

Genetic analysis of the Turkish gray wolf (*Canis lupus*) based on partial mitochondrial DNA sequences

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Accepted 19.vii.2016.

Published online at www.senckenberg.de/vertebrate-zoology on 13.xii.2016.

Abstract

In this study, we focused on determining the genetic variability of Turkish gray wolves and also to reveal phylogenetic relationships of the Eurasian wolf populations using mitochondrial DNA sequences. Partial mitochondrial DNA sequences (440 bp) were obtained from 12 Turkish gray wolves, including D-loop region (332 bp). We found seven D-loop haplotypes (332 bp) among the 12 Turkish gray wolves. The D-loop sequences of the Turkish gray wolf were compared to sequences registered in GenBank from Eurasia under the name *Canis lupus*. Five Turkish haplotypes were shared with conspecific sequences from other regions of Eurasia in the species' range. Two haplotypes were unique for the Turkish wolves. The existence of shared haplotypes suggests that the gray wolves in Turkey and other regions might be originated from the same gene pool. The present study reports significant data for determining the genetic variability and revealing the phylogenetic relationships of Turkish gray wolves; it is suggested that the genetic variability of Turkish gray wolves is relatively high.

Key words

Canis lupus, Mitochondrial DNA, D-loop, Turkey.

Introduction

The gray wolf (*Canis lupus* L., 1758) is a member of the family Canidae and one of the most important terrestrial predators in Northern Hemisphere (SILLERO-ZUBIRI *et al.* 2004). It is widely distributed across much of the Holarctic region (WILSON & REEDER 1993, 2005, MECH & BOITANI 2010). Gray wolves also occur in Turkey and its surrounding areas (SALVATORI & LINNELL 2005, WILSON & REEDER 1993, 2005, KRYŠTUFEK & VOHRALIK 2009).

The gray wolf has been considered the first mammal species domesticated by humankind (CLUTTON-BROCK 1995), with multiple origins (PANG *et al.* 2009, VON HOLDT *et al.* 2010). It is also known to be the most important wildlife species (SILLERO-ZUBIRI *et al.* 2004).

In order to manage and to conserve wild species, nature conservationists and researchers make an effort to draw a comprehensive picture of the genetic variability of populations in different regions. There are numerous studies on extant gray wolves on populations and on phylogenetic relationships throughout the species' range based on mitochondrial DNA (WAYNE *et al.* 1992, ELLEGREN *et al.* 1996, VILÁ *et al.* 1999, RANDI *et al.* 2000, FLAGSTAD *et al.* 2003, JĘDRZEJEWSKI *et al.* 2005, PILOT *et al.* 2006, 2010, 2014, PANG *et al.* 2009, FAIN *et al.* 2010, GOMERČIĆ *et al.* 2010, RUTLEDGE *et al.* 2010, WECKWORTH *et al.* 2010, 2011, ASADI-AGHBOLAGHI *et al.* 2014, BRAY *et al.* 2014, DJAN *et al.* 2014, FABRI *et al.* 2014, JANSSON



Fig. 1. Localities of the Turkish gray wolf samples (see Table 1 for numbers).

et al. 2014, MATSUMURA *et al.* 2014, ISHIGURO *et al.* 2016, *etc.*).

The present study is focused on the Turkish gray wolf (*Canis lupus*). This large-sized carnivore is widely distributed throughout Turkey, where forms a zoogeographical bridge between Africa, Asia, Europe and the Arabian Plate (JOHNSON 2002, KRYŠTUFEK & VOHRALÍK 2001, 2009). The gray wolf is one of well-known large carnivores like red fox (*Vulpes vulpes*), golden jackal (*Canis aureus*) and brown bear (*Ursus arctos*) in Turkey (JOHNSON 2002, CAN 2004, CAN & TOGAN 2004, ALBAYRAK 2011, AŞAN-BAYDEMİR *et al.* 2011, İLEMİN 2014, AKSÖYEK *et al.* 2016, AMBARLI *et al.* 2016).

The Turkish wolves inhabit all biomes, which include prey. The population size of the Turkish gray wolf has been estimated at around 5000–7000 individuals (CAN 2004, SALVATORI & LINNELL 2005). Due to the decrease of prey animals such as sheep, gazelle, red deer, roe deer, wild boar and brown hare, habitat fragmentation and illegal hunting *etc.*, the Turkish gray wolves have shown a decline in the last century; they occur in small subpopulations (CAN & TOGAN 2004, SALVATORI & LINNELL 2005, ŞEKERCİOĞLU *et al.* 2011). Therefore, measures for conservation management have been taken by the Ministry of Forestry and Water Affairs in Turkey (see www.ormansu.gov.tr).

