TÜBİTAK

Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2016) 40: 957-971
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doi:10.3906/zoo-1507-45

Mitochondrial DNA control region variability of wild boar *Sus scrofa* with various external phenotypes in Turkey

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Received: 31.07.2015 • **Accepted/Published Online:** 28.01.2016 • **Final Version:** 06.12.2016

Abstract: The wild boar (*Sus scrofa* L.) is distributed across most parts of Turkey, a major biogeographic crossroads connecting Southwest Asia and Southeast Europe. However, no information on genetic diversity and population structure of this species in Turkey is available. In this study, we report on mtDNA sequence variability and phylogenetic relationships among wild boars with variable external phenotypes from both its European (Turkish Thrace) and Asian (Anatolia) distributions in Turkey. Phylogenetic analyses of mtDNA D-loop sequences (413 bp) of 53 newly sequenced wild boars from different localities in Turkey and 432 wild boar sequences from various geographic origins downloaded from GenBank were performed to particularly compare the phylogeographic position of wild boars from the European part of the Turkish range with that of specimens from Anatolia and to explore a possible phylogeographic substructuring in Anatolia. Relatively high genetic diversity was found in the Turkish samples, with a total of 17 haplotypes. Phylogenetic analyses revealed partitioning of the currently found Turkish haplotypes into two haplogroups, which were, however, only partially concordant with the geographic origins of samples (central and southwestern Anatolia vs. Turkish Thrace and northeastern and southeastern Anatolia). A median-joining network grouped most Turkish haplotypes with those previously reported from the Near East, whereas the remaining two haplotypes were included in the European 1 haplogroup. The combined phylogenetic analysis of the currently obtained sequences and all sequences retrieved from GenBank supported the earlier findings of four major haplogroups. The present study will serve as a baseline for more comprehensive studies to understand phylogenetic relationships of wild boars in Turkey and the Near East.

Key words: *Sus scrofa*, wild boar, mtDNA control region, coat color, Turkey

1. Introduction

Among wild-living ungulates, the wild boar (*Sus scrofa* L.) is one of the most adaptable species with a wide natural distribution in the Palearctic; it has been successfully introduced in diverse regions globally, including the United States, Australia, Argentina, Uruguay, Brazil, and several parts of the tropics (Genov, 1999; Wilson and Reeder, 2005; Grossi et al., 2006; Oliver and Leus, 2008; Garcia et al., 2011). Currently, wild boar populations are increasing in their natural environments in many regions due to changes in agricultural practices, artificial feeding, and progressive decrease in predator numbers (Sáez-Royuela and Tellería, 1986; Ferreira et al., 2009; Apollonio et al., 2010; Scandura et al., 2011; Kusza et al., 2014). This increase may also, at least partially, be a result of the global increase in mean environmental temperatures (Root et al., 2003). The recent expansion of wild boar populations in Europe has stimulated studies of their genetic diversity and structure in order to develop adequate management strategies (Veličković et al., 2014). The increase of wild boar populations has been registered in hunting areas and rural areas in Turkish Thrace and Anatolia, as well. This has led to substantial damage to agricultural crops and forestry, and even problems in urban habitats. As a result, effective management strategies for wild boars are necessary, as this species is considered to be a pest in many parts of the world (Bieber and Ruf, 2005; Scandura et al., 2011; Hajji and Zachos, 2011).

Corbet (1978) stated that many races of *Sus scrofa* in the Palearctic were named based on differences in size and color; however, these subpopulations were never shown to have discrete boundaries. To date, 16 different subspecies have been recognized worldwide, including four geographical groups (Western, Indian, Eastern, and

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Indonesian) (Groves, 1981). The subspecies status of *S. scrofa* in Europe was discussed by Scandura et al. (2011). Groves and Grubb (1993) classified the European wild boar under a group of 'Western races', including the subspecies *S. s. scrofa* (Central-Western Europe), *S. s. meridionalis* (Sardinia and Corsica), *S. s. attila* (Eastern Europe), and *S. s. libycus* (Southern Balkans and Middle East). The taxonomic status of subspecies of wild boars still remains uncertain and several molecular studies have suggested the existence of more subspecies in both Europe and Asia (Alves et al., 2010).

Turan (1984) stated that *Sus scrofa* is the only taxon that is found across Turkey. Demirsoy (1996) recorded that the geographic variations of wild boars from Turkey were not known. Ellerman and Morrison-Scott (1951), Mohr (1960), and Mursaloğlu (1964) indicated that wild boars from Turkey were represented by *Sus scrofa libycus*. Genov (1999) included wild boars from Anatolia into *S. scrofa scrofa*. İnci et al. (2013) reported that morphometric data of specimens examined in Central Anatolia were consistent with those of Turkish wild boar belonging to the nominate subspecies, *S. scrofa scrofa*, but that its taxonomy needed further investigation. According to karyotypic results given by Albayrak and İnci (2007), the Turkish wild boar is different from Central and Western Continental European specimens in having 36 chromosomes and is identical to those from East and Southeast Europe and the Mediterranean islands, and to domestic pig.

Due to its position between the Eastern Mediterranean, the Black Sea, the Caucasus region, and eastern and southeastern parts of the Middle East, Turkey can be considered as a biogeographic crossroads connecting several major faunal regions. Hence, quite high levels of genetic polymorphism can be expected in wild boar populations from Anatolia and Thrace. Despite extensive literature data on its distribution (Steiner and Vauk, 1966; Huş, 1967; Ererçin, 1977; Kumerloeve, 1978), there is no information about the genetic diversity and structure of wild boar populations in Turkey. Wild boar is distributed across most areas in Turkey; however, information about possible regionally different phylogenetic lineages is not available. Moreover, there is only limited information on phylogenetic relationships of the Turkish populations with those from other regions in Europe and/or the Middle East (Larson et al., 2005; Alves et al., 2010; Alexandri et al., 2012; Meiri et al., 2013; Veličković et al., 2015). The aim of this study is to give a first account of the mitochondrial D-loop sequence variation and phylogenetic relationships of wild boars with different phenotypes in Turkey as a baseline for future comparative studies of wild boar, particularly in the Middle East.

