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Phylogenetic Status and Genetic Diversity of the Turkish Marbled Polecat, *Vormela peregusna*, (Mustelidae: Carnivora: Mammalia), inferred from the Mitochondrial Cytochrome *b* Gene

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Abstract

Previous genetic studies of marbled polecats did not include samples collected in Turkey. Therefore, the current knowledge is not sufficient to elucidate the phylogenetic relationships among marbled polecat populations. The aim of the present study was to determine the genetic diversity of the Turkish population and to reveal the phylogenetic relationships between the Turkish population and other marbled polecat populations. To accomplish this, we analyzed 17 cytochrome *b* gene sequences from Turkey and compared these with 10 cytochrome *b* gene sequences from the GenBank database. To construct the phylogenetic tree, we used the Neighbor-Joining, Maximum Likelihood, Maximum Parsimony and Bayesian methods, and also a median-joining network to assess relationships among haplotypes of the marbled polecat. The performed analyses, except for Bayesian method, yielded three main haplogroups within the marbled polecat. Haplogroup 1 contained haplotypes from Armenia, Azerbaijan, Russia, Turkey (Anatolia), Turkmenistan and Uzbekistan, Haplogroup 2 consisted of haplotypes from Turkey (Thrace), Turkmenistan and an unknown location, and Haplogroup 3 (Turkish Main Haplogroup) contained only the Turkish haplotypes. The present study shows that the Turkish population consists of individuals from all these three distinct phylogroups, and emphasized the importance of data obtained from the Turkish samples to reveal the phylogenetic relations among marbled polecat populations.

Key words

Marbled polecat, *Vormela peregusna*, Cytochrome *b*, Turkey.

Introduction

The marbled polecat *Vormela peregusna* (Güldenstaedt, 1770) is a medium-sized marten-like mustelid (Gorsuch & Larivière 2005), widely distributed from South-east Europe, through South-west Asia and Central Asia, to Mongolia and Northern China (Gorsuch & Larivière 2005, Wilson & Reeder 2005, Tikhonov *et al.* 2008). This species has an important role as predator in the ecosystem. It is threatened in its native range due to the loss of natural steppe and desert habitats, and has been labeled as a vulnerable (VU) species (Tikhonov *et al.* 2008).

Molecular analyses on intraspecific genetic diversity have been of specific value regarding phylogeographic structure of mammalian species. Global climatic fluctuations caused by glaciation cycles have been important in shaping the genetic structure of numerous extant

taxa and affected their distributions during Pleistocene. Possible genetic consequences of glaciations have been determined by DNA technology (HEWITT 2000). Based on DNA markers, *V. peregusna* is one of the least studied species in the family Mustelidae. An initial genetic study was performed by DNA sequencing of the mitochondrial cytochrome *b* gene of marbled polecats from Kalmykia and Rostov region in Russia, and Bukhara region in Uzbekistan (ROZHNOV *et al.* 2006). Also, other molecular genetic studies (ROZHNOV *et al.* 2006, KOEPFLI *et al.* 2008, SATO *et al.* 2012) have included marbled polecats in phylogenetic studies of the Mustelidae (ROZHNOV *et al.* 2006, KOEPFLI *et al.* 2008) and Musteloidea (SATO *et al.* 2012) using the mitochondrial cytochrome *b* gene (ROZHNOV *et al.* 2006), and mitochondrial and nuclear DNA data sets (KOEPFLI *et al.* 2008, SATO *et al.* 2012). These studies (ROZHNOV *et al.* 2006, KOEPFLI *et al.* 2008, SATO *et al.* 2012) showed that the marbled polecat assigned into the Tribus Ictonychini with the genera *Ictonyx* and *Poecilogale*. However, ROZHNOV *et al.* (2008) only presented the genetic variation of marbled polecat using the mitochondrial cytochrome *b* sequences. As noted in previous studies, the analyses of phylogenetic relationships and genetic variation were restricted to a small number of regional samples (ROZHNOV *et al.* 2006, 2008, KOEPFLI *et al.* 2008, SATO *et al.* 2012). The use of a limited number of regional samples may not be representative to the overall phylogenetic relationships and intraspecific genetic structure of marbled polecat. Therefore, a larger sample size from different regions is required in order to evaluate the different phylogeographic hypotheses. To date, the genetic structure of Turkish marbled polecat populations has not been investigated.

Currently, more than 150 mammalian species were listed from Turkey (KRYŠTUFEK & VOHRALIK 2001, 2009, WILSON & REEDER 2005). Until now, the studies have been based on the ecology, distribution, karyology and morphology of the Turkish marbled polecats (ÖZKURT *et al.* 1999, 2000, TEZ *et al.* 2001, YIĞIT *et al.* 2006, ARSLAN & ZIMA 2013). Turkey is one of the major unsampled areas to determine the genetic status of the marbled polecat. Hence, there is a lack of genetic data from an important part of the species' range. Such genetic data would be crucial to reveal the intraspecific genetic structure of the marbled polecat and will provide new insights into the phylogeographic processes of this species, data that may be essential for a better understanding of the evolution of the marbled polecat. In addition, as the marbled polecat populations are faced with the loss of habitats and hunting by human (TIKHONOV *et al.* 2008), it is urgent to clarify the genetic structure of the species from a conservation genetics perspective.

