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RESEARCH ARTICLE

Phylogenetic relationships of *Podarcis siculus* (Rafinesque-Schmaltz, 1810) and *Podarcis tauricus* (Pallas, 1814) in Turkey, based on mitochondrial DNA

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ABSTRACT

The Italian wall lizard and the Balkan wall lizard have a series of taxonomic revisions. However, their phylogenetic relationships still remain uncertain in Turkey. In the present study, we have assessed taxonomic relationships, both of *Podarcis siculus and Podarcis tauricus* through estimation of phylogenetic relationships among 43 and 42 specimens, respectively, using mtDNA (16 S rRNA and cytb) from great main populations in Turkey. The genetic distances among the populations of *P. siculus* in Turkey were very low and they were ranged from 0.2 to 1.6% in 16 S rRNA while they were ranged from 0.0% to 3.3% in cytb. On the other hand, the p-distances among the populations of *P. tauricus* were ranged from 0.0 to 0.6% in 16 S rRNA while they were 0.2% cytb in Turkey. Finally, most of the topologically identical trees of phylogenetic analyses and p-distances showed that monophyly was found in extant populations of *P. siculus* and *P. tauricus*. The nominate subspecies, *P. s. siculus* and *P. t. tauricus* are representatives of these lizards in Turkey.

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Introduction

Wall lizards of the genus *Podarcis* (Wagler 1830) comprise currently 23 recognized species (Sindaco et al. 2013; Uetz and Hošek 2016). The origin of the genus is western European (Oliverio et al. 2000) and the genus is distributed in Europe, North Africa, and North America. Most species of the genus are restricted to the Mediterranean basin (Harris 1999; Harris and Arnold 1999; Speybroeck et al. 2010; Silva-Rocha et al. 2012). Currently, the predominant reptile group in southern Europe is distributed from Northwestern Africa through the Iberian and the Italian peninsulas to the Balkans, northwestern Asia Minor and the Crimean peninsula (Arnold 1973).

Taxonomy of *Podarcis* genus is complicated and continuously needs revision, due to the substantial intra-specific variability (Arnold et al. 1978). The first phylogenetic studies on the genus were conducted by Harris and Arnold (1999) and Oliverio et al. (2000). The genus was separated into four geographic groups (the Western island group, the Balkan group, the Italian group and the Southwestern group) but relationships were mainly unresolved. It may be due to a large distribution area of the genus.

The Italian wall lizard, *Podarcis siculus*, is one such species that has a large distribution area in the central Mediterranean region (Corti 2006). It is widespread in Italy (on many Adriatic islands, the large islands Sicily, Sardinia and Corsica) and the northern part of the east Adriatic coast. Apart from this distribution, it is also distributed in the Mediterranean region (in Portugal, Spain, France, Montenegro, Turkey, Libya and Tunisia) and the USA (Behler and King 1979; Conant and Collins 1991). Italy is thought to be the area of origin and the expansion center of the species (Radovanovic 1956; Schneider 1971; Gorman et al. 1975). In this large distribution area, *P. siculus* has 23 subspecies.

In Turkey, the first specimens of *P. siculus* were recorded from Anatolian part of the İstanbul province by Berhold (1942) and he described the specimens as the representatives of *P. s. hieroglyphicus* based on their morphological characters. Other records were given from İstanbul and islands of Marmara (Bird 1936; Bodenheimer 1944; Mertens and Wermuth 1960; Clark and Clark 1973; Başoğlu and Baran 1977; Franzen 1990; Çevik 1999; Jablonski and Stloukal 2012), Bursa (Uğurtaş and Yıldırımhan 2000; Mollov 2009; Arslan et al. 2013) and Çanakkale (Hür et al. 2008; Tok and Cicek 2014; Tok et al. 2015) provinces in the Marmara Region and Zonguldak (Ilgaz et al. 2013) and Samsun (Tok et al. 2015) provinces in the Black Sea Region of Turkey. According to current literature, the lizards belonging to *Podarcis sicula cettii*, *Podarcis sicula ragusae* and *Podarcis siculus hieroglyphicus* were approved as synonyms of *P. s. siculus* and the Turkish specimens of *P. siculus* are considered as the representatives of *P. s. siculus* (http://www.lacerta.de; Silva-Rocha et al. 2014). However, there is no phylogenetic study on these specimens belonging to Turkey populations.

The Balkan wall lizard, P*odarcis tauricus* is another species of *Podarcis* genus that has a smaller distribution area than *P. siculus* (ranging mainly in the southern Balkans and eastern Europe). Currently, it is subdivided into three recognized subspecies (Sindaco and Jeremčenko 2008). The first one is *P. t. tauricus* (Pallas 1811); the second one is *P. t. ionicus* (Lehrs

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1902); and the last one is *P. t. thasopulae* (Kattinger 1942). The first two subspecies are geographically isolated by the Pindos mountain while the third one has a small inhabiting area on the islet of Thasopoula (north Aegean) (Psonis et al. 2017).

In Turkey, *Podarcis tauricus* is distributed in the provinces of Thrace Region and İstanbul (Schreiber 1912; Cyrén 1924; Bird 1936; Bodenheimer 1944; Mertens 1952; Clark and Clark 1973; Andren and Nilson 1976; Çevik 1999), Kocaeli and Sakarya (Baran 1977; Başoğlu and Baran 1977; Nilson et al. 1988; Bergmann and Norström 1990, Franzen 1990; Teynie 1991; Baran et al. 1992; Mulder 1995; Sindaco et al. 2000) and Çanakkale (Tok and Cicek 2014) provinces in the Marmara Region. Recently, Bülbül et al. (2015) reported these lizards from the western Black Sea Region of Turkey.