2. Materials and methods

2.1. Sampling and DNA extraction

This study was carried out with permission taken from of the General Directorate of Nature Conservation and National Parks of the Ministry of Forestry and Waterworks (permit number: 221163). Muscle and ear tissues of 70 wild boars were collected from 34 different sampling areas in Turkey during regular hunts between September 2013 and February 2015. Sequencing of 413 bp of the mtDNA control region was successful in 53 individuals (Figure 1; Table 1). Additionally, 432 D-loop sequences of wild boar were downloaded from GenBank (National Center for Biotechnology Information, USA) and their respective haplotype numbers were listed according to Veličković et al. (2015) (Table 2). The mitochondrial DNA sequences of *Sus barbatus* [GenBank accession number: AJ314540 (Randi et al., 2002)] and *Phacochoerus aethiopicus* [GenBank accession number: AB046876 (Okumara et al., 2001)] were included as two outgroup taxa in the analysis. Genomic DNA was isolated from tissues (stored at –20 °C prior to the analysis) by using standard phenol chloroform extraction (Sambrook and Russel, 2001). During field work, coat colors and patterns of 70 individuals were also recorded. Age determination of individuals was based on external body measurements and carnassial length according to İnci (2003). Only adults were used to evaluate phenotypic features.

2.2. DNA amplification and sequencing

In DNA amplification we followed the procedures mentioned by Veličković et al. (2015). A fragment of the mtDNA control region between sites 15378 and 15900 [based on the reference sequence with GenBank accession number AJ002189 (Ursing and Arnason, 1998)] was amplified with primers 5′-GGAGACTAACTCCGCCATCA-3′ (forward) and 5′-TGGGCGATTTTAGGTGAGATGGT-3′ (reverse). PCR reactions were performed in a total volume of 20 µL, containing 50 ng of DNA, 1X reaction buffer (Thermo Scientific, Vilnius, Lithuania), 2.5 mM $MgCl_2$, 100 µM of each dNTP, 0.10 µM of each primer, and 1 U of Taq DNA polymerase (Fermentas). Touchdown PCR was performed with the following conditions: 94 °C for 4 min; 20 cycles of 94 °C for 30 s, 65–55 °C for 30 s, and 72 °C for 30 s; 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 10 min. The PCR products were purified by using ExoSAP (Fermentas) following the manufacturer's recommendations. Sequencing was conducted on an ABI 3100 Genetic Analyzer.

2.3. Data analysis

The first analyses were conducted to evaluate the genetic diversity and population structure of wild boar in Turkey. These analyses included the newly generated sequences and 61 Turkey wild boar sequences retrieved

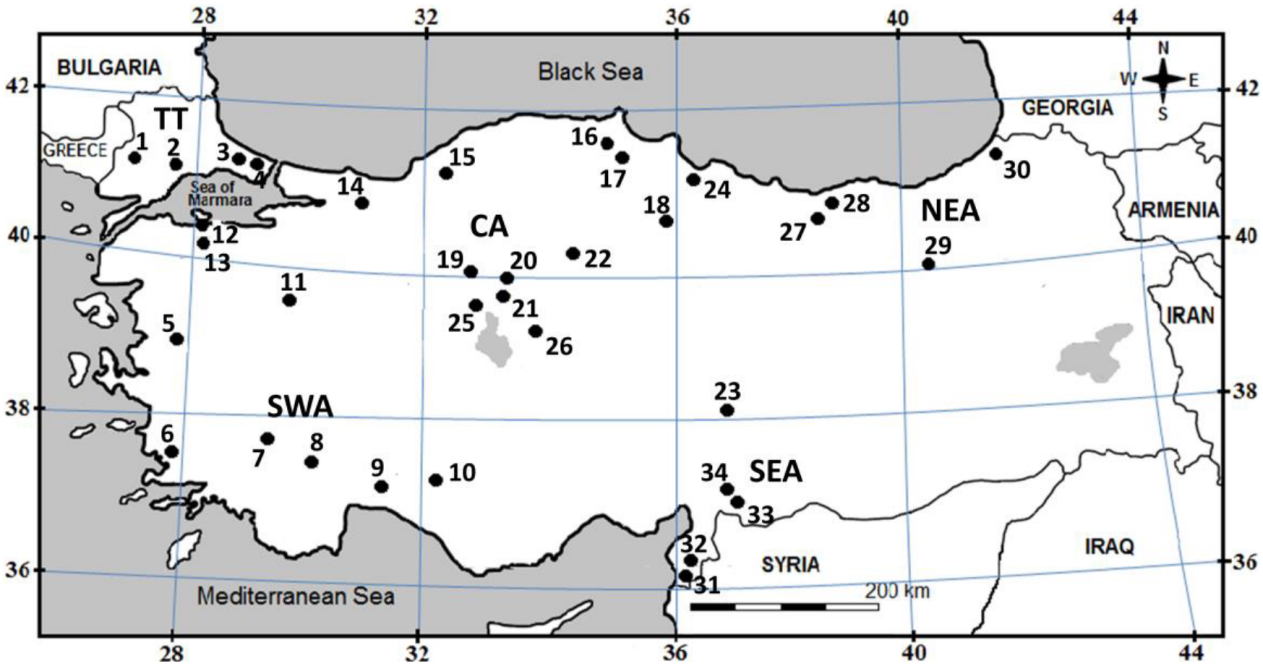

Figure 1. Location map of the 70 samples examined in this study: black dots indicate local samples of one or more individuals and numbers refer to locality names in Table 1. TT (Turkish Thrace, 1–4), SWA (Southwestern Anatolia, 5–11), CA (Central Anatolia, 12–26), NEA (Northeastern Anatolia, 27–30), and SEA (Southeastern Anatolia, 31–34).

Table 1. Specimens examined (n = 70) and the localities they were collected from. Asterisks indicate only localities of individuals sequenced.

