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Partial Characterization of the Mitochondrial DNA (mtDNA) in Gazelles (*Gazella marica*) in Şanlıurfa Province

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Abstract

Gazelles are one of the important species unique to Türkiye, as in most Asian and Middle Eastern countries. Gazelles in Türkiye belong to the Gazella subgutturosa species, which has two subspecies: Şanlıurfa gazelle (*Gazella marica*) and Hatay Mountain gazelle (Gazella gazella). Numbers of Şanlıurfa gazelle (*Gazella marica*) are decreasing in Türkiye as well as all over the world, and today they are found in a limited area on the southern border, mostly around the province of Şanlıurfa. The aim of this study is to examine the 400 bp region of the mitochondrial cytochrome-b (mtDNA *cyt-b*) gene, which is frequently used in phylogenetic studies in gazelles, by sequence analysis. Samples were taken from a total of 40 male and female gazelles from populations in four different holdings in Şanlıurfa province. Sequence analysis results did not reveal any nucleotide diversity in terms of the examined region. the samples examined in after constructed phylogenetic tree which was evaluated together with the sequences covering the same region in the database of the obtained sequence analysis result showed that the samples exanimated were clustered together with *Gazella marica* samples, in line with previous studies. This study implies that the *cyt-b* gene region of mtDNA is not promising to assess genetic variability and phylogenetic relationships among gazelle populations surviving in Şanlıurfa province. Due to its limitations on reveal genetic diversity in *Gazella marica* it should be screened genetic variability and phylogenetic relationships in this grup by using other regions of mtDNA as control region.

Keywords: *Gazella marica*, genetic variability, maternal lineage, mtDNA *cyt-b* gene, sequencing

Introduction

Huge increase in human population and excessive use of natural resources have accelerated climate change and habitat loss. This situation has become a growing threat to sustainable ecology and biodiversity. Türkiye is a geography where the Mediterranean, Iran-Anatolia and the Caucasus biodiversity hotspots intersect. Despite this advantage it has in terms of biodiversity, the risk of losing this advantage is also quite high (1, 2). It has been reported that there are 27 large mammal species in Türkiye and one of these is the gazelle, which is one of the first creatures that comes to mind when Şanlıurfa and the steppe are mentioned. Gazella, the most complex genus in the Antilopinae subfamily among the most diverse hoofed mammals in the Bovidae family, extends from North Africa through the Arabian Peninsula, the Middle East and India to the plains of China and Mongolia (3, 4, 5, 6).

It has been reported that there are 11 species belonging to the Gazella genus, most of which are considered to be at risk today (5, 7). There are two species of gazelle in Türkiye: Şanlıurfa gazelle (*Gazella marica*) and Hatay Mountain gazelle (*Gazella gazella*). Following the report of the IUCN SSC Antelope Specialist Group (2008) on the rapid extinction of gazelle species, breeding and conservation farms

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were established in Türkiye, as in many countries (8, 9, 10). However, since gazelles are one of the most complex and least studied mammal groups, the number of molecular genetic studies, despite their relatively increasing numbers in recent years, is less than the number of studies carried out in most mammal species. Even among the taxonomic classifications made by various research groups, there is still inconsistency. The same situation emerged for these two gazelle species in Türkiye each of them belong to *Gazella subgutturosa* (11, 12). Historical observations regarding the existence of the gazelle genus and its related species in Anatolia were well documented by 13. The Şanlıurfa gazelle (*Gazella marica*) was considered as a subspecies of the Hatay Mountain gazelle (*Gazella gazella*) and they were considered as a single species. In a study conducted with mtDNA sequences in 2011 (12), it was shown that *Gazella marica* is a separate species and immediately afterwards it was shown that there are two species in Türkiye as Şanlıurfa gazelle (*Gazella marica*) and Hatay Mountain gazelle (*Gazella gazella*) by 14 and 15. *Gazella marica*, known as the "Arabian sand gazelle", exits along Yemen, Oman, United Arab Emirates, Saudi Arabia and the southern border of Türkiye. Until the 20th century, gazelles were spreading from the South eastern border of Türkiye to the Northern border of Eastern Anatolia. However, due to reasons such as habitat destruction and illegal hunting, the living spaces of gazelles have narrowed and are limited to Şanlıurfa and surrounding provinces (16, 17).

It has been reported that the risk status of the *Gazella marica* species, whose individual number drops to around 1750-2150, has been included in the "vulnerable" category, like other gazelle species, in the IUCN Red List of Threatened Species within the scope of C2a (i) criteria (7). This means that the number of *Gazella marica* in nature is rapidly decreasing, and if the decrease continues, they will be in danger of extinction. To avoid this extinction, significant efforts are being made to protect the gazelle species. Conservation efforts for the species in Türkiye were initiated with the hunting ban enacted in 1957, the first official studies to collect information about gazelles in the Urfa Region were carried out by Ceylanpınar TİGEM in 1968, and between 1977 and 1982, gazelle breeding and breeding activities were carried out by the Republic of Türkiye Ministry of Agriculture and Forestry General Directorate of Nature Conservation and National Parks has been carried out. Since that date, the gazelle breeding and production station is still run by TİGEM, and breeding sales are also carried out at the 75th Year Gazelle Breeding Station *Gazella marica* was released into the wild in 2021 and 2022 within the scope of reintroduction, allowing them to reproduce there (8).

