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Authors: Kankiliç, Tolga, Özüt, Deniz, Gürler, Şükrü, Kence, Meral, Bozkaya, Faruk, et al.

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Rediscovery of a new mountain gazelle population and clarification of taxonomic status of the genus *Gazella* in Turkey using mtDNA sequencing

Tolga KANKILIÇ[1]*, Deniz ÖZÜT[2], Şükrü GÜRLER[3], Meral KENCE[4], Faruk BOZKAYA[3] and Aykut KENCE[4]

[1] *Department of Biology, Faculty of Science and Letters, Aksaray University, 68100 Aksaray, Turkey; e-mail: tkankilic@gmail.com, kankilic06@hotmail.com*
[2] *Nature Conservation Centre, 1065 Cad, 1293 Sok. 9/32, Öveçler, Ankara,Turkey*
[3] *Department of Animal Genetics, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Turkey*
[4] *Department of Biological Sciences, Middle East Technical University, Ankara, Turkey*

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Abstract. The fauna of Turkey lists the goitered gazelle (*Gazella subgutturosa*) as the only species of the genus *Gazella,* but older records reported another species of gazelle in Anatolia (either *G. dorcas* or *G. gazella*). However, the species status and the distribution of the second gazelle species have never been confirmed, and the species has been regarded as extirpated. We studied the nucleotide differences in a 400 bp region of cytochrome-b gene for Şanlıurfa population and a recently discovered gazelle population in Hatay. A total of 36 sequences were found with 23 variable sites. All individuals from Hatay and the Golan Heights, Israel (*G. gazella*) revealed an identical monophyletic lineage and the results of phylogenetic analyses suggest that the population in Hatay belongs to *G. g. gazella*. Şanlıurfa individuals were grouped with Genbank sequences of *G. subgutturosa marica*, which a recent study has suggested to be a distinct species, *G. marica*. Our findings have resolved an ongoing debate on the taxonomic status of the *Gazella* in Turkey and call for the urgent need of improved conservation efforts for the wild gazelles.

Key words: *Gazella gazella*, *Gazella marica*, cytochrome-*b*, taxonomy, conservation

Introduction

Gazella species have a fairly wide distribution, but their populations have decreased in size and become highly fragmented in the past 60 years (e.g. IUCN 2008). Many of the *Gazella* species are threatened today and most species survive only in captive breeding programmes (Ryder 1987, Saleh 1987, Thoules et al. 1991, Baillie & Groombrdige 1996, Hammond et al. 2001, IUCN 2008). Taxonomically correct identification of populations of endangered species is essential for the success of conservation programmes (Avise 1989, Wronski et al. 2010).

Taxonomically, the most complex group within the Bovidae is the Antilopinae (Groves 1997). Primarily, morphological characters such as body size, horn-shape, and pelage coloration have been used to describe different taxa. However, due to high intraspecific variation and a high degree of interspecific similarities, differentiation of taxa has been problematic (Groves 1996). Mitochondrial DNA (mtDNA) variation has been widely used in the description of taxa which can barely be distinguished morphologically. Many studies have utilized mtDNA sequence variation in quantification of genetic variation and in solving problems in classification or conservation of *Gazella* species (Rebholz & Harley 1997, 1999, Hammond et al. 2001, Lorenzen et al. 2008, Wronski et al. 2010, Wacher et al. 2011).

Formerly, *G. subgutturosa* was distributed widely, ranging from Oman across the Arabian Peninsula to southeastern Anatolia (Mallon & Kingswood 2001), following the steppes of central Asia through Iran and Turkmenistan to Mongolia and NW China.

* *Corresponding Author*

More recently, it was suggested that *G. subgutturosa* is polyphyletic and that *G. s. marica* is considered a separate species (sister taxon to *G. leptoceros*) from the Arabian Peninsula, Iraq, Jordan, Syria and Turkey different from *G. subgutturosa*, a genetically diverse larger clade from central Asia (Hammond et al. 2001, Wacher et al. 2011). Similarly, two reciprocally monophyletic genetic lineages exist within the presumed species *G. gazella*: the northern clade (the Golan Heights, Israel/Syrian border) and the genetically diverse larger clade from the Arabian Peninsula and the Negev, Israel (Rebholz & Harley 1999, Wronski et al. 2010).