Map number	Locality	D-loop haplotype	n	Map number	Locality	D-loop haplotype	n
1	Keşan, Edirne*	TR1	2 ??	18	Centrum, Amasya*	TR8	1 ?
2	Süleymanpaşa, Tekirdağ	-	1 ?	19	Kalecik, Ankara*	TR8	3 ♀♀
3	Arnavutköy, İstanbul*	TR2,TR3	2 ♀♀	20	Balışeyh, Kırıkkale*	TR8	7 ♀♀, 5 ♂♂
4	Sarıyer, İstanbul*	TR2	1 ♀	21	Keskin, Kırıkkale*	TR8	1 ♂
5	Akhisar, Manisa*	TR4, TR5	1 ♀, 1 ♂	22	Sungurlu, Çorum	-	1 ?
6	Milas, Muğla*	TR4	1 ♀, 1 ♂	23	Göksun, Kahramanmaraş*	TR8	2 ♀♀, 1 ♂
7	Centrum, Denizli	-	1 ♂	24	Tekkeköy, Samsun*	TR10	2 ♀♀
8	Tefenni, Burdur*	TR6	3 ??	25	Bala, Ankara*	TR11	1 ♂
9	Manavgat, Antalya*	TR7	2 ??	26	Kaman, Kırşehir*	TR12	1 ♀, 1 ♂
10	Alacabel, Antalya	-	1 ?	27	Bulancak, Giresun*	TR13	1 ♂
11	Tavşanlı, Kütahya	-	1 ♂	28	Centrum, Giresun*	TR13	1 ?
12	Erdek, Balıkesir*	TR8	5 ♀♀, 2 ♂♂	29	Otlukbeli, Erzincan*	TR13	1 ♂
13	Bandırma, Balıkesir	-	1 ♂	30	Arhavi, Artvin*	TR8	1 ?
14	Kaynarca, Sakarya*	TR8, TR9	2 ♂♂, 2 ??	31	Yayladağı, Hatay*	TR14	1 ♀
15	Centrum, Zonguldak*	TR8	2 ♂♂	32	Centrum, Hatay	-	1 ♀
16	Ayancık, Sinop*	TR8	1 ♀, 2 ??	33	Musabeyli, Kilis*	TR14	1 ♂
17	Erfelek, Sinop	-	1 ♂	34	Islahiye, Gaziantep*	TR15	1 ♂

Table 2. List of wild boar (*Sus scrofa*) control region sequences obtained in this study and downloaded from GenBank.

Haplotype number	Haplotypes from GenBank	Haplotypes from Turkey (present study)	Haplogroup	Source
1	H93	-	A	Larson et al. (2005)
2	H103	-	A	Larson et al. (2005)
3	H112	-	A	Larson et al. (2005)
4	H125	-	A	Larson et al. (2005)
5	H130	-	A	Larson et al. (2005)
6	H131	-	A	Larson et al. (2005)
7	H148	-	A	Larson et al. (2005)
8	H154	-	A	Larson et al. (2005)
9	H48*/**, H156*	TR4	NE	Larson et al. (2007)*, Alexandri et al. (2012)**
10	H49	TR5	NE	Larson et al. (2007), Alexandri et al. (2012)
11	H50	-	NE	Alexandri et al. (2012)
12	H100	TR8	NE	Larson et al. (2007), Ottoni et al. (2013)
13	H102	-	NE	Larson et al. (2007), Ottoni et al. (2013)
14	H133	-	NE	Larson et al. (2005)
15	H135	-	NE	Larson et al. (2005)
16	H139	-	NE	Larson et al. (2005), Larson et al. (2007)
17	H152	-	NE	Larson et al. (2007)
18	H160	-	NE	Kusza et al. (2014)
19	H164	TR13	NE/E1***	Ottoni et al. (2013), present study***
20	H165	-	NE	Ottoni et al. (2013)
21	H168	-	NE	Ottoni et al. (2013)
22	H69	-	E2	Larson et al. (2005), Scandura et al. (2008)
23	H70	-	E2	Giuffra et al. (2000), Larson et al. (2005), Scandura et al. (2008)
24	H71	-	E2	Scandura et al. (2008)
25	H79	-	E2	Larson et al. (2005), Scandura et al. (2008)
26	H138	-	E2	Larson et al. (2005)
27	H1	-	E1	Alexandri et al. (2012), Kusza et al. (2014)
28	H2	TR15	E1	Larson et al. (2005), Larson et al. (2007), Scandura et al. (2008), Alexandri et al. (2012), Ottoni et al. (2013), Veličković et al. (2015)
29	H9	TR1	E1	Alexandri et al. (2012)
30	H10	-	E1	Alves et al. (2003), Larson et al. (2005), Fang et al. (2006), Scandura et al. (2008), Alves et al. (2010), van Asch et al. (2011), Alexandri et al. (2012), Kusza et al. (2014), Veličković et al. (2015)
31	H11	-	E1	Alexandri et al. (2012)
32	H12	-	E1	Alexandri et al. (2012)
33	H13	-	E1	Alexandri et al. (2012)
34	H15	-	E1	Alexandri et al. (2012)
35	H17	-	E1	Alexandri et al. (2012)

Table 2. (Continued).

36	H20	-	E1	Alexandri et al. (2012)
37	H21	-	E1	Alexandri et al. (2012)
38	H22	-	E1	Alexandri et al. (2012)
39	H31	-	E1	Larson et al. (2005), Fang et al. (2006), Larson et al. (2007), Scandura et al. (2008), Alves et al. (2010), Alexandri et al. (2012), Veličković et al. (2015)
40	H34	-	E1	Alexandri et al. (2012)
41	H43	-	E1	Alexandri et al. (2012), Veličković et al. (2015)
42	H52	-	E1	Alexandri et al. (2012), Veličković et al. (2015)
43	H55	-	E1	Alexandri et al. (2012)
44	H57	-	E1	Larson et al. (2005), Scandura et al. (2008), Veličković et al. (2015)
45	H59	-	E1	van Asch et al. (2011), Veličković et al. (2015)
46	H60	-	E1	Larson et al. (2007), Scandura et al. (2008), Alves et al. (2010), Ottoni et al. (2013), Kusza et al. (2014), Veličković et al. (2015)
47	H64	-	E1	van Asch et al. (2011)
48	H65	-	E1	Alves et al. (2010), van Asch et al. (2011)
49	H67	-	E1	Larson et al. (2005), van Asch et al. (2011)
50	H73	-	E1	Scandura et al. (2008)
51	H75	-	E1	Larson et al. (2005), Fang et al. (2006), Scandura et al. (2008), Alves et al. (2010), Kusza et al. (2014)
52	H80	-	E1	Scandura et al. (2008)
53	H84	-	E1	Alves et al. (2010)
54	H86	-	E1	Alves et al. (2003), Fang et al. (2006), Alves et al. (2010), Kusza et al. (2014)
55	H87	-	E1	Alves et al. (2010)
56	H141	-	E1	Larson et al. (2005), Larson et al. (2007), Fang et al. (2006)
57	H143	-	E1	Fang et al. (2006)
58	H157	-	E1	Kusza et al. (2014)
59	H166	-	E1	Ottoni et al. (2013)
60	-	TR6	NE	Present study
61	-	TR11	NE	Present study
62	-	TR12	NE	Present study
63	-	TR10	NE	Present study
64	-	TR9	NE	Present study
65	-	TR14	NE	Present study
66	-	TR2	E1	Present study
67	-	TR3	E1	Present study
68	-	TR7	NE	Present study
Outgroup1	*Sus barbatus*, AN:AJ314540	-	-	Randi et al. (2002)
Outgroup2	*Phacochoerus aethiopicus*, AN:AB046876	-	-	Okumura et al. (2001)

