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

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**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 Sahelian 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 phylogenetic reconstruction at several taxonomic levels. Phylogenetic 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 phyleogeographic analyses focusing on *Gazella dorcas* have been conducted until now [19]. Mitochondrial DNA is suitable for phylogenetic 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 (cyt) 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 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
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 epipodifl 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 epipodifl tubes and put to QIA spin column. Spin at 8000 rpm 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. Discarded the deposit and put the QIAamp column in epipodifl 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 epipodifl 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 (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**

**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 species of 17 blood samples from Gazella dorcas published 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. Used the sequences for phylogenetic analysis of Gazella dorcas in Sudan compared with other species of Gazella dorcas published 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).

**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 epipodifl tube, then 200 µl of tissue lyses buffer were added, then, 200 µl from the supernatant sample were added, vortexed and mixed 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 epipodifl tubes and put to QIA spin column. Spinning was performed at 8000 rpm 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. Discarded the deposit and put the QIAamp column in epipodifl 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 epipodifl tube was kept at freezing degree.

**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 epipodifl tube and diluted with added 300 µl distilled water, vortexed hardly. The tube was kept into -20°C in deep freezer for 10 minutes, finely to form homogenous extract, the whole volume was moved to an epipodifl tube and diluted with added 300 µl distilled water, vortexed hardly. The tube was kept into -20°C in deep freezer for 10 minutes, finely to form homogenous extract, the whole volume was moved to an epipodifl tube and diluted with added 300 µl distilled water, vortexed hardly. The tube was kept into -20°C in deep freezer for 10 minutes, finely to form homogenous extract, the whole volume was moved to an epipodifl tube and diluted with added 300 µl distilled water, vortexed hardly. The tube was kept into -20°C in deep freezer for 10 minutes, finely to form homogenous extract, the whole volume was moved to an epipodifl tube and diluted with added 300 µl distilled water, vortexed hardly. Then, centrifuged at 300 rpm for 1 minute. Aspirated 200 µl from supernatant for DNA extraction.

**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 [140247.1: 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 gazelles of 17 blood samples from 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.
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.
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 Gazella 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

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

<table>
<thead>
<tr>
<th>Name of Sequence</th>
<th>1R- GZ1</th>
<th>2R- GZ1</th>
<th>3Q- GZ1</th>
<th>4R- GZ1</th>
<th>5R- GZ1</th>
<th>6R- GZ1</th>
<th>7R- GZ1</th>
<th>8R- GZ1</th>
<th>9R- GZ1</th>
<th>10R- GZ1</th>
<th>11Q- GZ1</th>
<th>12R- GZ1</th>
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<th>14Q- GZ1</th>
<th>15Q- GZ1</th>
<th>1Q- GZ1</th>
<th>2Q- GZ1</th>
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<tr>
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<td>79 T A</td>
<td>82 A C</td>
<td>86 C T</td>
<td>92 C T</td>
<td>101 T A</td>
<td>145 G T</td>
<td>381 G A</td>
<td>294 T A</td>
<td>262 T G</td>
<td>65 T A</td>
<td>76 T A</td>
<td>79 T A</td>
<td>82 T C</td>
<td>87 A C</td>
<td>91 C T</td>
<td>92 C T</td>
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<tr>
<td>The number of different ion between query and subject</td>
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<td>93 T* A</td>
<td>99 T* A</td>
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</tr>
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</table>
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 saudiy rather 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 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 the important for the enforcement of wildlife legislation, which recognizes and protects groups that are classed as ‘species’ or ‘subspecies’.

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References