In this study, to determine the genetic diversity of the Turkish marbled polecat population and to reveal the phylogenetic relationships between this and other populations within its distribution range, we analyzed the complete cytochrome *b* gene sequences from the 17 Turkish samples and compared them with 10 sequences obtained from the GenBank database.

Material and Methods

Sampling, DNA isolation, PCR conditions and sequencing

Tissue samples (ear, tail, skin and skeletal muscle) from the 17 marbled polecats, which were road kills, were collected from different localities in Turkey (Table 1; Fig. 1). Tissue pieces fixed in 99% ethanol were used as sources for total DNA extraction by using the DNeasy Blood and Tissue Kit (QIAGEN), following the manufacturer's instructions. The samples had been stored in the corresponding author's private collection at the Department of Biology, Faculty of Sciences, Erciyes University in Kayseri, Turkey. We sequenced the complete cytochrome *b* (Cyt *b*) gene for a total of 17 marbled polecats. The Cyt *b* gene was amplified by using Hot Start Touch Down PCR with the primers

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L14724 (5' CGAAGCTTGATATGAAAAACCATCGTTG 3),
H15494 (5' TAGTTGTCAGGGTCTCCTAG 3) and
L15162 (5' GCAAGCTTCTACCATGAGGACAAATATC 3),
H15915 (5' AACTGCAGTCATCTCCGGTTTACAAGAC 3)

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(IRWIN *et al.* 1991, KOEPFLI & WAYNE 1998). PCR amplifications were performed in 50 µl reaction mixtures (1 × Taq buffer with $(NH_4)_2SO_4$, 200 µM dNTP mix, 1.5 u Hot Start Taq DNA polymerase (Thermo Scientific), 1.5 mM $MgCl_2$, 0.8 µM of each primer, 1 µl DNA extract). The Hot Start Touch Down PCR program was comprised of a pre-denaturation procedure consisting of 15 min. at 95°C by 1 cycle, a denaturation step of 30 sec. at 94°C by 14 cycles, an annealing step of 90 sec. at 60°C (decreasing 0.5°C at per cycle) by 14 cycles, an extension step of 60 sec. at 72°C by 14 cycles, a denaturation step of 30 sec. at 89°C, an annealing step of 90 sec. at 53°C, an extension step of 60 sec. at 72°C by 33 cycles and an ending step of 30 min. at 60°C by 1 cycle. To verify the quality of total DNA and PCR products, 1% and 1.5% agarose gels were run and stained with ethidium bromide, respectively.

PCR products were purified using High Pure PCR Product Purification Kit (Roche)**.** Sequencing was performed by using forward and reverse primers, resulting in sequences readable for 1140 bp. The purified PCR products were sequenced with an ABI 3100 Genetic Analyzer (RefGen, METU, Technopark-Ankara, Turkey).

Sequence analysis

To determine the phylogroups of Turkish population, we compared Turkish marbled polecat sequences with those from the GenBank database (NCBI: The National Center for Biotechnology Information) that were deposited by ROZHNOV *et al.* (2008) (Armenia: EF581357_ARM; Azerbaijan EF581358_AZ), ROZHNOV *et al.* (2006) (Uzbekistan: DQ138079_UZB; Russia: AY789052_RUS,

Table 1. List of the Turkish samples/haplotypes used in the present study (* Map numbers are locality codes in Figure 1).

No	Map number*	Cyt *b* haplotype/sequence	Locality	Coordinate/Altitude
1	2	VPETR1	Dazkırı, Afyonkarahisar	N 37° 52.260′ E 29° 46.430′ H – 1025m
2	3	VPETR1	Anayurt, Şuhut, Afyonkarahisar	N 38° 27.963′ E 30° 34.674′ H – 1132m
3	4	VPETR1	Kıreli, Hüyük, Konya	N 37° 53.461′ E 31° 33.105′ H – 1157m
4	8	VPETR1	Kızören, Konya	N 38° 08.621′ E 33° 11.152′ H – 1024m
5	10	VPETR1	Saratlı Kasabası, Aksaray	N 38° 28.321′ E 34° 12.984′ H – 1188m
6	13	VPETR1	Erciyes University Campus, Kayseri	N 38° 04.390′ E 35° 34.160′ H – 1070m
7	14	VPETR1	Hamit, Akpınar, Kırşehir	N 39° 31.181′ E 33° 45.544′ H – 900m
8	15	VPETR1	Ağcın, Sorgun, Yozgat	N 39° 53.000′ E 35° 05.000′ H – 1220m
9	9	VPETR2	Yenice, Konya	N 38° 10.441′ E 33° 17.232′ H – 1004m
10	16	VPETR2	Ilgaz, Çankırı	N 40° 52.367′ E 33° 38.172′ H – 1259m
11	7	VPETR3	Zincirli, Konya	N 38° 01.745′ E 32° 53.018′ H – 1010m
12	5	VPETR4	Bayındır, Beyşehir, Konya	N 37° 43.926′ E 31° 46.635′ H – 1187m
13	17	VPETR5	Kars	N 40° 33.300′ E 43° 04.320′ H – 1780m
14	11	VPETR6	6 Km. W, Ulukışla, Niğde	N 37° 32.260′ E 34° 30.300′ H – 1500m
15	1	VPETR7	Azatlı, Havsa, Edirne	N 41° 29.000′ E 26° 41.000′ H – 120m
16	12	VPETR8	Yeşilhisar, Kayseri	N 39° 13.261′ E 35° 01.867′ H – 1362m
17	6	VPETR9	Konya	N 37° 52.210′ E 32° 35.090′ H – 1005m