The fauna of Turkey lists the goitered gazelle, *G. subgutturosa*, as the only species of the genus *Gazella* to occur in Turkey, but in the older records two species of gazelle were reported from Anatolia (Kumerloeve 1975a, Turan 1984, Kasparek 1986, Kryštufek & Vohralík 2005). It was also suggested that the species occurring in Hatay and Adana could be *G. gazella* (Kumerloeve 1969, 1975) or *G. dorcas* (Danford & Alston 1877, Harrison & Bates 1991).

Until the 20th century, the distribution of the genus *Gazella* in Turkey included a large area extending from Çukurova-Adana to Eastern Anatolia. However it shrank to several isolated locations in Şanlıurfa, Hatay and Adana during the last century, due to overhunting, live-trapping of juveniles for trade, heavy pesticide use and habitat degradation (Turan 1977). The numbers of individuals were reported to be around 1500-3000 in 1968 in Ceylanpınar-Şanlıurfa (Kumerloeve 1969, Turan 1990) and by 1977, only around 300 animals were left in the same region (Turan 1977). Apart from the still existing populations in Ceylanpınar and Kızılkuyu districts in Şanlıurfa province, the remainder of the genus *Gazella*'s populations in Turkey went locally extinct.

As a protective measure, the Turkish General Directorate of Nature Protection and National Parks (GDNP) established a 26 ha fenced captive breeding station in Ceylanpınar-Şanlıurfa in 1977. The gazelles reared in this station were later used to establish three further captive breeding stations in Şanlıurfa, Gaziantep and Adana (Turan 1977, Erkan & Göksu 1978, Turan 1984). At present, one of the two known wild populations of *G. marica* in Turkey occurs in Kızılkuyu Wildlife Protection Site near Şanlıurfa, with an estimated population of around 500 individuals, including 86 which were released for supplementation in 2005 from the captive breeding station in Şanlıurfa (Gürler 2009). During field studies started in 2007, a new gazelle population was discovered near Kırıkhan district of Hatay province, in hilly terrain extending to the Turkey-Syria border. The morphology of the individuals was observed in the field using spotting scopes and by examining photographs taken during field studies. Certain morphological features of this population showed a marked difference from the *G. marica* individuals in Şanlıurfa, with a darker and more distinct facial, flank and pygal stripes. Additionally, male horns rather resemble those of *G. gazella* being much wider apart at their bases than in *G. marica*. Horns grow more or less parallel, turning slightly forward at the tips, as described for *G. g. gazella*, while the horns of other subspecies of *G. gazella* are shorter and more bend outwards (Groves 1996). Due to absence of skull samples from the Hatay population, no comparisons of actual morphological measurements could be made.

In this study, the two remaining wild gazelle populations in Şanlıurfa and Hatay provinces of Turkey were studied genetically for the first time by sequencing the mtDNA cytochrome-*b* gene. The primary objective of the study is to determine the species status of the Hatay population using molecular markers, since the morphology was observed to have key differences from *G. marica*. The validity of the observation records from previous studies and their conclusions were tested by comparing mtDNA cytochrome-*b* gene sequences from Hatay and sequences of other *Gazella* obtained from GenBank. Convincing evidence was needed to settle the debate on the presence of a second gazelle species in Turkey. It was also important to confirm the specific status of the Şanlıurfa population, which is listed as *G. subgutturosa*, but recent studies suggest it belongs to *G. marica* (Wacher et al. 2011).