Under the leadership of the institution, they strive at national and international levels to protect ecological systems and biodiversity in Türkiye. On the other hand, it is seen that research examining gazelles in both Şanlıurfa and Hatay regions in Türkiye, in terms of molecular markers 13, 14, 18, 19 as well as morphometric and reproductive characteristics and habitat needs, has gained momentum after the first decade of the twenty-first century (8, 18, 20, 21). "Conservation and Sustainable Management of Türkiye'sSteppe Ecosystems" project which was also carried out between 2019-2020 with the cooperation of the United Nations Food and Agriculture Organization (FAO) and the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (DKMPGM), General Directorate of Plant Production (BÜGEM) and General Directorate of Forestry (OGM) and with the support of the Global Environment Facility (GEF). In Şanlıurfa Steppe Conservatıon Strategy and Action Plan 2021-2030 prepared within the scope of the project (22), advisory targets for measuring genetic diversity were put forward in order to protect the Şanlıurfa steppe biological diversity at the levels of ecosystem, species, genetic and ecological process diversity. It is already known that estimating genetic diversity at regular intervals with reliable markers is of great importance in determining the risk status of the species, monitoring it, determining the conservation strategy and observing the results of conservation activities.

Although molecular studies have been carried out by various genetic markers to understand the genetic background of the species and to elucidate taxonomic confusion, these are very few in number when compared to domestic farm animals (13, 14, 19, 23, 24 25).

Our aim in this study is to examine the 400 bp long region of the mtDNA cyt b gene by sequence analysis in the blood sample obtained from forty Gazelle *marica* individuals located within the borders of Şanlıurfa province, and to compare it with the sequences belonging to the same region in the database to both review its phylogenetic location and to determine the current genetic diversity through nucleotide diversity.

Material and Method

The research permissions were obtained with the letter dated 29.09.2023 and numbered E-21264211-288.04-11423456 from the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks. Ethical approval was also obtained by Harran

University Animal Experiments Local Ethics Committee Presidency with decision number 2023/07/20 and ethics committee permission dated 12/09/2023 and numbered 244460.

All blood samples were taken from the Vena jugularis of male (n=16) and female (n=24) gazelles belonging to the *Gazella marica* species, found in four different locations within the borders of Şanlıurfa Province. DNA isolations from these 40 blood samples were performed using commercial kits, according to the manufacturing instructions. Isolated DNA samples were used for PCR amplifications. The 400 bp located on 5′ -region of the mtDNA *cyt-b* gene belonging to gazelle mtDNA genome was amplified by using primers L14724: 5'- TGACTAATGATAGAAAAACCATCGTTG and H15149: 5'- TAACTGTTGCTCCTCAAAAAGATATTTGTCCTCA (26, 27, 28). Following to amplifications PCR products were purified and sequenced. The sequencing results were aligned and trimmed by using BioEdit and CLUSTALW (http://www.ebi.ac.uk/clustalw/). Using the sequence obtained from sequencer and 105 sequences (KM978962, GU384826, GU384835, GU384836, GU384840, GU384844, GU384856,GU384864, GU384866, GU384867, GU384869, AF187715, AF187716, DQ269164, AF187696, AF187718, AF187719, AF187699, AF187698, MH360717, KU560654, JN632643, MH360718, MH360720, MF180128, MF179979, KU560636, KM582095, NC_020704, MF179985, MF179984, MF179983, MF179982, MF179981, MF179980, MF179978, MF179977, MF179976, MF179975, MF179974, MF179973, MF179972, MF179971, MF179970, KU560635, KM582114, KM582113, KM582112, KM582102, KM582101, KM582100, KM582098, KM582097, KM582096, JN410259.1:11-424, KM582111, NC_020708, KU560634, MT811637, AF187702, MT811636, MT811635, MT811634, MT811633, MT811632, MT811631, MT811626, MT811625, MT811623, AF187696, KP729619, KP729618, KM387302, KM387301, KM387300, KM387299, KM387298, KM387297, KM387296, KM387295,KU560633, MT811638, MT811630, MT811628, MT811627, NC_020703,MT811629, KU560631, NC_020709, KM582103,AF187699, KU560632, KM978968, KU560653, OQ595233, KM978991, KM978990, KM978989, KM978984, KM978980, KM978977, KM978972, KM978971, KM978967, KM978964, KM978962) from public database (https://www.ncbi.nlm.nih.gov/nucleotide/) phylogenetic tree was constructed by MEGA version 10.2.6 according to the neighbour-joining method using Tamura-Nei model (29, 30, 31).