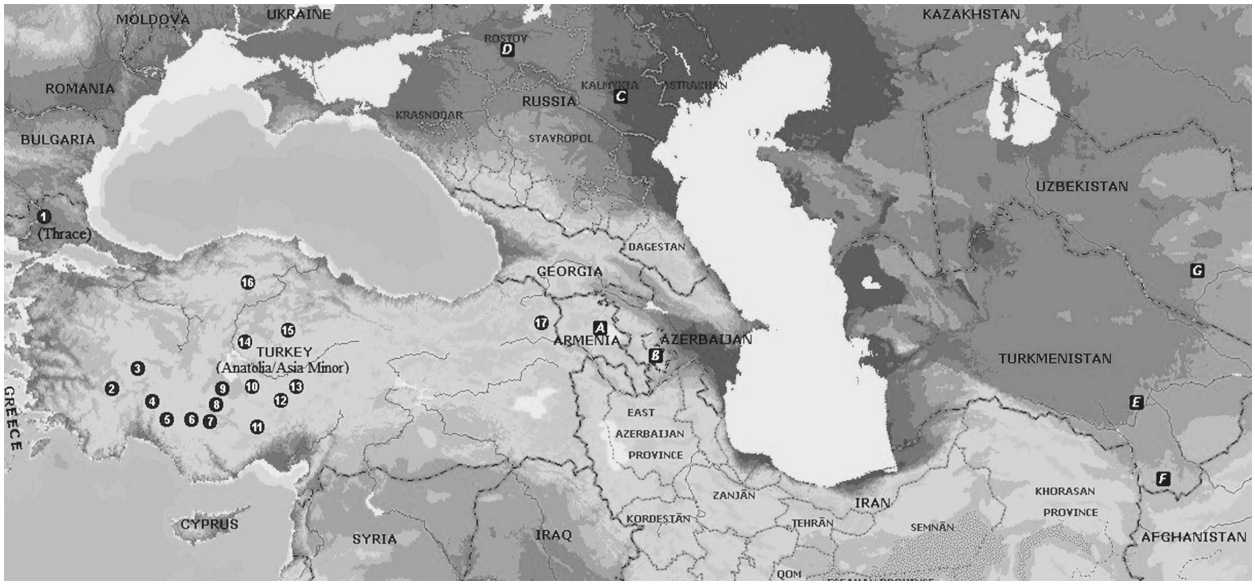

Fig. 1. Sampling localities of the marbled polecat. Map letters and numbers are locality codes (see Table 1 for 1 – 17 numbers, and **A**) EF581357 and AB564108: Armenia, Sevan Lake region; **B**) EF581358: Azerbaijan, Lachin region; **C**) AY789052: Russia, Kalmykia; **D**) AY789053: Russia, Rostov region; **E**) EF581359 and EF581360: Turkmenistan: SE Karakumy; **F**) EF581361: Turkmenistan: Kushka region **G**) DQ138079: Uzbekistan: Bukhara region).

AY789053_RUS), Rozhnov *et al.* (2008) (Turkmenistan: EF581359_TUR, EF581360_TUR, EF581361_TUR), and Sato *et al.* (2012) (Armenia: AB564108_ARM). As the location of EF987740_UL was not reported by Koepfli *et al.* (2008), it was pointed out as „unknown location" in our analyses. The greater grison, *Galictis vittata* [Accession number: AB498155 (Koepfli & Wayne 2003)], and the pine marten, *Martes martes*, [Accession number: EF987751 (Koepfli *et al.* 2008)] were used as out-group.

We used the program Geneious v.6.1 (available from http://www.geneious.com) to edit the sequences and to construct a sequence alignment of 1140 bp. Haplotype diversity (*h*) and nucleotide diversity (π) were calculated using DnaSP v. 5.10.01 (Librado & Rozas 2009). Genetic

distances were estimated under the Kimura 2-parameter (K2P) nucleotide substitution model (Kimura 1980) in MEGA5 v. 5.01 (Tamura *et al.* 2011).

A haplotype network was constructed using the median-joining (MJ) network with the Network 4.6.1.1 software (Bandelt *et al.* 1999, http://www.fluxus-engineering.com) to represent the intra-specific genealogy of the haplotype dataset.

Phylogenetic trees were generated using the Maximum Likelihood (ML) and Neighbor-Joining (NJ) and Maximum Parsimony (MP) methods with MEGA5 software (Tamura *et al.* 2011), and Bayesian method with Mr.Bayes v.3.2.1 (Ronquist & Huelsenbeck 2003). Before generating ML and Bayesian trees, the HKY (Hasegawa-Kishino-Yano) + G (gamma) model of nucleotide substitution was determined as the most appropriate model according to Bayesian Information Criterion (BIC) using the program jModelTest2 (Guindon & Gascuel 2003, Darriba *et al.* 2012). NJ tree was generated using the Kimura 2-parameter (K2P) nucleotide substitution model (Kimura 1980).