Material and Methods

Sampling and DNA extraction

Gazelle samples (n = 36) were collected from three populations: 1) captive-bred Kızılkuyu (Şanlıurfa) population whose founding individuals were brought from Ceylanpınar-Şanlıurfa captive breeding station in 1999, and used in supplementation of the remaining wild population in 2005, 2) wild Kızılkuyu-Şanlıurfa population (n = 5), 3) wild Hatay population (n = 3, Fig. 1). Sampling took place between 2005 and 2009. Samples were collected by cutting a small piece from the ear tips (≈ 0.2 cm) with a metal pincer. The samples were preserved in 96 % ethanol in 1.5 ml sterile tubes. DNA from ethanol-preserved tissue was extracted using DNeasy Tissue Extraction Kit (QIAGEN) according to the protocols of the manufacturer.

DNA amplification and sequencing

We amplified a 400 bp segment of the cytochrome-*b* gene of the mtDNA using the primers L14724 and H15149 (Kocher et al. 1989, Irwin et al. 1991). The DNA amplification was carried out in a total volume of 25 µl, containing 5 µl of template DNA (40 ng/µl), 25 mM $MgCl_2$, 1 µM of each primer, 0.2 mM of each dNTP, and 0.5 units of *Taq* polymerase (Fermantas). PCR cycles consisted of an initial denaturation at 95 °C for 4 minutes, followed by 35 cycles of 95 °C for 35 seconds, 50 °C for 35 seconds and 72 °C for 35 seconds. A final extension step was conducted at 72 °C for 5 minutes.

Sequencing of PCR products were done by using a BigDye Cycle Sequencing kit v 3.1 Applied Biosystems on an ABI PRISM 3130XL automated sequencer (Applied Biosystems). All PCR products were sequenced for both strands using L14724 and H15149 as sequencing primers. Sequencing PCR was performed in a total volume of 20 µl, containing 2 µl of each primer (2 µM), and 4 µl of mix containing fluorescent labelled dNTP, distilled water and template DNA. Twenty-five cycles were composed of denaturation at 96 °C, 30 s; hybridization at 50 °C, 30 s; and polymerization at 60 °C, 4 min. Dye terminators were removed by spin-column purification. Nucleotide sequence data reported are available in the GenBank databases under accession numbers JF719320-JF719321.

Genetic data analysis

The individual sequences were automatically aligned using ClustalW multiple alignment, implemented in the BioEdit package 7.0.9.0 (Hall 1999). Basic descriptive statistics and genetic diversity parameters such as haplotype diversity (h), nucleotide diversity (Pi), and number of polymorphic sites (Nei 1987) were calculated on the 400 bp sequences using the software DNASP v 4.5.0 (Rozas et al. 2003).

To trace the primary phylogenetic relationship among *Gazella* species, the 358 bp cytochrome-*b* gene sequences of seven *Gazella* species were obtained from GenBank (http://wwwncbinlmnihgov). The detailed information on GenBank sequences is as follows: *G. gazella* (Accesion number: GU384826, GU384835, GU384836, GU384840, GU384844, GU384856, GU384864, GU384866, GU384867, GU384869, GU384870), *G. subgutturosa* (AF187715, AF187716 and DQ269164), *G. marica* (AF187696 and AF187718), *G. dorcas* (AF187708 and AF187719), *G. saudiya* (AF187710 and AF187722), *G. leptoceros* (AF187699) and *G. bennettii* (AF187698). The downloaded sequences of five *Gazella* species were first aligned with those of Şanlıurfa and Hatay samples.

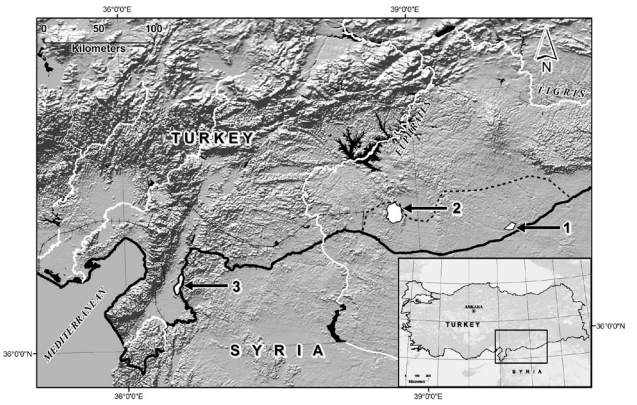

Fig. 1. *Map of southeastern Turkey, showing the locations of gazelle populations studied, 1: Ceylanpınar-Şanlıurfa, 2: Kızılkuyu-Şanlıurfa, 3: Hatay. Dotted lines surrounding the two populations of* G. marica *show the distribution of the species in Turkey around 1960s (Turan 1984).*