Mitochondrial DNA, indicative of maternal inheritance, is one of the important tools, which have been used in genetic analyses of Turkish carnivores (İBİŞ & TEZ 2014, İBİŞ *et al.* 2014, 2015a,b, AKSÖYEK *et al.* 2016, DEMİRBAŞ *et al.* 2016). While mitochondrial DNA of the gray wolf was analyzed for various populations throughout species' range (ELLEGREN *et al.* 1996, VILÁ *et al.* 1999, RANDI *et al.* 2000; FLAGSTAD *et al.* 2003; JĘDRZEJEWSKI *et al.* 2005, PILOT *et al.* 2006, 2010, 2014, PANG *et al.* 2009, FAIN *et al.* 2010, GOMERČIĆ *et al.* 2010, RUTLEDGE *et al.* 2010, WECKWORTH *et al.* 2010, 2011, ASADI-AGHBOLAGHI *et al.* 2014, BRAY *et al.* 2014, DJAN *et al.* 2014, FABRI *et al.* 2014, JANSSON *et al.* 2014, MATSUMURA *et al.* 2014, ISHIGURO *et al.* 2016, *etc.*), no wide-ranging and detailed mitochondrial DNA analysis of Turkish gray wolves has been performed up to now. Only a few samples of gray wolves from Turkey have been used in genetic analyses (VILÁ *et al.* 1997, 1999, RANDI *et al.* 2000, PILOT *et al.* 2006, 2010, 2014).

By presenting preliminary results of an analysis of partial mitochondrial sequences, we aim to contribute to the knowledge of the genetic variability of Turkish gray wolves and the relationships with other conspecific populations throughout Eurasia.

Materials and Methods

Sampling

The tissue samples (ear, skin, tail, *etc.*) were collected from the 12 road-killed gray wolves in the Asian part of Turkey (Table 1, Fig. 1).

DNA extraction and PCR conditions

Tissue samples stored in 99% ethyl alcohol were used to obtain genomic DNA, which were extracted by using a commercial extraction kit (The DNeasy Blood and Tissue Kit, Qiagen) according to the manufacturer's instructions. A short fragment of mitochondrial DNA including the D-loop (control region) was amplified by using DLH (Forward: 5'-CCTGAAGTAAGAACCAGATG-3') and LF15926F (Reverse: 5'-ATATAAAATACTTTGGTCTTGTAAACC-3') primers (KIRSCHNING *et al.* 2007).

A total of 50 µl reaction mixture were used for PCR (Polymerase Chain Reactions) amplifications; 10 × Taq

Table 1. A list of grey wolf samples (sequences/haplotypes) used in this study.