The four Monte Carlo Markov chains were used to calculate the Bayesian posterior probabilities for 0.1 million generations with trees sampled every 100 generations; the first 25 % of samples were discarded as burn-in (average standard deviation of split frequencies <0,01). After discarding burn-in trees and evaluating convergence, the remaining samples were retained for generating consensus trees (50 % majority rule), calculating 95 % Bayesian credible intervals and posterior probabilities. Bayesian tree diagrams were obtained with tree figure drawing tool, FigTree v1.3.1 (Rambaut 2009).

Additionally, to obtain genetic divergence among the haplogroups, we used K2P distance estimator (Kimura 1980) implemented in MEGA5 (Tamura *et al.* 2011). Avise's (2000) formula was used to estimate divergence time among the haplogroups, based on the genetic divergence obtained with K2P; $P_{net} = P_{AB} - 0.5(P_A + P_B)$, where P_{net} is the corrected distance between the A and B haplogroups, P_{AB} is the mean genetic divergence in pairwise comparisons of haplotypes A versus B, and P_A and P_B are the mean genetic divergence among haplotypes within these haplogroups. In order to date branching events, we assumed a Cyt *b* divergence rate of 2 % per million years as is the commonly used rate for large mammals (Brown *et al.* 1979, Avise *et al.* 1998).

Results

Genetic variability in the Turkish marbled polecat

From the 17 Turkish samples we obtained complete sequences (1140-bp) of the mitochondrial Cyt *b* gene, and observed nine haplotypes (VPETR1-9) (Table 1, Fig. 1). One of nine haplotypes, VPETR1, was identical to two sequences (AB564108 and EF581357) already reported from Armenia (Rozhnov *et al.* 2008, Sato *et al.* 2012). The other haplotypes, VPETR2–9 have not previously been reported. The sequences of the Turkish haplotypes (VPETR1–9) were deposited in the GenBank database (Accession numbers: KM068802–KM068810).

Haplotype diversity (*Hd*) (0.7868) and nucleotide diversity (π) 0.00273) demonstrated relatively low levels of genetic diversity for the 17 Turkish marbled polecats, respectively. The sequence divergences, using K2P distance, ranged from 0.007 to 0.001, with an average of 0.004 among the Turkish haplotypes.

Phylogenetic relationships of the marbled polecat Cyt *b* haplotypes

To reveal phylogenetic relationships among the haplotypes, we analyzed the complete cytochrome *b* gene sequences from the 17 Turkish samples with 10 sequences obtained from the GenBank database, and observed 16 haplotypes from the 27 Cyt *b* gene sequences (1140 bp).

The NJ and ML trees showed similar topologies and had similar branching structures (Figs. 2, 3). In both trees, the Cyt *b* haplotypes were separated into three groups; Haplogroup 1, Haplogroup 2 and Haplogroup 3. In ML tree (Fig. 3), Phylogroup 3 was the same as that of the NJ tree (Fig. 2). In contrast to the NJ tree (Fig. 2), one haplotype from Turkmenistan (EF581359) clustered in Haplogroup 1 of the ML tree (Fig. 3). However, the MP and BI trees differed from the NJ and ML trees (Figs. 4, 5). The MP tree (Fig. 4) consisted of three haplogroups, as shown in the NJ and ML trees (Figs. 2, 3) and haplogroup 3 in the MP tree (Fig. 4) contained the Turkish haplotypes (Anatolia), similar to the NJ and ML trees (Figs. 2, 3). However, Haplogroup 1 comprised haplotypes from Azerbaijan, Russia, Turkey (Thrace and Anatolia), Turkmenistan, Uzbekistan and an unknown location, and Haplogroup 2 included haplotypes from Armenia and Turkey (Anatolia) (Fig. 4). Furthermore, the Bayesian tree (Fig. 5) exhibited four haplogroups (Haplogroup 1, Haplogroup 2, Haplogroup 3 and Haplogroup 4), supported by higher posterior probability values (59–99%) than the bootstrap values (13–80%) of the ML, MP and NJ trees (Figs. 2–4). Of the haplogroups in BI tree (Fig. 5), Haplogroup 1 included haplotypes occurring in Azerbaijan, Russia, Turkmenistan and Uzbekistan, Haplogroup 2 consisted of haplotypes from Turkmenistan, Turkey (Thrace and Anatolia) and an unknown location, Haplogroup 3 was composed of one Turkish haplotype (Anatolia), and Haplogroup 4 comprised haplotypes occurring in the Turkey (Anatolia) and Armenia.

Network relationships of the marbled polecat Cyt *b* haplotypes

As shown in the all trees (Figs. 2–4), except for Bayesian tree (Fig. 5), the median-joining network also formed