The average genetic distances between individuals and between groups were estimated by using MEGA version 4.0.2 (Tamura et al. 2007) and the Kimura's two parameter (K2P) model with a gamma correction (shape parameter = 0.5) for among site rate variation (Kimura 1980). Phylogenetic relationships were estimated by Maximum Likelihood (ML) and Bayesian analysis. The best-fitting models of sequence evolution were determined using Modeltest 3.7 (Posada & Crandall 1998) for reconstructions and MrModeltest 2.2 (Nylander 2004) for Bayesian Inference (BI). The GTR + GAMMA + P – Invar model parameters for DNA substitution were selected by Modeltest 3.7 under the AIC, BIC and hLRT criteria. The substitution model was incorporated into ML analysis using PAUP 40b10 (Swofford 1998). Support values were estimated by 1000 bootstrap replicates. MrModeltest 2.2 selected the HKY + G model under both the hLRT and AIC criteria. Using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), we generated a BI tree by two independent runs with four chains (three heated chains and a cold chain) each of which were run simultaneously for one million generations and trees were sampled every 100. Convergence of the chains was assessed by looking at a standard deviation of split frequencies (< 0.01) in MrBayes and average log-likelihood values analyzed in Tracer 1.5 (Rambaut & Drummond 2007). A burn-in period of 2500 was discarded before calculating the consensus

Table 1. *Kimura 2 - parameter genetic distances (lower semi matrix) and standard deviation (upper semi matrix) among the studied lineages of Gazella (Şanlıurfa, Hatay and GenBank samples) estimated from fragment of the mtDNA cytochrome-b gene (358 bp).*

Populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1. *G. marica* Şanlıurfa (34)	****	0.018	0.018	0.018	0.018	0.021	0.020	0.020	0.020	0.020	0.022	0.022	0.020	0.022	0.000	0.003	0.016	0.016	0.016	0.020	0.019	0.020	0.019	0.007	0.012
2. *G. gazella* Hatay (4)	0.073	****	0.000	0.003	0.003	0.009	0.008	0.007	0.008	0.008	0.008	0.008	0.008	0.008	0.018	0.018	0.014	0.014	0.014	0.016	0.015	0.014	0.014	0.015	0.012
3. *G. gazella* Wadi Sarek, Golan	0.073	0.000	****	0.003	0.003	0.009	0.008	0.007	0.008	0.008	0.008	0.008	0.008	0.008	0.018	0.018	0.014	0.014	0.014	0.016	0.015	0.014	0.014	0.015	0.012
4. *G. gazella* Wadi Sarek, Golan	0.077	0.003	0.003	****	0.000	0.010	0.009	0.007	0.008	0.009	0.009	0.009	0.009	0.009	0.018	0.019	0.015	0.015	0.015	0.016	0.015	0.015	0.015	0.016	0.013
5. *G. gazella* Afik, Golan	0.077	0.003	0.003	0.000	****	0.010	0.009	0.007	0.008	0.009	0.009	0.009	0.009	0.009	0.018	0.019	0.015	0.015	0.015	0.016	0.015	0.015	0.015	0.016	0.013
6. *G. gazella* Farasan Zifaf	0.086	0.025	0.025	0.028	0.028	****	0.005	0.007	0.004	0.003	0.008	0.008	0.003	0.008	0.021	0.022	0.016	0.016	0.016	0.018	0.017	0.016	0.016	0.018	0.014
7. *G. gazella* Al Khunfah	0.082	0.021	0.021	0.025	0.025	0.009	****	0.006	0.003	0.004	0.008	0.008	0.004	0.008	0.020	0.021	0.015	0.015	0.015	0.017	0.016	0.014	0.015	0.017	0.013
8. *G. gazella* Tabalah Bishah	0.082	0.015	0.015	0.018	0.018	0.015	0.012	****	0.005	0.006	0.004	0.004	0.006	0.004	0.020	0.021	0.015	0.015	0.015	0.017	0.016	0.015	0.015	0.017	0.013
9. *G. gazella* Al Wabra, Quatar	0.077	0.018	0.018	0.021	0.021	0.006	0.003	0.009	****	0.003	0.007	0.007	0.003	0.007	0.020	0.020	0.015	0.015	0.015	0.016	0.015	0.015	0.014	0.017	0.013
10. *G. gazella* Wadi Khulagb	0.082	0.021	0.021	0.025	0.025	0.003	0.006	0.012	0.003	****	0.007	0.007	0.000	0.007	0.020	0.021	0.015	0.015	0.015	0.017	0.016	0.016	0.015	0.017	0.013
11. *G. gazella* Farasan Kebir	0.091	0.021	0.021	0.025	0.025	0.022	0.018	0.006	0.015	0.018	****	0.000	0.007	0.004	0.022	0.023	0.016	0.016	0.016	0.018	0.017	0.017	0.016	0.019	0.015
12. *G. gazella* Al Hayla Tihama	0.091	0.021	0.021	0.025	0.025	0.022	0.018	0.006	0.015	0.018	0.000	****	0.007	0.004	0.022	0.023	0.016	0.016	0.016	0.018	0.017	0.017	0.016	0.019	0.015
13. *G. gazella* Makshush	0.082	0.021	0.021	0.025	0.025	0.003	0.006	0.012	0.003	0.000	0.018	0.018	****	0.007	0.020	0.021	0.015	0.015	0.015	0.017	0.016	0.016	0.015	0.017	0.013
14. *G. g. muscatensis* Oman	0.091	0.021	0.021	0.025	0.025	0.022	0.018	0.006	0.015	0.018	0.006	0.006	0.018	****	0.022	0.023	0.016	0.016	0.016	0.016	0.015	0.017	0.016	0.019	0.015
15. *G. marica* Abu Al Jir Iraq	0.000	0.073	0.073	0.077	0.077	0.086	0.082	0.082	0.077	0.082	0.091	0.091	0.082	0.091	****	0.003	0.016	0.016	0.016	0.020	0.019	0.020	0.019	0.007	0.012
16. *G. marica* Ramlat Fas	0.003	0.077	0.077	0.081	0.081	0.091	0.086	0.086	0.082	0.086	0.095	0.095	0.086	0.095	0.003	****	0.015	0.015	0.015	0.020	0.019	0.021	0.020	0.008	0.013
17. *G. subgutturosa* Iran	0.060	0.056	0.056	0.060	0.060	0.064	0.060	0.060	0.056	0.060	0.068	0.068	0.060	0.068	0.060	0.056	****	0.000	0.000	0.019	0.018	0.018	0.017	0.013	0.009
18. *G. subgutturosa* Samarra	0.060	0.056	0.056	0.060	0.060	0.064	0.060	0.060	0.056	0.060	0.068	0.068	0.060	0.068	0.060	0.056	0.000	****	0.000	0.019	0.018	0.018	0.017	0.013	0.009
19. *G. subgutturosa* Mongolia	0.060	0.056	0.056	0.060	0.060	0.064	0.060	0.060	0.056	0.060	0.068	0.068	0.060	0.068	0.060	0.056	0.000	0.000	****	0.019	0.018	0.018	0.017	0.013	0.009
20. *G. dorcas* Sudan	0.082	0.057	0.057	0.061	0.061	0.065	0.061	0.061	0.057	0.061	0.070	0.070	0.061	0.061	0.082	0.086	0.081	0.081	0.081	****	0.003	0.008	0.008	0.018	0.016
21. *G. dorcas* Egypt	0.077	0.053	0.053	0.057	0.057	0.061	0.057	0.057	0.054	0.057	0.065	0.065	0.057	0.057	0.077	0.082	0.077	0.077	0.077	0.003	****	0.008	0.007	0.017	0.016
22. *G. saudiya* Wadi Markha	0.086	0.053	0.053	0.057	0.057	0.061	0.050	0.057	0.054	0.057	0.065	0.065	0.057	0.065	0.086	0.091	0.073	0.073	0.073	0.022	0.018	****	0.003	0.019	0.016
23. *G. saudiya* Dhalm	0.082	0.049	0.049	0.053	0.053	0.057	0.054	0.054	0.050	0.054	0.061	0.061	0.054	0.061	0.082	0.086	0.068	0.068	0.068	0.018	0.015	0.003	****	0.018	0.015
24. *G. leptoceros* Egypt	0.018	0.057	0.057	0.061	0.061	0.070	0.065	0.065	0.061	0.065	0.074	0.074	0.065	0.074	0.018	0.021	0.045	0.045	0.045	0.074	0.070	0.078	0.074	****	0.010
25. *G. bennettii* Turbat Pakistan	0.041	0.045	0.045	0.049	0.049	0.053	0.049	0.049	0.045	0.049	0.056	0.056	0.049	0.056	0.041	0.045	0.028	0.028	0.028	0.064	0.060	0.064	0.060	0.028	****