| Map no. (Fig. 1) | Nr. of Turkish samples | Code of Turkish haplotypes | Accession number | Locality | Reference | Conspecific sequences | Reference |
|------------------|------------------------|----------------------------|------------------|---------------------------------|------------|---------------------------------------|-------------------------------------|
| 1 | 1437 | Tr.C1.D1 | KY039989 | Sankaya, Yozgat, Turkey | This Study | AY570178-AY570181 | Ardalan <i>et al.</i> 2004 |
| 2 | 1516 | Tr.C1.D1 | KY039989 | Kars, Turkey | This Study | AF008136-AF008142 | Vila <i>et al.</i> 1997 |
| 3 | 1518 | Tr.C1.D1 | KY039989 | Nebiöglü, Köyü, Ardahan, Turkey | This Study | KJ195895-KJ195898, KJ490942-KJ490944 | Pilot <i>et al.</i> 2014 |
| 4 | 1543 | Tr.C1.D1 | KY039989 | Suveren Köyü, Iğdır, Turkey | This Study | AF115667-AF115703, AF115707, AF115714 | Randi <i>et al.</i> 2000 |
| 5 | 1446 | Tr.C1.D2 | KY039990 | Sankamış, Kars, Turkey | This Study | AY916804-AY916822 | Luo <i>et al.</i> 2005 |
| 6 | 1336 | Tr.C1.D3 | KY039991 | Sorgun, Yozgat, Turkey | This Study | AF338803-AF338811, AF487754 | Valiere <i>et al.</i> 2003 |
| 7 | 1542 | Tr.C1.D3 | KY039991 | Tüney Köyü, Çankırı, Turkey | This Study | KF723519-KF723526 | Jansson <i>et al.</i> 2014 |
| 8 | 1448 | Tr.C1.D4 | KY039992 | Kıbrıscık, Bolu, Turkey | This Study | FJ978005-FJ978035 | Pilot <i>et al.</i> 2010 |
| 9 | 1334 | Tr.C1.D5 | KY039993 | Tekman, Erzurum, Turkey | This Study | DQ480503-DQ480507 | Björnerfeldt <i>et al.</i> 2006 |
| 10 | 1559 | Tr.C1.D5 | KY039993 | Sankamış, Kars, Turkey | This Study | JN182019-JN182092 | Hindrikson <i>et al.</i> 2011 |
| 11 | 1460 | Tr.C1.D6 | KY039994 | Abant Gölü, Bolu, Turkey | This Study | AB007372-AB007373 | Tsuda <i>et al.</i> 1997 |
| 12 | 1441 | Tr.C1.D7 | KY039995 | Karakurt, Kars, Turkey | This Study | AY172676-AY172677 | Yang <i>et al.</i> 2002 |
| | | | | | | KF857179 | Deng and He 2016 |
| | | | | | | KF661038-KF661055, KF661078-KF661082 | Thalmann <i>et al.</i> 2013 |
| | | | | | | KF661087, KF661091, KF661095 | Thalmann <i>et al.</i> 2013 |
| | | | | | | AB499818-AB499825 | Matsumura <i>et al.</i> 2014 |
| | | | | | | AM711902 | Amason <i>et al.</i> 2007 |
| | | | | | | KC540917- KC540929 | Asadi-Aghbollahi <i>et al.</i> 2014 |

Haplotype codes in Figures 2–3; lu.1: AF338807, AF115689, lu.2: KF723519, FJ978017, lu.3: FJ978027, AF115688, AF115687, FJ978028, lu.4: AF115687, FJ978028; lu.5: Tr.C1.D3; lu.6: Tr.C1.D4; lu.7: Tr.C1.D6, AF115696, KJ490942, KC540923, KC540924; lu.8: AF338806, KF723523, AF115694, AF115695, FJ978020, FJ978019; lu.9: FJ978034; lu.10: AY916808, AY916807, FJ978016, KJ195895, FJ978015, AF008142; lu.11: AF115697; lu.12: AY916810; lu.13: Tr.C1.D7, KJ195897; lu.14: AY916805, AY916814, lu.15: AY916819; lu.16: AY916815; lu.17: FJ978006, AF487754, KF723521, KF723520, AF115693, AF115692, FJ978005, DQ480504, JN182058, JN182032, JN182074, JN182036, JN182033, JN182030, JN182075, JN182085, JN182084, JN182049, JN182050, JN182020, JN182026, JN182023, JN182022, JN182088, JN182086, JN182087, JN182041, JN182043, JN182039, JN182037, JN182025, JN182024, JN182021, JN182078, JN182066, JN182064, JN182047, JN182056, JN182046, JN182088, JN182081, JN182051, JN182052, JN182042, JN182039, JN182037, JN182044, JN182035, JN182034; lu.18: KF723526, FJ978022; lu.19: KF723525; JN182073, JN182072, JN182071, JN182062, JN182059, JN182054, JN182055, JN182040, JN182019, JN182029, JN182025, JN182024, JN182037, JN182044, JN182035, JN182034; lu.20: AF338809, AF115702; lu.21: AF338808, AF115703, AF008137, DQ480505; lu.22: AF338810, KF723524, FJ978025; lu.23: KF723522, AF115698, FJ978012, FJ978011, DQ480503; lu.24: FJ978032, FJ978031, KF857179, KC540917; lu.25: AY916816, AY916817, AY916804; lu.26: FJ978026; lu.27: FJ978021, KC540925; lu.28: FJ978018; lu.29: AY570181, KC540928; lu.30: AY916811, AY916812; lu.31: AF338805, AY172677; lu.32: AY570179, KJ490943, KC540919; lu.33: Tr.C1.D5, AF115690, AY916813, FJ978010, AF008136, KC540918, AM711902, KC540926, KC540922; lu.34: JN182089, JN182063, JN182068, JN182081, JN182079, JN182077, JN182070, JN182073, FJ978024, FJ978023; lu.35: AF115691, AB007373, FJ978024, FJ978023; lu.36: FJ978007, FJ978008; lu.37: AF008140, DQ480506; lu.38: AY916818; lu.39: AY916809; lu.40: FJ978033; lu.41: Tr.C1.D1, AY570178, AY570180, AF008141, DQ480507, KC540920, KC540927, KC540929, KC540921; lu.42: Tr.C1.D2, KJ195896, KJ490944; lu.43: AY916806; lu.44: AY172676; lu.45: KF661095; lu.46: KF661087; lu.47: FJ978030; lu.48: AF338804, FJ978014, FJ978013, AF115701, KF661045; lu.49: AF338803, FJ978035, AF115699, KF661048, AF008138; lu.50: FJ978029; lu.51: AF115700, AB007372; lu.52: KF661053, KF661041; lu.53: KF661078; lu.54: KF661091; lu.55: KF661039, KF661038; lu.56: KF661081; lu.57: KF661049, KF661044, KF661052, KF661040; lu.58: AB499819, AB499820; lu.59: KF661053, KF661041; lu.60: KF661042; lu.61: KF661054; lu.62: KF661043; lu.63: KF661046; lu.64: KF661047; lu.65: KF661044, KF661050; lu.67: AB480739, LC064093, AB499823; lu.68: LC064091, AB480737, AB480742, AB499825, AB499828, AB499822; lu.69: AB480736, AB480741, AB499824, AB499818; lu.70: AB480740; lu.71: KF661051; lu.72: KF661080; lu.73: LC064094, LC064095, AB480738, LC064096, AB499821; lu.74: AY916822; lu.75: AY916820; lu.76: AY916821.