from GenBank (Larson et al., 2005, 2007; Ottoni et al., 2013). A multiple alignment of the D-loop sequences was performed using the Clustal W algorithm implemented in MEGA 6 (Tamura et al., 2013), and final adjustments were done by eye. The total sequence information obtained for the alignment was 395 bp. Our samples were divided into five regional groups according to their geographical proximity and biogeographical features of the study area (abbreviation for each group is given in parentheses): Turkish Thrace (TT), Central Anatolia (CA), Southwestern Anatolia (SWA), Northeastern Anatolia (NEA), and Southeastern Anatolia (SEA) (Figure 1). Basic molecular indices such as number of haplotypes (nh), haplotype diversity (h), nucleotide diversity (π), mean number of pairwise differences (k), and number of segregating sites (S) were calculated using DnaSP 5.10.01 (http://www.ub.edu/dnasp/). Population structure between and within the analyzed groups was estimated by analysis of molecular variance (AMOVA), as implemented in ARLEQUIN 3.5 (Excoffier and Lischer, 2010). ARLEQUIN 3.5 was also used for neutrality analysis as Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997). Bayesian analysis with MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) was based on the best model of nucleotide substitution for all datasets as found by Modeltest 3.7 (Posada and Crandall, 1998). The Bayesian tree was conducted for 1 million generations, with sampling every 1000 and with a burn-in of 25%. A maximum likelihood (ML) phylogenetic tree of Turkish haplotypes based on the HKY+I model was constructed by MEGA 6. The robustness of the trees was determined by bootstrap resampling (1000 replicates) (Felsenstein, 1985).

Phylogenetic analyses were also performed with an additional 432 wild boar sequences downloaded from GenBank (Table 2). The final alignment of the combined dataset comprised 395 bp. A Bayesian phylogenetic model was developed with MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) using the best model of nucleotide substitution for all datasets as found by Modeltest 3.7 (Posada and Crandall, 1998). The Bayesian tree was conducted for 18 million generations, with sampling every 1000 generations and with a burn-in of 25%. In addition, a ML phylogenetic tree based on the HKY+G+I model was constructed using MEGA 6, and a median-joining (MJ) network of haplotypes was created using NETWORK 4.5.1.0 (Bandelt et al., 1999) to represent the intraspecific genealogy of the haplotype dataset, by allowing for potential alternative evolutionary pathways.

3. Results

A fair number of external phenotypes (i.e. coat colors) were recorded among specimens from different regions of Turkey. Based on our observations in the field, the dominant coat colorations in different regions were as follows: grayish brown and pale yellowish brown in Turkish Thrace; slightly grayish/blackish or yellowish light brown in Central Anatolia; light gray/grayish light brown/pale brownish yellow/black and brown in Western Anatolia; slightly blackish light brown/pale brownish yellow in the Black Sea region, and slightly yellowish pale light brown/pale light yellowish brown or blackish brown in the western part of Southeastern Anatolia. Furthermore, distinct coat coloration differences did occur within wild boar populations. Notably, in Western Anatolia, the Black Sea region, and Kahramanmaraş Province (with a specific microclimate between Central and Southeastern Anatolia) with warm climates, wild boars had very short hairs during the hot season and, as a result, they had a different phenotype, resembling the external body view of the domestic pig (Figure 2).

Among all 485 sequences analyzed for the D-loop, 68 haplotypes (H1–H68) were defined by 45 variable sites and 34 parsimony-informative sites. The average ratio of transition/transversion (R) was 9.569 (nucleotide composition was A = 35.67%, T = 26.65%, C = 24.71%, and G = 12.98%). The sequences from 114 individuals of Turkish wild boar represented 17 haplotypes (TR1–TR15, H165, and H168) based on variation at 15 variable sites and 14 parsimony-informative sites. The average ratio of transition/transversion (R) was 6.772 (nucleotide composition was A = 35.28%, T = 26.59%, C = 24.94%, and G = 13.19%). Of the 17 haplotypes from Turkey, nine were new (TR2, TR3, TR6, TR7, TR9–TR12, and TR14) and so far had not been reported in any wild boar population (Tables 1 and 2). Haplotype diversity (h) of the Turkish wild boars was 0.723 ± 0.03, nucleotide diversity (π) was 0.0117 ± 0.0003, and mean number of pairwise differences (k) was 4.528.

The ML and Bayesian trees revealed two main haplogroups of the Turkish wild boars. One haplogroup was composed of haplotypes from CA, SWA, and Artvin in NEA. The second haplogroup was composed of haplotypes from TT, SEA, and NEA (Figures 3 and 4). Both phylogenetic trees showed similar topologies, but the Bayesian tree supported the topology by higher posterior probability values (75%–100%) than the bootstrap values (31%–75%) of the ML tree (Figures 3 and 4). AMOVA provided strong support for the differentiation into those two haplogroups (85.90%, $P < 0.0001$, $F_{ST} = 0.86$) (Table 3).

The ML tree for the complete dataset (including all downloaded sequences) did not clearly reveal four main haplogroups (Asian (A), Near Eastern (NE), European 1 (E1), and European 2 (E2)), as might have been expected according to previously published data (e.g., as summarized by Veličković et al. (2015)), and the Bayesian tree did not convincingly resolve the Asian (A), Near Eastern (NE/CA & SWA), and European (E2) lineages (Figures 5 and 6).

Figure 2. Various phenotypes of obtained Turkish wild boar individuals from different localities studied presently.