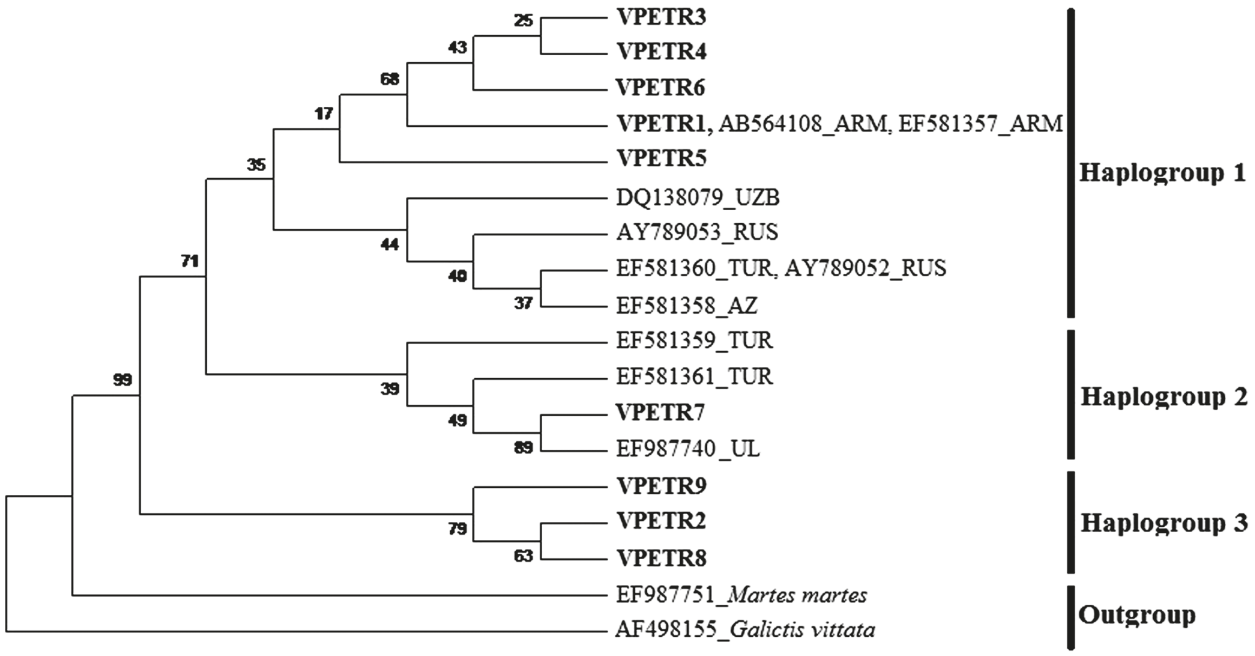

Fig. 2. Neighbor-Joining tree constructed from haplotypes of *Vormela peregusna*, based on the complete sequences of mitochondrial cytochrome *b* gene. Numbers above branches show the Bootstrap values. Bold indicates the Turkish haplotypes.

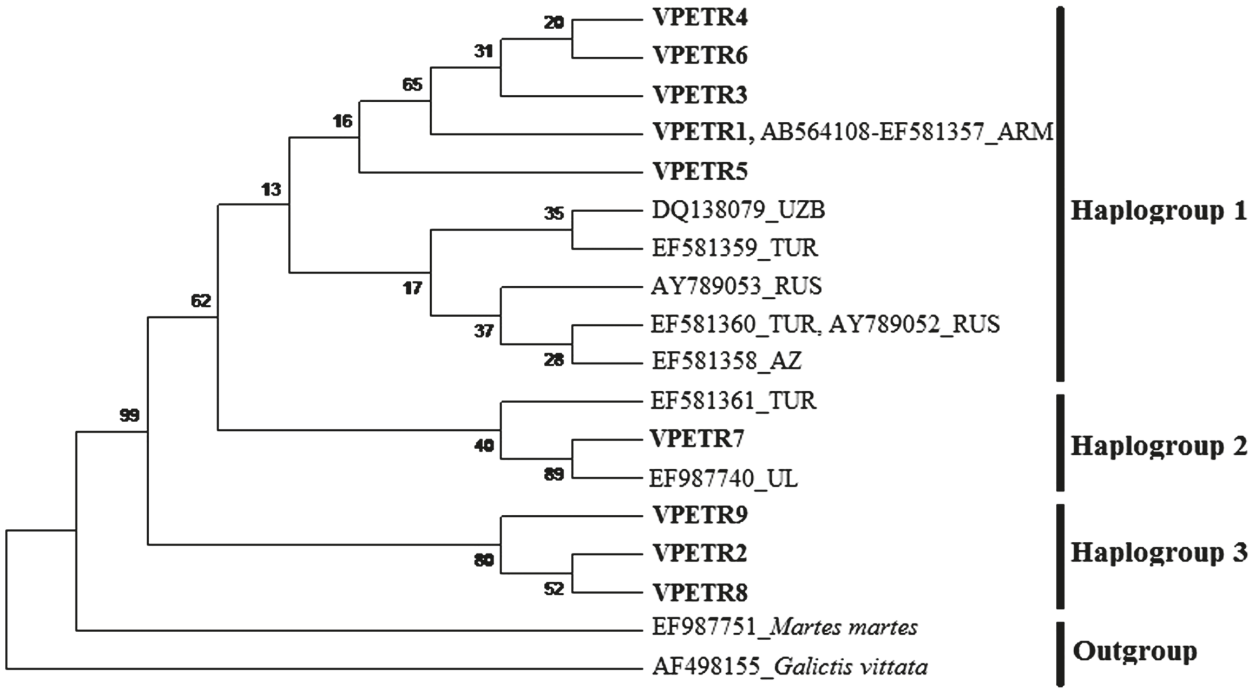

Fig. 3. Maximum Likelihood tree constructed from haplotypes of *Vormela peregusna*, based on the complete sequences of mitochondrial cytochrome *b* gene. Numbers above branches show the Bootstrap values. Bold indicates the Turkish haplotypes.

three haplogroups (Fig. 6). The haplotype network (Fig. 6) and the NJ tree (Fig. 2) matched. However, the haplotype network matched partly to the ML (Fig. 3) and MP trees (Fig. 4). Furthermore, the median-joining network (Fig. 6) and Bayesian tree (Fig. 3) did not match.