buffer with $(\text{NH}_4)_2\text{SO}_4$: 5 μl , 10 mM dNTP mix: 1 μl , 5u/ul Taq DNA polymerase (Thermo Scientific): 0.3 μl , 25 mM MgCl_2 : 3 μl , 10mg/ml BSA: 3 μl , 10uM each primer: 5 μl , genomic DNA extract: 1 μl , dH_2O : 26.7 μl). The PCR program comprised of a pre-denaturation procedure consisting of 5 min. at 95°C by 1 cycle, a denaturation step of 40 sec. at 95°C, an annealing step of 1 min. at 54°C, an extension step of 90 sec. at 72°C by 30 cycles and an ending step of 10 min. at 72°C by 1 cycle. The quality of genomic DNA and PCR products was verified by running in 1% agarose gel and by staining with ethidium bromide.

Sequencing, Aligning and Analyzing

The Macherey-Nagel Nucleospin Gel and PCR Clean-up kit were used to purify the PCR products, and sequencing of which was performed in forward and reverse directions with PCR primers; DLH and LF15926F (KIRSCHNING *et al.* 2007), by using a sequencer (ABI 3100 Genetic Analyzer).

We used Geneious v.R6.1.6 (<http://www.geneious.com>), in which MAFFT v7.017 was used for the multiple sequence alignment with default parameters (KATOH *et al.* 2002), to align the mitochondrial DNA sequences, and DnaSP v.5.10.01 (LIBRADO & ROZAS 2009) to determine mitochondrial haplotypes and to estimate haplotype and nucleotide diversities of gray wolf. MEGA 6.0 (TAMURA *et al.* 2013) was used to calculate genetic distances among the Turkish haplotypes of gray wolf, based on the K2P (Kimura 2-parameter) model of DNA substitution (KIMURA 1980).

Phylogenetic and network analyses were performed with mitochondrial D-loop sequences of 317 bp from Turkish gray wolves and GenBank (NCBI: The National Center for Biotechnology Information), including D-loop sequences registered under the name *Canis lupus* from Eurasia (Table 1). The golden jackal (*Canis aureus*) (AY289997: AGGARWAL *et al.* 2007) was used as out-group in phylogenetic analysis.