On the other hand, the MJ network resolved the four main haplogroups (A, NE, E1, and E2) convincingly, with the majority of the haplotypes generated in this study clustering in the NE haplogroup, and two in the E1 haplogroup (Figure 7; haplotypes TR13 (H19) and TR15 (H28) were assigned to the E1 haplogroup, although they were found in Northeastern and Southeastern Anatolia). Additionally, the network showed that the Turkish haplotypes TR2 (H66) and TR14 (H65) connected between the E1 haplogroup and the NE haplogroup. Those two haplotypes have so far been found only in the European part of the İstanbul region (TR2: H66) and in Southeastern Anatolia (TR14: H65).

Tajima's D and Fu's Fs values were calculated to determine the demographic history of the observed Turkish haplogroups. Both of the haplogroups had negative values for Tajima's D and Fu's Fs, indicating population expansion (Table 4).

4. Discussion

Many cases of intraspecific subdivisions are known to exist within Anatolia as well as between Anatolia and Thrace as a result of reduced gene flow due to migration barriers such as mountain chains (the Anatolian Diagonal, the Taurus Mountains, and the Black Sea Mountains), the Central Anatolian Plateau, the Central Anatolian Lake system, and the Sea of Marmara (Bilgin, 2011). For the regions examined so far in Turkey, distinct genotypes are present in many species, and in a number of cases more easterly genome elements do occur from the Black Sea to the Caspian Sea (Hewitt, 1999). In addition to the Balkans, Turkey has acted as an ice-age refugium for many temperate mammalian species (Alexandri et al., 2012).

For wild boar, demographic trends and the genetic composition of populations have been affected by human-induced habitat transformations, game practices, and agricultural development in Europe (Randi, 2005). For wild boar in Turkey, however, no large-scale geographic data exist that may allow conclusions on the distribution of its genetic diversity. In this study we present the first geographically meaningful data analyses for assessing the genetic diversity and phylogenetic status of Turkish wild boar.

The current variability of partial mitochondrial control region sequences (h = 0.723, among 114 individuals) of

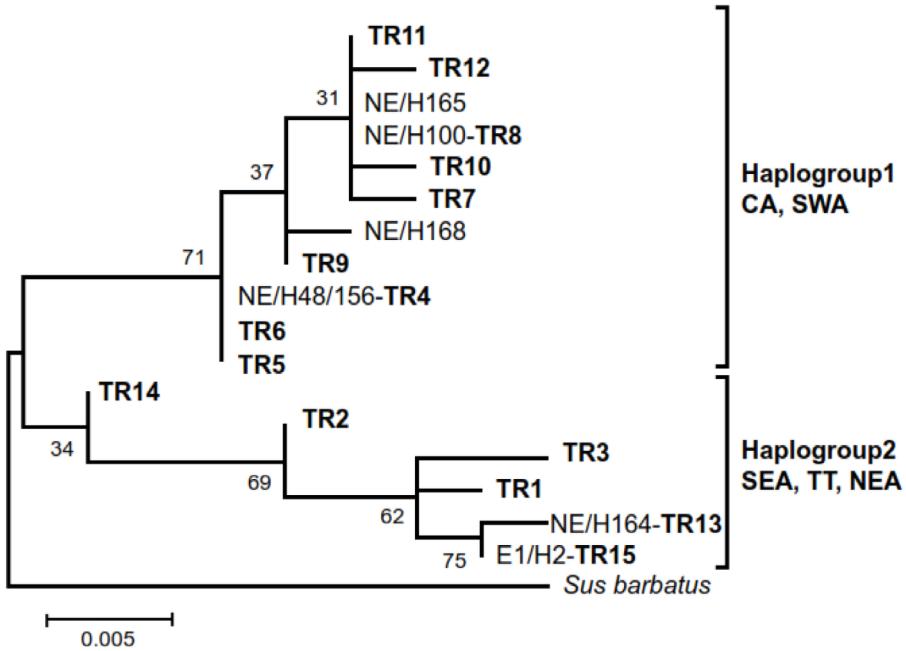

Figure 3. Maximum likelihood phylogenetic tree of haplotypes of the Turkish wild boars (*Sus scrofa*) obtained in the present study, based on the partial D-loop sequences of mtDNA. Numbers above or below branches indicate bootstrap values. TR numbers refer to current haplotype numbers in Table 1 and H numbers refer to the published haplotype labels downloaded from GenBank (Table 2). E1: European 1 haplogroup/clade in Figure 5, NE: Near East haplogroup/clade in Figure 5.

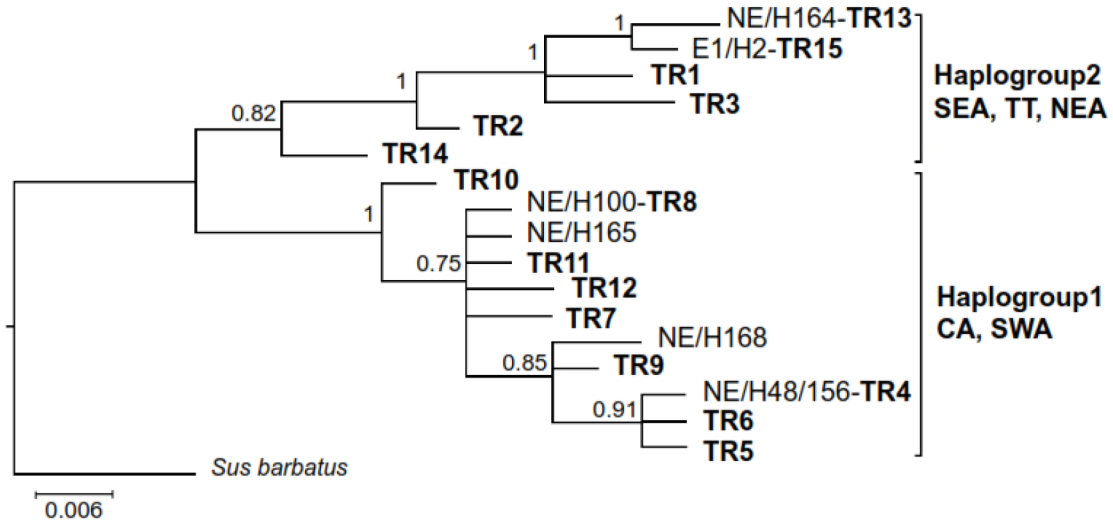

Figure 4. Bayesian phylogenetic tree constructed from haplotypes of the wild boar (*Sus scrofa*) samples collected in this study, based on the partial D-loop sequences of mtDNA. Posterior probabilities are indicated at nodes. TR numbers refer to current haplotype numbers in Table 1 and H numbers refer to the published haplotype labels downloaded from GenBank (Table 2). E1: European 1 haplogroup/clade in Figure 5, NE: Near East haplogroup/clade in Figure 5.