The mean genetic distances and the divergence times among haplogroups

By assuming a divergence rate of 2 % per million years, the mean genetic distance between the Haplogroup 1

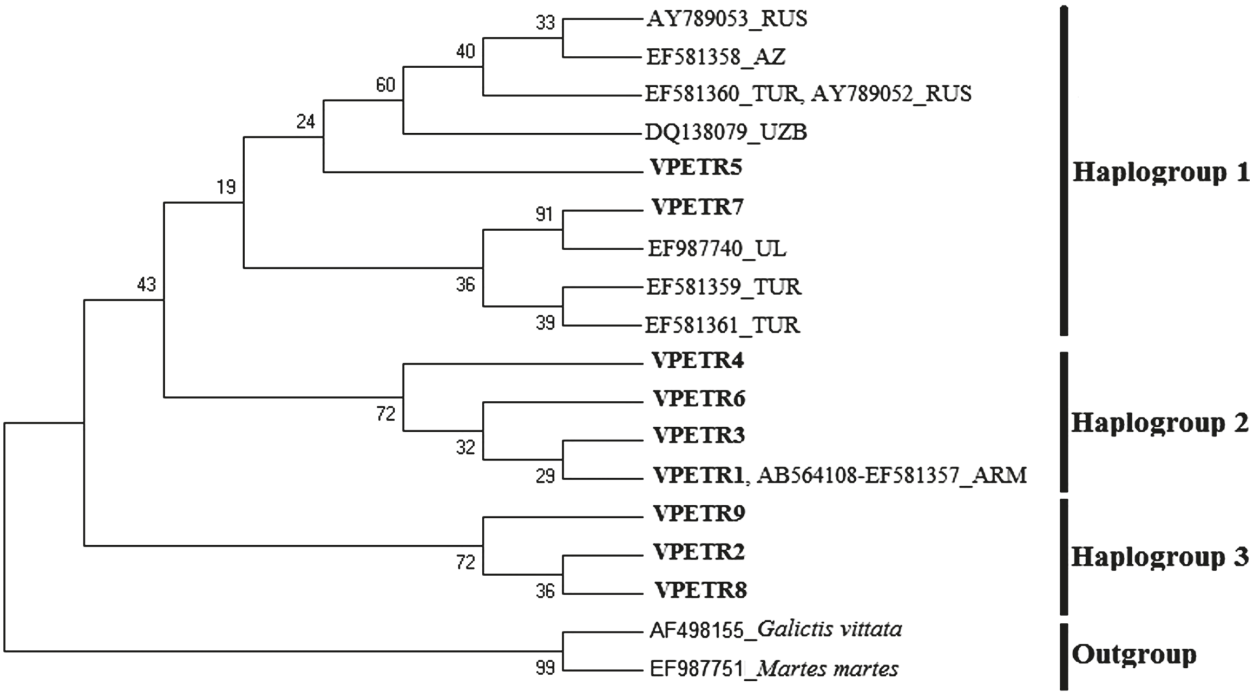

Fig. 4. Maximum Parsimony tree constructed from haplotypes of *Vormela peregusna*, based on the complete sequences of mitochondrial cytochrome *b* gene. Numbers above branches show the Bootstrap values. Bold indicates the Turkish haplotypes.

and Haplogroup 2 of 0.0014 corresponds to a divergence time of about 0.07 myr. The mean genetic distance and divergence time were 0.0038 and 0.19 myr between the Haplogroup 1 and Haplogroup 3 and 0029 and 0.14 myr between the Haplogroup 2 and Haplogroup 3.

Discussion

This study was performed to determine the genetic diversity of Turkish marbled polecat and to reveal the phylogenetic relationships between the Turkish population and other populations within the distribution range of the species. The Turkish population has not previously been studied by genetic analyses. Therefore, our results present new insights into the phylogenetic relationships of marbled polecat populations. Cluster analyses of the complete Cyt *b* gene sequences suggested that the marbled polecat haplotypes form three main mitochondrial Cyt *b* phylogroups (Figs. 2–4, 6). Based on the mitochondrial sequences obtained from the current distribution of marbled polecat, we estimated divergence times of the three identified groups for the marbled polecat in Figure 6. Our estimates suggest that Haplogroup 1 and Haplogroup 2 diverged at the middle of Late Pleistocene (about 0.07 myr), and Haplogroup 1 and Haplogroup 3 (about 0.19 myr), and Haplogroup 2 and Haplogroup 3 (about 0.14 myr) at the end of Middle Pleistocene.