tree. Bayesian posterior probabilities (BPP) were used to assess the branch support of the BI tree. The tree was rooted with *Gazella subgutturosa* (DQ269164).

Results

Genetic studies

A fragment of 400 bp of cyctochrome-*b* for the samples of *G. marica* (Şanlıurfa, n = 33) and *G. gazella* (Hatay, n = 3) was revealed in this study. There was no nucleotide diversity within both of the populations. Analysis of 36 sequences yielded two haplotypes, one for each population with 23 variable sites. The transition: transversion ratio between two haplotypes were k_1 = 13233 (purines) and k_2 = 15431 (pyrimidines). Base composition of cyctochrome-*b* sequences of 33 samples in Şanlıurfa (A = 0.305, C = 0.260, T = 0.285, G = 0.150) was similar to that of the Hatay samples (A = 0.310, C = 0.275, T = 0.268, G = 0.147). The overall transition/transversion bias was *R* = 5885. The two haplotypes differed by three transitions at the 400 nucleotides. The overall genetic distance between the Şanlıurfa and Hatay populations was around 7 % (0073 ± 0018) according to the K2P with gamma correction (shape parameter = 0.5).

To estimate the primary phylogenetic relationship among *Gazella* species, the cytochrome-*b* gene sequences (n = 23) of seven *Gazella* species were obtained from GenBank and from the sequences (n = 2) obtained in this study. A total of 25 sequences were aligned, with 52 variable sites and 18 haplotypes. Distance values among *Gazella* species obtained by using the K2P with gamma correction (shape parameter = 0.5) are shown in Table 1. Genetic distances (D) between Şanlıurfa and *G. marica* sequences were 0-0.003, and with *G. leptoceros* were 0.014, while those between Şanlıurfa and *G. s. subgutturosa* from Iraq, Iran and Mongolia were 0.060. The genetic distance between the Şanlıurfa population and other *Gazella* species was found to range from 0.041 to 0.910. No, or very small differences were detected between Hatay samples and *G. g. gazella* from Golan, but the Hatay samples differed from all other *G. gazella* subspecies by D = 0.015-0.025 (Table 1).