Table 3. Results of the analysis of molecular variance (AMOVA) for the two distinguished haplogroups of wild boar in Turkey.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	F_{ST}	Significance (P*)
Between groups	1	202.780	3.55715 Va	85.90	0.85881	<0.0001
Within groups	112	65.413	0.58404 Vb	14.10	0.85914	<0.0001
Total	113	268.193	4.14120			
Fixation index F_{ST} = 0.85897						

*After 10,000 random permutations.

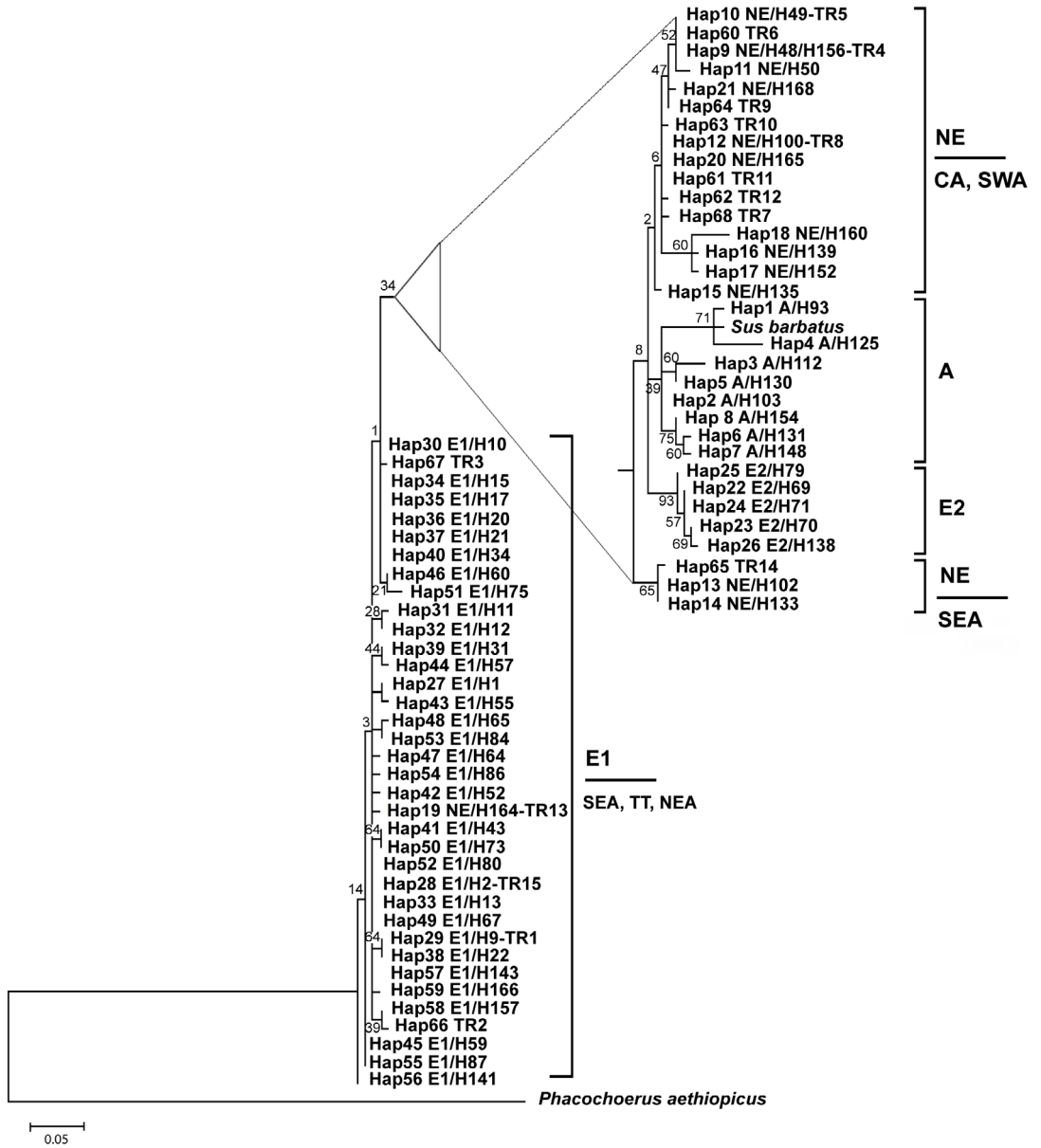

Figure 5. Maximum likelihood tree based on 68 haplotypes from 485 wild boar D-loop region sequences (both obtained in this study and downloaded from GenBank). Numbers above or below branches indicate bootstrap support values. Haplogroups: A, Asian; NE, Near Eastern; E1, European E1; E2, European E2. Outgroup taxa are *Sus barbatus* and *Phacochoerus aethiopicus*.