The HKY (Hasegawa-Kishino-Yano) + I (Invariant) + G (Gamma) was the most suitable model of nucleotide substitution according to the corrected Akaike Information Criterion (AICc) and the Bayesian Information Criterion (BIC) using jModeltest2 (DARRIBA *et al.* 2012), and this model was used in phylogenetic analysis to reconstruct Bayesian (Bayesian Inference) tree by means of MrBayes v.3.2 (RONQUIST *et al.* 2012). In Bayesian analysis (RONQUIST *et al.* 2012), the MCMC (Markov Chain Monte Carlo) technique was used to calculate the Bayesian posterior probabilities for 2.6 million generations with tree sampled every 100 generations, and to discard the first 25% of samples as burn-in (The Average Standard Deviation of split Frequencies < 0.01: 0.009400). The remaining samples after discarding burn-in, were retained and to estimate posterior probability and 95% Bayesian credibility interval, and to create the consensus tree (50% majority rule). FigTree v1.3.1 soft-

ware (RAMBAUT 2009) was used to picture the Bayesian phylogenetic tree.

A haplotype network was drawn by using median-joining method by means of the Network v.4.6.1.1 software (BANDELT *et al.* 1999, <http://www.fluxus-engineering.com>).

Results

A fragment of the 440 bp of mtDNA (tRNA^{Thr}+tRNA^{Pro}+D-loop) was amplified successfully for the 12 Turkish gray wolves, including 332 bp for D-loop (control region). A comparison of the 440 bp fragments revealed the presence of seven haplotypes (Tr.Cl.D1–Tr.Cl.D7) with 12 segregating sites and 10 parsimony informative sites. The haplotype Tr.Cl.D1 was the most common, and it was found in four out of the 12 samples. Distribution of Turkish haplotypes was given in Table 1 and Figure 1. Seven haplotypes of the Turkish gray wolf (Tr.Cl.D1–Tr.Cl.D7) are deposited in the GenBank database (Accession numbers: KY039989–KY039995). The haplotype (*Hd*) and nucleotide (π) diversities were 0.8788 and 0.01088, respectively. The Turkish wolves were polymorphic. Mean sequence divergence of the seven Turkish wolf haplotypes was 0.012, ranging from 0.002 to 0.021, based on K2P. When sequences including D-loop region (332 bp) of the Turkish samples were analyzed, seven D-loop haplotypes were found (Table 1). The haplotype (*Hd*) and nucleotide (*Pi*) diversities for the D-loop region of Turkish wolf were 0.8788 and 0.01392, respectively.

Based on the 12 sequences in this study and 244 sequences in the different lengths available in the GenBank database under the name *Canis lupus* from Eurasia (Table 1), a total of 76 haplotypes (317 bp) were found. The sequences under the other names of *C. lupus*, such as *C. lupus chanco* and *C. lupus pallipes*, were not included in the genetic analyses. Five Turkish haplotypes were shared with sequences registered in GenBank; Tr.Cl.D1: AY570178 (Iran), AY570180 (Iran), AF008141 (Saudi Arabia), DQ480507 (Saudi Arabia), KC540920, KC540921, KC540927 and KC540929 (Iran); Tr.Cl.D2: KJ195896 and KJ490944 (Caucasia); Tr.Cl.D5: AF115690 (Greece), AY916813 (China), FJ978010 (Belarus, Bulgaria, Greece, Romania, Russia, Sweden, Ukraine), AF008136 (Romania, Russia), KC540918 (Iran), AM711902 (Sweden), KC540926 and KC540922 (Iran); Tr.Cl.D6: AF115696 (Israel), KJ490942 (Caucasia), KC540923 and KC540924 (Iran); Tr.Cl.D7: KJ195897 (Georgia) (Table 1, Figs. 2–3).

Phylogenetic relationships of the mitochondrial D-loop haplotypes (317 bp) are shown in the Bayesian tree (Fig. 2). Branch nodes of the Bayesian tree (Fig. 2) were supported with relatively high posterior probabilities (0.5–1). In the Bayesian tree (Fig. 2), Tr.Cl.D1 and Tr.Cl.D2 were clustered together with haplotypes from