Phylogenetic analyses

The phylogenetic relationships among *Gazella* species estimated by using ML and BI are presented in Fig. 2. Four monophyletic lineages were found in the phylogenetic trees, supported by bootstrap values (Fig. 2). A monophyletic lineage which includes *G. gazella* and its subspecies and the Hatay samples is well supported [ML bootstrap (MLb) = 96 %, BI posterior probabilities (pp) = 100 %]. However, the sub-cluster group is diverged into two reciprocally monophyletic groups. While one of these two clades (*G. g. gazella*) includes all individuals from Hatay and the Golan Heights (MLb = 96 % and BI pp = 100 %), the second clade consisted of all other *G. gazella* individuals from different localities on the Arabian Peninsula (MLb = 62 % and BI pp = 98 %). The second monophyletic lineage comprising samples from *G. dorcas* and *G. saudiya* is well supported (MLb = 99 % and BI pp = 100 %). The third clade including *G. subgutturosa* and *G. bennettii* is slightly supported (MLb = 64 %, BI pp = 84 %). The latter clade comprising samples from *G. leptoceros*, *G. marica* and Şanlıurfa is well supported (MLb = 99 % and BI pp = 100 %). One of these lineages contains individuals of *G. marica* and the Şanlıurfa samples and this taxon shows a closer relationship to individuals from *G. leptoceros* (MLb = 99 %, BI pp = 100 %).

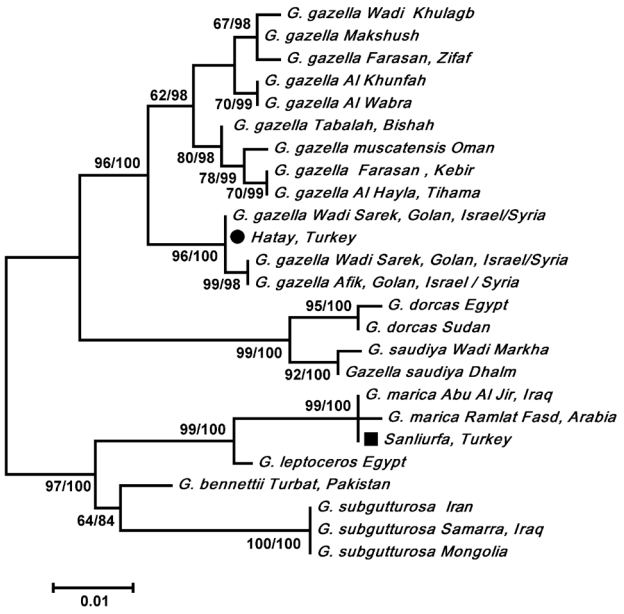

Fig. 2. *Phylogenetic tree reconstructed by the Maximum likelihood (ML) methods based on the cytochrome-*b *nucleotide sequences. Bootstrap support values (left) from ML and posterior probabilities (right) from Bayesian inference are given on branches.*

Discussion

The results of this study suggest that *Gazella* individuals from the Hatay region belong to a different lineage than the *G. marica* individuals from Şanlıurfa. In the phylogenetic analyses, *G. gazella* comprises

a monophyletic genetic lineage and the sub-cluster group is diverged into two reciprocally monophyletic clades. Hatay individuals were grouped with samples from the Golan Heights (Fig. 2). Wronski et al. (2010) remarked that *G. gazella* diverged in two reciprocally monophyletic genetic lineages. These clades were a northern clade from the Golan Heights and a clade comprising all other *G. gazella* from the Arabian Peninsula and the Arava Valley in the southern Negev. Similarly, Rebholz & Harley (1999) found a genetic difference of 19-25 % between *G. g. gazella* and other *G. gazella* subspecies and mentioned the geographical and reproductive isolation between them.

Palestine mountain gazelle (*G. g. gazella*) phenotypically differs in coloration, size, horn and skull morphometrics from other subspecies of *G. gazella* (Groves 1996). Currently, these animals have a distribution from Northern Israel (Galilee to southern Jerusalem), via the Golan Heights along the Dead Sea Valley and previously into southern Lebanon and southeastern Syria (Mendelssohn et al. 1995, Groves 1997). While Groves (1997) reported that the largest population of *G. g. gazella* is found in northern Israel, and that the species once inhabited southern Lebanon, the IUCN Antelope specialist group report (IUCN 2008) considered the species to be extinct in Lebanon. Mallon & Kingswood (2001) reported that *G. g. gazella* was found in mountains of southwest Syria, but no records have been obtained from the region since the 1970s due to extensive poaching. However, records of *G. g. gazella* were last reported from northwestern Syria (Choula and Jabal Shuah; Green et al. 1991, Kingswood et al. 2001). These records are in close proximity to the Hatay population, i.e. the same mountain range (Lebanon Mountains and Nuşayriyah Mountains) on the Syrian side of the border. The Hatay region comprises hilly terrain as a continuation of the low mountain range that extends from Lebanon to southwest Syria. Our K2P test and phylogenetic results indicated that the Hatay population is closely related to the *G. g. gazella* found in Northern Israel (Fig. 2 and Table 1). The Hatay individuals therefore became an isolated population of *G. g. gazella* once the geographically intermediate Syrian population was extirpated.