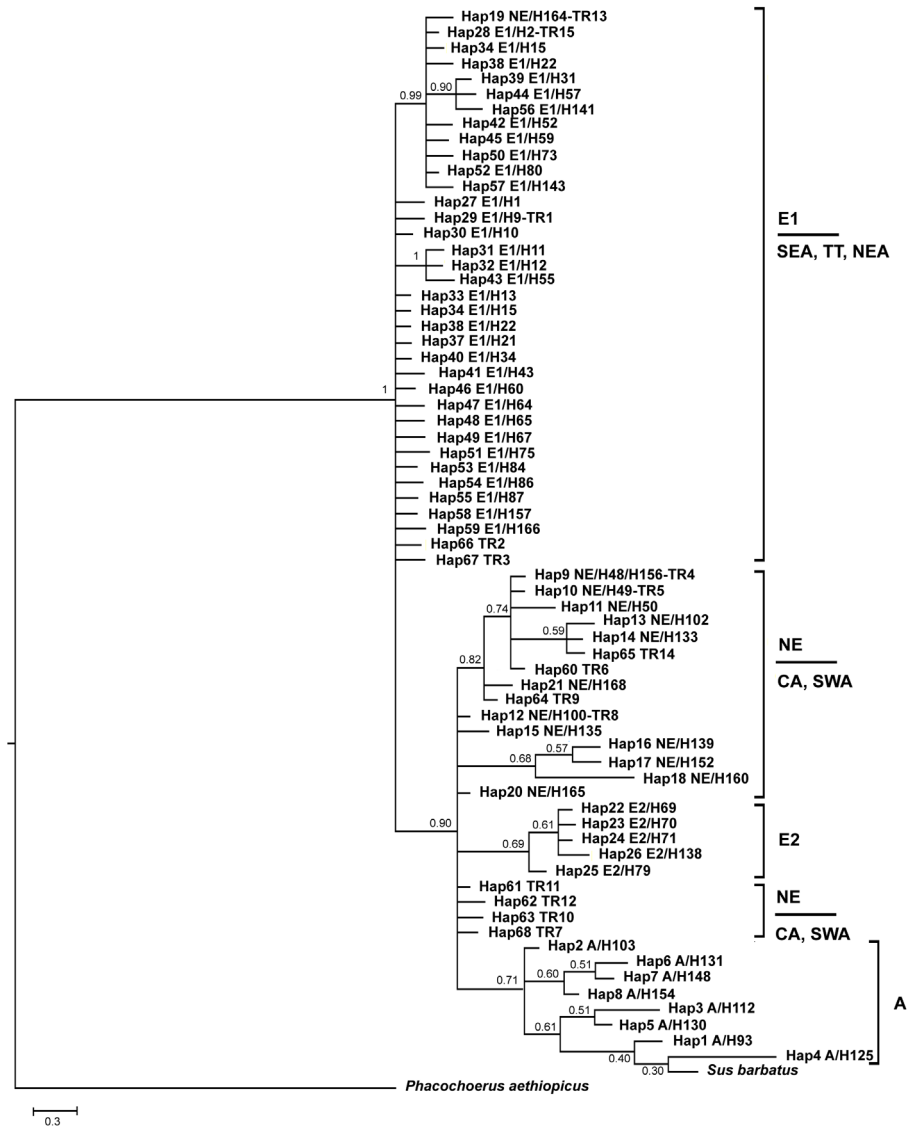

Figure 6. Bayesian inference tree based on 68 partial D-loop haplotypes of 485 wild boars (both obtained in this study and downloaded from GenBank). Posterior probabilities are indicated at nodes. Haplogroups: A, Asian; NE, Near Eastern; E1, European E1; E2, European E2. Outgroup taxa are *Sus barbatus* and *Phacochoerus aethiopicus*.

Turkish wild boar was somewhat lower than for wild boar samples from Europe (h = 0.947, Alexandri et al., 2012), the Balkan Peninsula (h = 0.892, Alexandri et al., 2012), the Near East (h = 0.894, Veličković et al., 2015), and Asia (h = 0.967, Veličković et al., 2015). However, for somewhat smaller geographic areas, such as the Northern Dinaric Balkans, haplotype diversity of wild boar partial control region sequences (h = 0.6909, n = 163, Veličković et al., 2015) was similar to the presently found values. The somewhat lower haplotype diversity of the presently analyzed samples from Turkey might be due to the not comprehensive geographical sample coverage of this study, i.e. no samples were available from quite large parts of Turkish Thrace, the entire Eastern Anatolia, and some parts of Southeastern Anatolia. However, nucleotide diversity of Turkish wild boar (π = 0.0117) was similar to that found in wild boar populations from the Balkan Peninsula (π = 0.011, Alexandri et al., 2012) and the Near East (π = 0.012, Veličković et al., 2015), but higher than for samples from the Northern Dinaric Balkans (π = 0.0031, Veličković et al., 2015) and other samples of Europe (π = 0.005, Alexandri et al., 2012), and clearly lower than for

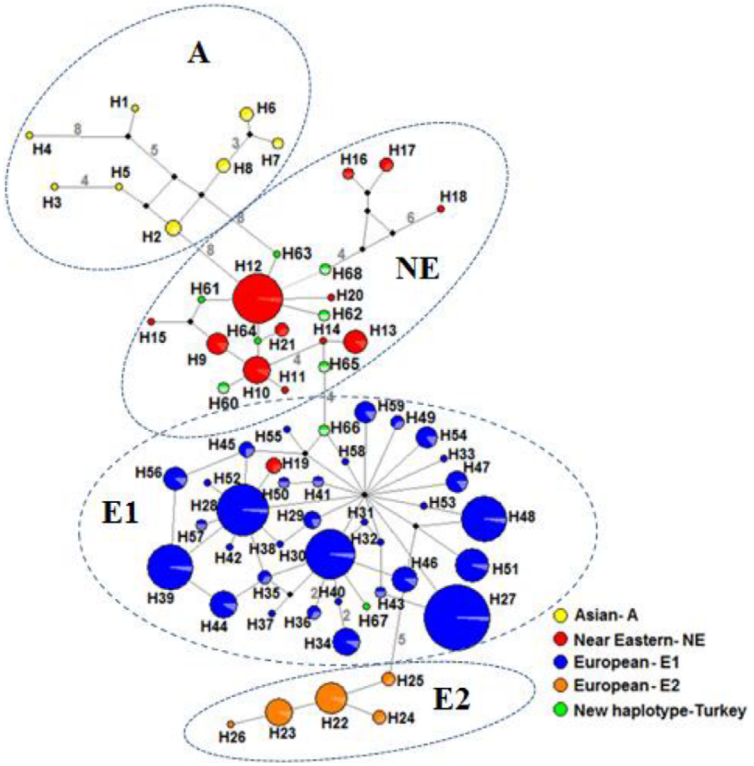

Figure 7. Median-joining network of 68 haplotypes from 485 wild boars from different regions of the world. Circle sizes are proportional to haplotype frequencies and numbers refer to haplotype codes in Table 2. Numbers on the branches indicate the number of nucleotide substitutions, if more than one. Major haplogroups are delimited by dashed lines. A, Asian; NE, Near Eastern; E1, European E1; E2, European E2. Haplotypes exclusive to Turkey are labeled in green.

Table 4. Basic molecular diversity indices for each Turkish wild boar haplogroup and associated neutrality test results.

