
9R- GZ1	262 T* A	266 T* G	271 -* G	275 A* -	276 A* G	278 A* C	281 T* -																	
10R- GZ1	65 T* A	89 -* A																						
11Q- GZ1	76 T* A	79 T* A	82 T* C	87 A* T	91 C* T	92 C* G	94 T* A	100 T* A	106 T* C	111 G* C	112 T* A	113 T* A	382 G* A	384 -* A										
12R- GZ1	75 T* A	90 C* T	99 T* A																					
13Q- GZ1	74 T* A	77 T* A	85 A* T	89 C* T	90 C* G	92 T* A	98 T* A	110 T* A	380 G* A	382 -* A														
14Q- GZ1	75 T* A	90 C* T	91 C* G	99 T* A	111 T* A	174 G* A																		
15Q- GZ1	74 T* A	77 T* A	80 T* C	85 A* T	90 C* G	92 T* A	96 T* G	98 T* A	110 T* A	380 G* A	382 -* A													
1Q- GZ1	234 T* G	265 T* A	304 T* C	329 C* T	363 G* A																			
2Q- GZ1	221 T* G	224 C* A	235 G* T	259 C* A	260 T* C	268 G* T	276 G* A	279 T* A	298 G* T	332 T* A														
*: Query/A: Adenine/ T: Thymine/ C: Cytosine/ G: Guanine/the nitrogenous base without star: subject.																								

Table 1: Comparison between *Gazella dorcas* isolated from Sudan with reference from GenBank.

Preservation, Mali and Israel). For phylogeny used Mega 4 software. Figure 3 shows the tree of *Gazella dorcas* compared with other species from GenBank. In this study the isolated species from Sudan were as follows: (1R, 2R, Q3, 4R, 5R, 6R, 7R, 8R, 9R, 10R, Q11, 12R, Q13, Q14, Q15, Q1 and Q2) by forward primer. Figure 4 shows the same samples read by using reverse primer GZ2 for the same species (1, 2, Q3, 4, 5, 6, 7, 8, 9, 10, Q11, Q12, Q13, Q14, Q15, Q1 and Q2). All species were much closer to *Gazella saudiya* more than other species isolated from other countries.

Discussion

Lack of detailed information about phylogenetic relationships among and within threatened groups of animals can hamper conservation efforts [31-33]. For instance, unrecognized differentiation within a putative species can lead to admixture of independent evolutionary entities in captivity. In this context, conservation genetic approaches are valuable tools for captive breeding and *in situ* conservation programs as morphologically indiscernible (cryptic) animal species appear to exist throughout taxonomic groups and biogeographic regions [25,27,30].

In this study we used PCR on the *Gzaella dorcas* in Sudan using 421 bp of mitochondrial DNA (mtDNA) cytochrome- b gene derived from blood and tissues samples collected from the *Gazella dorcas*. The

polymerase chain reaction analysis of blood samples collected from living gazelles in Bahry and East of Nile in Sudan.

We conduct another experiment it was sequencing and phylogeny of *Dorcas gazelles* which were examined in this study compared with other species of *Gazella dorcas, Gazella saudiy* and *Gazella benetti* which were found in GenBank. The examined gazelles were closer to *Gazella saudiya* more than other species and this in agreement with findings of [23,26]. May be the *Gazella dorcas* in Sudan was transported to Saudi Arabia or from the last one to Sudan.

Conclusion and Recommendation

In conclusion the phylogeny of *Gazella dorcas* will be apply by use complete mitochondrial cytochrome- b gene alternative from partial fragments of the gene which were used in this study.

There is, therefore, need to conduct research that can determine the practicability of LAMP method being applied for diagnosis in the field, such as conducting LAMP experiments at field ambient temperatures, and determining simple DNA template preparations which could also be easily applied in the field. Ecological, biological, and genetic knowledge of wildlife has traditionally been covered in the disciplines of wildlife ecology, physiology and conservation genetics and it is this research that forms the foundation for the interpretation of genetic data for forensic applications.

We argue that for forensic science to advance in the field of wildlife biology, cross-disciplinary collaborations with ecologists, biologists and conservation geneticists are essential. Phylogenetic, phylogeographic and population genetic studies are required for species, population and individual identification of wildlife, respectively. The objectives of conservation research are often complimentary with forensic outcomes. For example, phylogenetics can be used to delineate species boundaries and this is important for the enforcement of wildlife legislation, which recognizes and protects groups that are classed as 'species' or 'subspecies'.

Acknowledgement

Thanks for Imadeldeen. E.E. Aradaib for prepared his Lab for help and fund to make the experiment completely.

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