The marbled polecat is widely distributed in the steppe and desert parts of Eurasia (Gorsuch & Larivière 2005, Wilson & Reeder 2005, Tikhonov *et al.* 2008). The genetic diversity and phylogenetic relationships of marbled polecat populations have been poorly investigated (Rozhnov *et al.* 2006, 2008, Koepfli *et al.* 2008, Sato *et al.* 2012). The first molecular study (Rozhnov *et al.* 2006) including the marbled polecat was performed by sequence analyses of the complete Cyt *b* gene. It defined the position of the genus *Vormela* in the family Mustelidae (Rozhnov *et al.* 2006). Rozhnov *et al.* (2008) analyzed sequences from the mitochondrial Cyt *b* and control region and reported no genetic difference among the marbled polecat samples obtained from southern Russia, Transcaucasia, Turkmenistan and Uzbekistan. Furthermore, sequencing of mitochondrial DNA (Cyt *b* and the central domain of control region) indicated that the marbled polecat had a high degree of genetic homogeneity (Rozhnov *et al.* 2008).

The intraspecific genetic structure and phylogenetic relationships of marbled polecat populations have not be known in detail due to the small sample size in previous studies (Rozhnov *et al.* 2006, 2008, Koepfli *et al.* 2008, Sato *et al.* 2012). Hence, the present study was important to determine the genetic diversity and to reveal phylogenetic relationships within the Turkish population, and furthermore, to assess the intraspecific genetic structure of the marbled polecat. There are many previous studies on the genetic diversity and the phylogeographic structure of various species occurring in the surrounding regions of the Mediterranean Basin (Ludt *et al.* 2004, Bardakçı *et al.* 2006, Çiplak *et al.* 2005, Gündüz *et al.* 2007, Joger *et al.* 2007, Macholan *et al.* 2007, Rajabi-Maham *et al.* 2008, Kryštufek *et al.* 2009, Gvozdik *et al.*

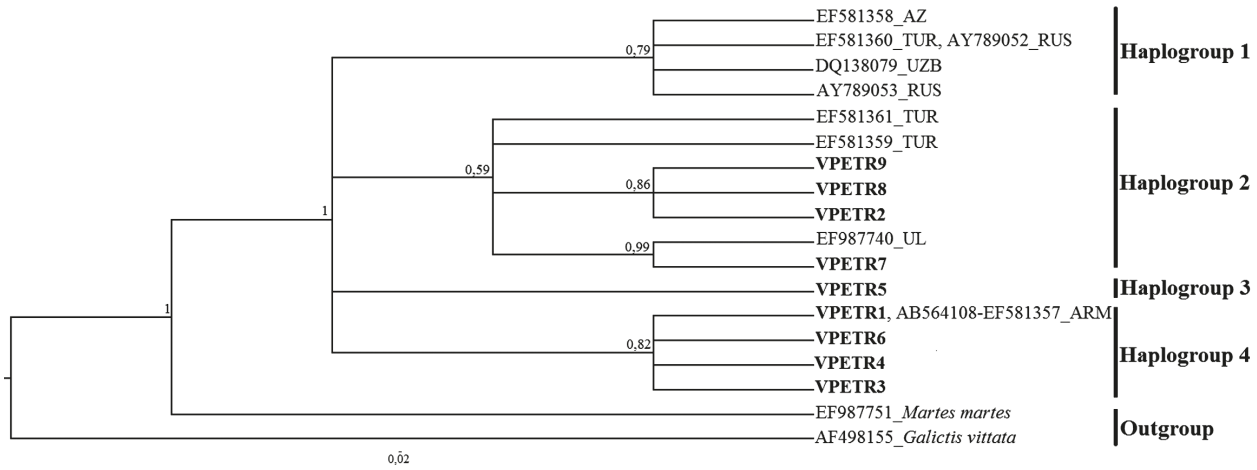

Fig. 5. Bayesian phylogenetic tree constructed from haplotypes of *Vormela peregusna*, based on the complete sequences of mitochondrial cytochrome *b* gene. Numbers above branches show the Bayesian posterior probabilities. Bold indicates the Turkish haplotypes.