Following some observations from west of the Euphrates Valley, Adana, Ceyhan and Hatay regions, the Hatay population was previously assigned to the species *G. dorcas* (Danford & Alston 1877, Misonne 1957, Harrison & Bates 1991, Kryštufek & Vohralík 2001, Albayrak et al. 2007). However, the Hatay population is the only *Gazella* population present in this area and our study found no genetic relationship between the individuals studied here and *G. dorcas*. Further, records indicate that the distribution of *G. dorcas* is restricted to the south of the Sinai Peninsula and Israel (Yom-Tov et al. 1995, Groves 1997). We therefore, propose that also the populations that were once found in the Adana and Ceyhan regions, but no longer exist today, belonged to *G. g. gazella*.

In the literature, the Şanlıurfa population has been assigned to *G. subgutturosa* (Kumerloeve 1975a, Turan 1984, Kasparek 1986, Kaya & Dikmenli 2000, Ölçer 2001). The phylogenetic analyses of Şanlıurfa samples and *G. subgutturosa* sequences from GenBank showed a polyphyletic structure for *G. subgutturosa* (Fig. 2). Şanlıurfa individuals were grouped with samples of *G. marica* and *G. subgutturosa* is more distantly related than previously thought. Similarly, Wacher et al. (2011) stated that the species *G. subgutturosa* is a polyphyletic assemblage composed of two distinct clades. These clades are *G. marica* from the Arabian Peninsula, Iraq, Jordan, Syria and Turkey and *G. s. subgutturosa* in Iraq, Iran, Afghanistan, Azerbaijan and Chinese Turkestan. Therefore Wacher et al. (2011) suggested that *G. subgutturosa marica* appear to form a reasonably distinct conservation unit and recommended to render full species status under the name *G. marica*. Our findings support this recommendation and the suggestion of Wacher et al. (2011), proposing that *G. s. subgutturosa* and *G. marica* have evolved independently.

Implications for conservation and management

Our results suggest that the population in the Şanlıurfa region belongs to the newly proposed species *G. marica* rather than *G. subgutturosa*. Additionally a second species, *G. gazella*, was genetically rediscovered in Hatay region. Our results thus indicate two distinct conservation units, which should be the subjects of individually directed conservation efforts.

Until today all the conservation efforts were directed to the captive breeding of individuals in the Şanlıurfa region, which our results show to be *G. marica*. However, we consider that maintaining a viable population in the wild should be one of the main goals in conservation of *G. marica*. There have also been translocations of individuals from the Şanlıurfa population – which were thought to belong to *G. subgutturosa* – to Georgia, where *G. subgutturosa* occurred in the past. Since the Şanlıurfa population has now been confirmed to belong to *G. marica*,

further translocation of individuals to Georgia or other northern regions within and outside Turkey, or vice versa, should be discontinued.

The *G. gazella* population in Hatay is the northernmost population of the mountain gazelle and urgent field studies are needed to determine the major threats. It seems likely that this population has been able to survive only because it uses an area on the border between Turkey and Syria, where local human activities such as herding and farming are much reduced. Relevant conservation measures are needed through collaboration between the Turkish and Syrian authorities and local people. In the short term, it is recommended to establish a protected area in the border region between Turkey and Syria as then active measures against illegal hunting are more practicable. Fortunately, our results have already been noted by those responsible in government agencies, and translocation of individuals from Hatay to Şanlıurfa has been prevented.

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