Haplogroups	Sample size	Number of haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)	Average number of nucleotide differences (k)	Tajima's D	Fu's Fs
Haplogroup 1 (CA/SWA)	60	11	0.508	0.00234	0.907	–0.72423	–4.94079
P value						0.26 (P > 0.05)	0.01 (P < 0.05)
Haplogroup 2 (TT/SEA/NEA)	54	6	0.363	0.00262	1.014	–1.51716	–0.70516
P value						0.04 (P < 0.05)	0.38 (P > 0.05)

the Asian samples ($\pi = 0.0240$) reported by Veličković et al. (2015). The slightly elevated sequence diversity might be interpreted as reflecting a certain admixture of divergent phylogenetic lineages in Turkey, due to its biogeographic position between Southwest Asia and Southeast Europe. On the other hand, for another terrestrial mammal with a wide geographic distribution in Turkey, the brown hare (*Lepus europaeus*), a high variability (h = 97.6%) of D-loop sequences was recorded and haplotype diversity for hares from Anatolia was significantly higher than for hares from the Balkan Peninsula or other parts of Europe (Sert et al., 2009). Similarly, Turkish red fox (*Vulpes vulpes*) exhibited high levels of genetic diversity compared to data from other parts of the species' range (İbiş et al., 2014).

For wild boar, so far no Anatolian haplotypes were detected in the Balkans or other parts of Europe. However, mtDNA sequences from the island of Samos off the Anatolian coast clustered with the Near Eastern clade, consistent with the island's geographical proximity to Asia Minor (Alexandri et al., 2012). The haplotype distribution found in this study corroborates the findings of Alexandri et al. (2012). Notably, the three haplotypes within Turkish Thrace (TR1–TR3) were not found in Anatolian wild boar, and the twelve haplotypes found in Anatolia (TR4–TR15) were not detected in wild boar from Turkish Thrace. On the other hand, the currently found widespread distribution of some wild boar mtDNA haplotypes could be a result of natural (demographic and range expansion) or anthropogenic processes, such as translocations and reintroductions (Alves et al., 2010; Meiri et al., 2013). Haplotype TR8 was a common haplotype in Turkey (Table 1) and was found in 31 newly sequenced individuals in a wide geographic range, from Central Anatolia to Northwestern Anatolia and from the Central Black Sea region to Northeastern Anatolia.

The geographic haplotype distribution from this study suggested some phylogeographic structure of mitochondrial lineages of wild boar from Turkey; however, several phylogenetically very divergent haplotypes were also found within single regions (in Turkish Thrace and Southwestern and Southeastern Anatolia). Notably, a number of haplotypes (TR2, TR3, TR6, TR7, TR9–TR12, and TR14) have so far not been reported in wild boar.

The combined MJ network analysis of the presently obtained sequences and all those retrieved from GenBank yielded in essence the same overall evolutionary pattern as reported by Alexandri et al. (2012), Meiri et al. (2013), Kusza et al. (2014), and Veličković et al. (2015), namely differentiation into four major clades of A, NE, E1, and E2 phylogroups; this pattern was previously detected by Larson et al. (2005) and Scandura et al. (2008) and was summarized by a MJ network for the worldwide dataset by Veličković et al. (2015). However, our ML analysis did not at all convincingly reflect such a phylogenetic partitioning, and the Bayesian tree only partly reflected a differentiation into the four expected phylogroups. The majority of Turkish haplotypes clustered with the NE phylogroup, and only two haplotypes clustered with the E1 phylogroup (Figures 5–7). While all haplotypes of wild boar collected in Turkish Thrace clustered with E1 haplotypes, wild boars collected from Giresun and Erzincan, in Northeastern Anatolia, also surprisingly carried a haplotype (TR13) that clustered with the E1 haplotypes. Whether this reflects the natural presence of European lineages in wild boar from Northeastern Anatolia or possible historic anthropogenic translocations of wild boar (e.g., during the Byzantine Empire) remains to be investigated by more samples from, e.g., Turkish Thrace and Northern Anatolia.

According to Ferreira et al. (2009), Nikolov et al. (2009), and Veličković et al. (2012, 2015), geographical barriers such as rivers or mountain ranges may not fully explain genetic divergence between wild boar populations, and genetic clusters may also be related to habitat structuring or to evolutionary changes over longer periods in addition to historic population processes related to (anthropogenic) landscape changes. Due to its continental position, and connecting several major faunal regions, Turkey can be considered as a biodiversity hotspot (Bilgin, 2011; Şekercioğlu et al., 2011). Our AMOVA results confirmed the two phylogroups identified particularly by the Bayesian tree and tentatively also by the ML tree for the Turkish sequences (Table 3), and revealed significant variation within the two major phylogroups. This, together with the encountered geographical distribution of haplotypes, suggests a closer phylogenetic relationship of wild boar from Central and Southwestern Anatolia on the one hand and among wild boar from Turkish Thrace and Northeastern and Southeastern Anatolia on the other. Thus, some phylogeographic structure that cannot be readily explained by plain geographic relations seems to occur in Turkish wild boar. However, more geographically meaningful samples are necessary for detailed conclusions. In addition, comprehensive population genetic analyses based on multilocus nuclear markers will help to refine genetic relationships among wild boar in Turkey (see, e.g., Scandura et al., 2008; Veličković et al., 2015) and sequence analysis of coat color genes may help to uncover evolutionary patterns of the currently detected external coat polymorphism, a phenomenon that has been reported for wild boar by, e.g., Harrison and Bates (1991) and Garcia et al. (2011).

Acknowledgments

We are very grateful to Prof Dr Nevena Veličković (Department of Biology and Ecology, University of Novi

Sad, Serbia) for her advice and valuable comments. We thank all those who helped us in collecting samples from Turkey: Ahmet Yaşar Yıldız (1st Region Directorate of the Turkish Forestry and Water Affairs Ministry, İstanbul), Neslihan Akdemir (Kırıkkale Post of the Turkish Forestry and Water Affairs Ministry), and several local hunters and hunting associations. This study was financially supported by the Kırıkkale University Scientific Research Projects Coordination Unit (project number: BAP-2014/35). The Turkish Nature Protection and National Parks Directorate (Turkish Forestry and Water Affairs Ministry, Ankara) issued a research permit (permit number: 221163). We thank the editors Prof Dr Nusret Ayyıldız, Prof Dr Haluk Kefelioğlu, and Prof Dr Mustafa Sözen, and three anonymous referees for their valuable comments and constructive suggestions on the manuscript.

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