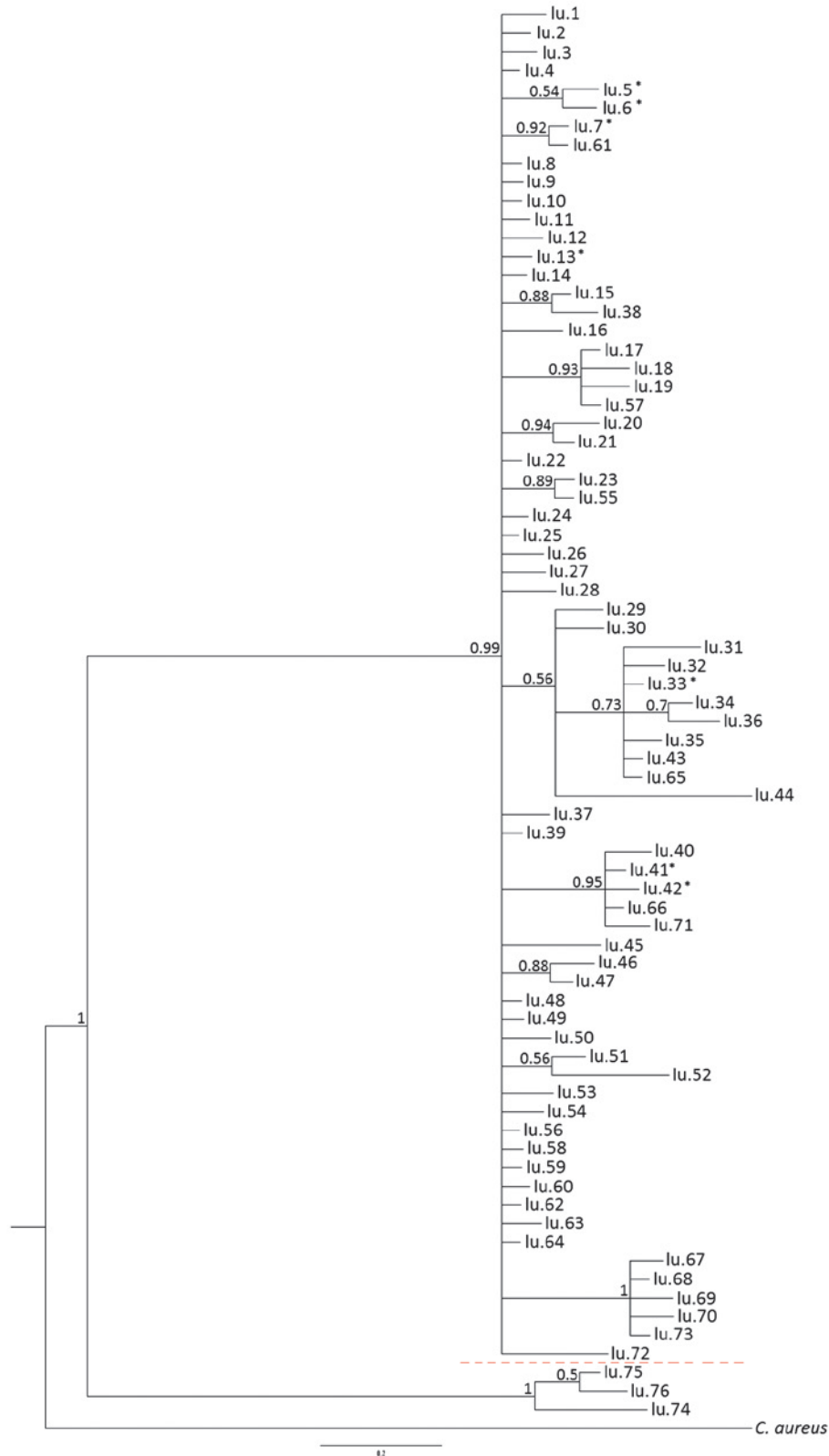


Fig. 2. Bayesian tree constructed from the mitochondrial D-loop haplotypes (317 bp) of gray wolves, rooted with *Canis aureus*. The asterisks indicate the Turkish gray wolf haplotypes (see Table 1 for haplotype codes).

Turkey (Trakia), Iran and Oman, whereas Tr.Cl.D3 and Tr.Cl.D4 were clustered together. Tr.Cl.D5 was grouped with haplotypes from Belarus, Bulgaria, Caucasus, China, Croatia, France, Iran, Latvia, Lithuania, Poland, Portugal, Russia, South Korea, Spain, Ukraine and Yugo-

slavia. Furthermore, Tr.Cl.D6 was clustered with haplotype from Israel, whereas Tr.Cl.D7 was branched alone in the Bayesian tree.