Results and Discussion

All of the DNA samples obtained were amplified by PCR (Figure 1), but the sequence analysis results of 37 of them were found suitable for evaluation.

Figure 1. Electropherogram of 400 bp PCR products

However, unfortunately, all the sequences of the 37 samples obtained were identical to each other. For this reason, the diversity parameters of the population could not be estimated. Those who investigated before (13, 14) did not find any nucleotide diversity in Şanlıurfa gazelle population either. However, examination of the mtDNA cyt b region in Iranian *Gazella subgutturosa* in 2019 revealed diversity although it was low (24). According to phylogenetic tree constructed the sample was grouped with sequences obtained from *Gazella subgutturosa* and *Gazella subgutturosa marica* species, Gazella *dorcas* x *leptoceros*, aswell (AF187702, AF187718, AF187696, MH360720, KM582098, KM58210) (Figure 2). This clustering was consistent with the findings of researchers who previously clarified the taxonomic status of gazelles in the city of Şanlıurfa (13, 14).

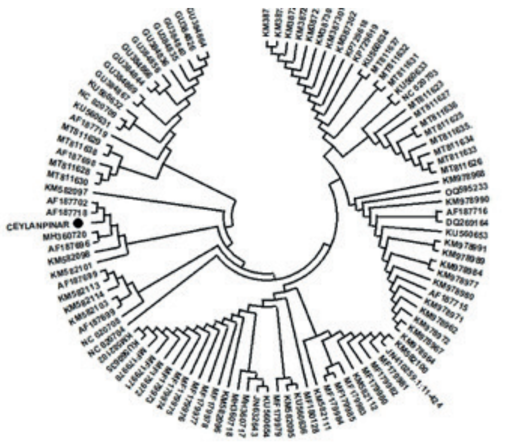

Figure 2. Neighbour-joining phylogenetic tree constructed with the previous reported sequences from database

Sequence analysis of the 400 bp region in the mtDNA *cyt-b* gene, which is most used in researching maternal diversity and lineages in the Gazelle breed, did not reveal any nucleotide diversity as in previous research results (13, 14). Examination of the Turkish gazelle population with autosomal markers also shows that diversity is significantly restricted. A more recent study examined an exonic region of the MHC class II DRB gene and found the estimated diversity parameters to be lower than the diversity estimated from the same region of the bovine species (19). This situation strengthened the impression that genetic diversity is low in the gazelle population in Türkiye (19). By examining the mtDNA cyt b in studies conducted in China (25, 32) and Iran (24), low genetic diversity was determined, although the region examined was wider in some of them (25, 32). Additionally, a low level of genetic diversity was observed in the examination of Mitochondrial Cytochrome-b, Cytochrome-c and d-loop in *Antilope cervicapra* (33). Previous studies carried out by using first generation markers based on biochemical and karyotypic variations also revealed low diversity in the specie (34, 35). This may be due to the underlying homogeneity of the species, or a recent bottleneck may have led to this reduced diversity, considering that their numbers have dropped to around 300 in the recent past in Türkiye (16). However, it also should be kept in mind that different results may be obtained by examining the control region of mtDN*A*. As a matter of fact, 36 in their study on *Gazella marica* in the wild, determined a low nucleotide diversity and a medium level of haplotype diversity with seven different haplotype groups. Although more than 70% of the samples in the study carried the predominant haplotype, it was greater than the diversity revealed by the mtDNA cyb gene.

As a result, it was shown by 14, that the gazelles in Şanlıurfa belong to the *Gazella marica* specie. Around the same time 15, analysed the samples obtained from Ceylanpınar in terms of the same mitochondrial region and reported that these samples were grouped together with *Gazella marica*. Following these, 13's study also gave this result. Our study confirms these previous studies. In these previous studies it was shown that the mtDNA *cyt-b* region of *Gazella marica* and *Gazella gazella* are clearly separated by 23 variable regions. However, its success in reflecting the genetic diversity within the species should be evaluated together with the results from studies with the control region, which is a more protected region of mtDNA in the same populations. To determine appropriate conservation strategies, genetic diversity both within and between populations must be evaluated with appropriate molecular and phenotypic markers. Both 14's showing that gazelles in Türkiye are diphylogenic by using the mtDNA *cyt-b* region and Saatoglu's study (13) show that the C′ T substitution in the INRA126 microsatellite on the Y chromosome can be used to distinguish species have paved the way for the pure conservation of these species. However, to create successful conservation strategies, genetic diversity in both wildlife and the captive breeding gazelle population at the institute must be monitored with autosomal neutral markers. As it was underlined by 24, microsatellite markers may provide a more objective estimate of genetic diversity and have the potential to make a significant contribution to conservation studies due to their power to show the possible structuring in populations.

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