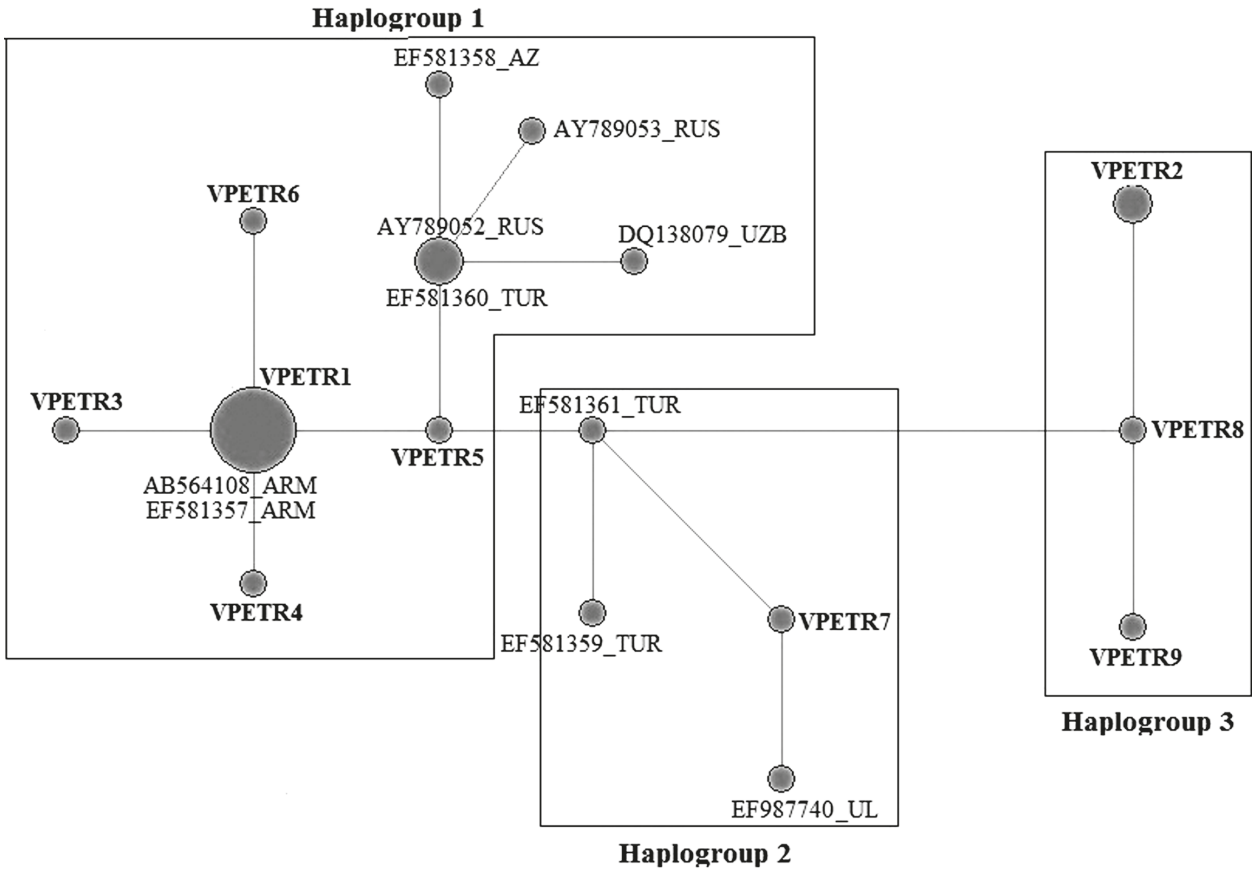

Fig. 6. Median-joining network constructed from haplotypes of *Vormela peregusna*, based on the complete sequences of mitochondrial cytochrome *b* gene. Bold indicates the Turkish haplotypes.

2010, Thanou *et al.* 2012, Kaya *et al.* 2013, Poulakakis *et al.* 2013). Heptner *et al.* (1967) suggested that the genus *Vormela* might have originated from the Anatolian part of Turkey (Asia Minor) in the Pliocene. During the Middle Pliocene (3.6–2.5 mya), the climate of the Northern Hemisphere was hotter and wetter than nowadays. However, during the Late Pliocene (2.5–1.84 mya), the climate was more different than the Middle Pliocene, and it was both drier and colder (Willis *et al.* 1999, Fauquette & Bertini 2003).

The climate changes during the Pliocene might have led to the genetic differentiation within the genus *Vormela*.

The marbled polecat (*V. peregusna*) might have evolved from *V. petenyii* (Spassov 2001), and fossil remains of the marbled polecat were found in the late Pleistocene localities from the Caucasus (Azerbaidjan) and the Middle East (Palestine) (Kurtén 1968). At the beginning of the Pleistocene (1.84 mya) (Webb & Bartlein 1992) climatic fluctuations led to glacial and interglacial periods which influenced the distribution and diversification process of species (Hewitt 2000). In Europe, most of temperate species have survived in refuge populations around the Mediterranean Basin during the Pleistocene (Hewitt 1996). Anatolia, situated in Southwest Asia at the north eastern part of the Mediterranean Basin, is also considered to be a refuge for some European species during the climatic cycles of the Pleistocene (Hewitt 1996, 1999, 2000, 2004, Taberlet *et al.* 1998, Schmitt 2007).

Considering the distribution and phylogenetic positions of the marbled polecat haplotypes (Figs. 2–4, 6), all phylogenetic analyses based on the mitochondrial Cyt *b* sequences (Figs. 2–4), except the Bayesian analysis (Fig. 5), revealed that the Haplogroup 3, which contains haplotypes restricted to the Central Anatolia-Turkey, was the most basal cluster (Figs. 2–4). This seems to indicate that the marbled polecat might have spread from an ancestral population in the Asian part of Turkey, as suggested by a previous study (Heptner *et al*. 1967). However, a closer inspection of the phylogenetic trees (Figs. 2–5) reveals that the phylogenetic relationships are poorly resolved and not well supported (particularly groups I and II) and this is consistent with the very low nucleotide diversity. Even though Haplogroup 3 comprised only the Turkish haplotypes (Anatolia), it may not be a sufficient evidence to support the supposed origin of the marbled polecat as mentioned above. Given that glacial and interglacial periods affected significant portions of the Palaearctic region and led to genetic subdivisions of most taxa (Hewitt 2000), the present phylogenetic relationships and genetic divergence of marbled polecat populations may be considered the genetic consequences of climatic fluctuations having occurred during the Middle and Late Pleistocene.e.

Finally, to clarify in detail the intraspecific phylogenetic relationships and genetic structure of marbled polecat, it is important to include more samples from the whole range of the species, and in addition to mitochondrial DNA analysis, such a study should also be designed to analyze multiple and nuclear DNA markers.

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