In the median joining haplotype network (Fig. 3), the Turkish haplotypes of this study grouped together with

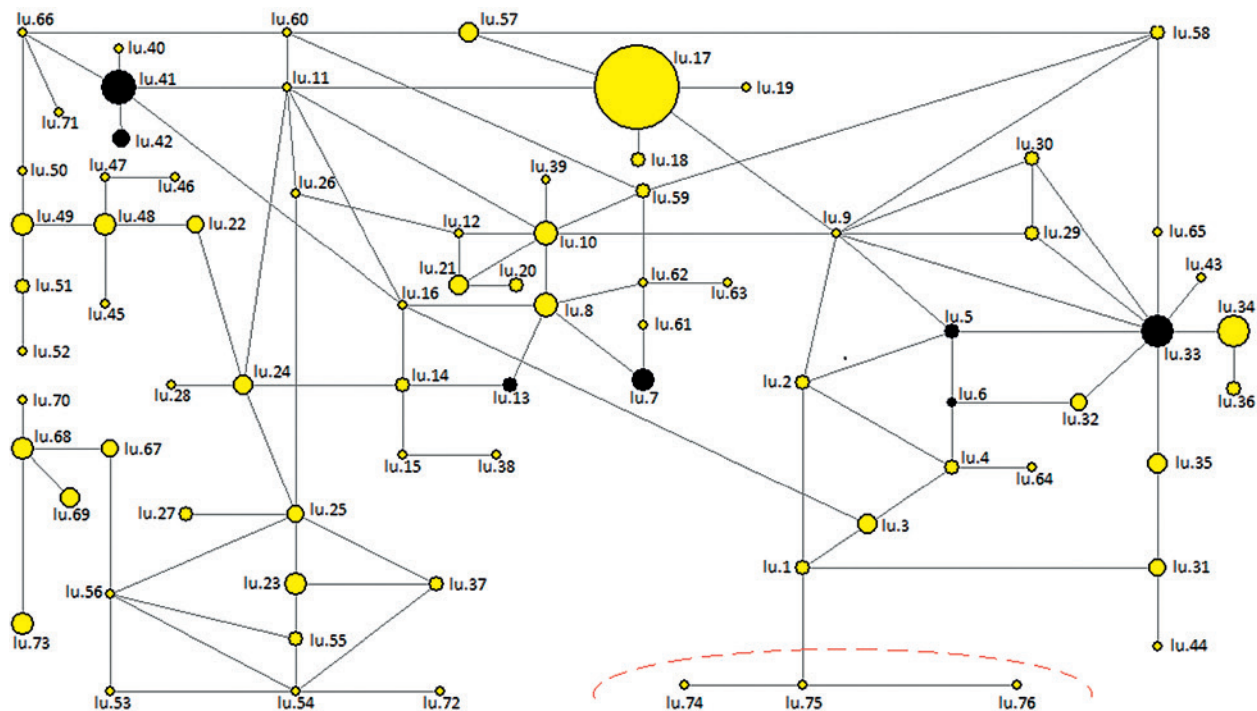


Fig. 3. Median-joining network constructed from the mitochondrial D-loop haplotypes (317 bp) of gray wolves. The bold circles indicate the Turkish gray wolf haplotypes (see Table 1 for haplotype codes).

conspecific haplotypes in the inner parts; this grouping was not entirely compatible with the Bayesian tree (Fig. 2).

Discussion

The gray wolf, *Canis lupus*, is a widespread canid species in Turkey (KRYŠTUFEK & VOHRALIK 2009, AMBARLI *et al.* 2016). Although there are several genetic studies including Turkish gray wolves, the genetic characterization of the Turkish gray wolves is still scarce. To date, the samples of Turkish gray wolf have only been included in six studies that used mitochondrial DNA sequences (VILÁ *et al.* 1997, 1999, RANDI *et al.* 2000, PILOT *et al.* 2010, 2014, AKSÖYEK *et al.* 2016).

In the present study, genetic variability and phylogenetic relationships of Turkish gray wolves were researched by using partial sequences of mitochondrial DNA (tRNA^{Thr} + tRNA^{Pro} + D-loop: 440 bp). The analysis revealed seven haplotypes, two of them (Tr.Cl.D3 and Tr.Cl.D4) were unique and new for the Anatolian part of Turkey, when compared to the other geographical regions Eurasia. One postulates that this may be an indication of a relatively high genetic diversity of the Turkish gray wolf.

The mutation rate in the mitochondrial D-loop region of wolves is relatively high (SAVOLAINEN *et al.* 2000), which might explain a high genetic variability, as had been previously reported in other two Turkish canids,

Canis aureus (İbiş *et al.* 2015a) and *Vulpes vulpes* (İbiş *et al.* 2014). Similarly, a high genetic diversity in gray wolves was also reported in some studies (JĘDRZEJEWSKI *et al.* 2005, PILOT *et al.* 2010, ASADI-AGHBOLAGHI *et al.* 2014, BRAY *et al.* 2014, DJAN *et al.* 2014, PILOT *et al.* 2014), including packs in Białowieża Primeval Forest on the border between Poland and Belarus, and in the modern European, Iranian, Saudi Arabian Dinaric-Balkan and Caucasus populations.

In the dataset including 317 bp fragments composed of sequences of the present study and those available in the GenBank from Eurasia under the name *Canis lupus* (Table 1), we found two new haplotypes (Tr.Cl.D3 and Tr.Cl.D4) in the 12 Turkish gray wolves (Table 1). The five remaining haplotypes (Tr.Cl.D1, Tr.Cl.D2, Tr.Cl.D5, Tr.Cl.D6 and Tr.Cl.D7) were shared with sequences derived from the GenBank (Table 1). Four D-loop sequences of Turkish gray wolves analyzed in previous studies (VILÁ *et al.* 1997, 1999, RANDI *et al.* 2000, PILOT *et al.* 2006, 2010, 2014) are of different lengths (257 bp, 542 bp, 658 bp and 659 bp). Of these sequences, three sequences (AF115693: RANDI *et al.* 2000, FJ978020 and FJ978033: PILOT *et al.* 2010, 2014) were included in the analyses of present study. On the other hand, the sequence AF005296 (257 bp) (VILÁ *et al.* 1997, 1999, PILOT *et al.* 2006) was not used in the current analyses due to the shortage of the mentioned sequence.

Our analyses indicate that the Turkish haplotypes in this study were relatively distant from each other and clustered into distinct sublineages in the Bayesian tree (Fig. 2). In Figure 2, the previous Turkish sequence, FJ978033 (PILOT *et al.* 2010, 2014), was grouped with the

haplotypes Tr.Cl.D1 and Tr.Cl.D2 of this study, whereas the remaining previous Turkish sequences, AF115693 (RANDI *et al.* 2000) and FJ978020 (PILOT *et al.* 2010, 2014), were clustered relatively distant from the haplotypes of this study. The phylogenetic resolution in the Bayesian tree (Fig. 2) was relatively low, which might be due to the high mutation rate in mitochondrial D-loop region of wolves. Similarly, a low phylogenetic resolution was also reported by LEBARBENCHON *et al.* (2010) and RODRIGUES *et al.* (2016) for least weasels (*Mustela nivalis*), based on the D-loop (control region) sequences.

The Turkish wolf haplotypes were grouped together with conspecific haplotypes in distinct subgroups in the inner parts of the haplotype network (Fig. 3), which is considered to show the intraspecific phylogenetic relationships better than phylogenetic trees (CRANDALL *et al.* 2000). The Bayesian tree (Fig. 2.) and the haplotype network (Fig. 3) were partly in agreement with each other, and both of them (Figs. 2, 3) have shown that the gray wolf haplotypes were divided into two main haplogroups. However, a clear geographical pattern was not observed for the distribution of gray wolf haplotypes in the Bayesian tree (Fig. 2) and the haplotype network (Fig. 3). In this context, the result of present study was compatible with that of PILOT *et al.* (2006). The existence of shared D-loop haplotypes found in this study suggested ongoing and/or past gene flow within the gray wolves in Turkey and other regions of Eurasia and that they originated from the same gene pool. Therefore, this study supported the view of PILOT *et al.* (2014) suggesting the ongoing and/or past gene flow between the Caucasian and Eastern European wolf populations.

To confirm the high genetic diversity and to elucidate the phylogeography of Turkish gray wolves, additional analysis is needed to obtain more data from the Turkish gray wolves in a multilocus study carried out by using two molecular markers, mitochondrial and nuclear DNA.

Acknowledgments

We would like to thank Donna Sue Özcan for English editing.

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