Mertens (1952) reported that all examined specimens in the literature from the European and Anatolian parts of Turkey belonged to *P. t. tauricus*. The current literature (based on the morphological investigations) approves this view. However, there is no phylogenetic study on the Turkish populations of *P. tauricus*.

The phylogenetic relationships and phylogeography of the *P. tauricus* subgroup found in Europe have previously been investigated on the basis of mitochondrial DNA loci (Podnar et al. 2004, 2015; Poulakakis et al. 2005a,b; Psonis et al. 2017).

Although molecular studies were performed on the specimens in European populations of *P. siculus* and *P. tauricus*, the phylogenetic relationships of the Turkish populations of these species were not investigated. For this reason, the purpose of the present study is to appraise the phylogenetic relationships of the *P. siculus* and *P. tauricus* specimens from the great main distribution areas of Anatolia and to determine whether there is a potential new phylogenetic lineage of these lizards in Turkey based on the results of mitochondrial DNA for the first time.

Material and methods

Collection of the samples

A total of 43 specimens of *P. siculus* and 42 ones of *P. tauricus* were caught from different localities in Turkey (Figure 1) (Tables 1 and 2). For each lizard, the longest finger of the hind limb was clipped and preserved in 96% ethanol. After registration and toe-clipping, all lizards were released back into their natural habitats.

DNA extraction and PCR amplifications

The clipped toes obtained from lizards were stored in 96% ethanol. Later, the toes were treated with 180 µl ATL, 20 µl proteinase K and 4 µl RNAse in 2 ml eppendorf tubes overnight at 56 °C. Total genomic DNA of each specimen was extracted using the NucleoSpin tissue isolation kit following Manufacturer's instructions.

For *P. siculus*, a 501 base-pair-fragment of the 16 S rRNA gene (for 37 specimens) and a 469 base-pair-fragment of the cytb gene (for 39 specimens) were amplified while a 501 base-pair-fragment of the 16 S rRNA gene (for 40 specimens) and 425 base-pair-fragment of the cytb gene (for 40 specimens) were amplified for *P. tauricus*, using 16SarL and 16SbrH (Palumbi et al. 1991); L14724 and H15175 (Palumbi 1996) primers, respectively. Each 16 S rRNA gene amplification involved an initial incubation 3 min at 94 °C; 35 cycles of 30 s at 94 °C; 30 s at the appropriate annealing temperature (48–54 °C); and 1 min at 72 °C; followed by one cycle of 8 min at 72 °C. PCR amplifications for 16 S rRNA were conducted as described by (Guo et al. 2011). Each cytb gene amplification involved an initial incubation 5 min at 94 °C; 35 cycles of 60 s at 94 °C; 60 s at the appropriate annealing temperature (52–56 °C); and 1 min at 72 °C; followed by one cycle of 70 s at 72 °C. PCR amplifications for cytb were conducted as described by (Poulakakis et al. 2003). Amplified DNA segments were purified and sequenced by Macrogen Corporation in Netherlands.

Sequence alignment and phylogenetic analyses

The nucleotide sequences of each gene were aligned using the Bioedit (Thompson et al. 1997) program. Haplotypes were determined for each gene using TCS (Clement et al. 2000) program. (GenBank accession numbers for each haplotype sequence are given in (Tables 1 and 2). After confirming the suitability for the combination of all the sequences of two genes, we combined the data on these two genes for ML and BI. For a comparison of our haplotypes with other European *P. siculus* populations, we used six haplotypes from Italy (AY770920.1 and AY770919.1, Podnar et al. 2005), Spain (HM746963.1 and HM746964.1, Valdeon et al. 2010) and Croatia (EU362073.6 and EU362074.1, Herrel et al. 2008) for 16 S rRNA and eight haplotypes from Italy (KF372034.1, Salvi et al. 2013) and (AY770895.1, Podnar et al. 2005), Spain (JX072939.1 and JX072941.1, Silva-Rocha et al. 2012), Croatia (AY770882.1 and AY770892.1, Podnar et al. 2005), Portugal (JX072953.1, Silva-Rocha et al. 2012) and Turkey (KP036398.1, Silva-Rocha et al. 2014) for cytb gene. For similar aim, we compared our *P. tauricus* haplotypes with European populations of the species and we used five haplotypes from Greece (AY768728.1 and AY768727.1, Poulakakis et al. 2005a), Turkey (KX658353.1 and KX658354.1, Psonis et al. 2017) and Albania (KX658351.1, Psonis et al. 2017) for 16 S rRNA and three haplotypes from Greece (KX658062.1, KX658057.1, Psonis et al. 2017) and Albania (KX658038.1, Psonis et al. 2017) for cytb gene on Genbank.

Phylogenetic analyses based on the two genes (16 S rRNA and cytb) separately and combined data. We conducted multiple complementary methods of data analysis, such as neighbour-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) phylogenetic approaches using MEGA 6.0 v (Tamura et al. 2013) for NJ and MP and ML, and MrBayes 3.2.3 (Ronquist and Huelsenbeck 2003) for BI. Neighbour-joining (NJ), Maximum Parsimony (MP) and maximum likelihood (ML) analyses were carried out using a heuristic search method (10,000 random addition replicates tree-bisection-reconnection, TBR, branch swapping) and bootstrap analyses with 1000 replications for NJ, MP and ML (Felsenstein 1985) were applied. Transitions and transversions were equally weighted, and gaps were treated as

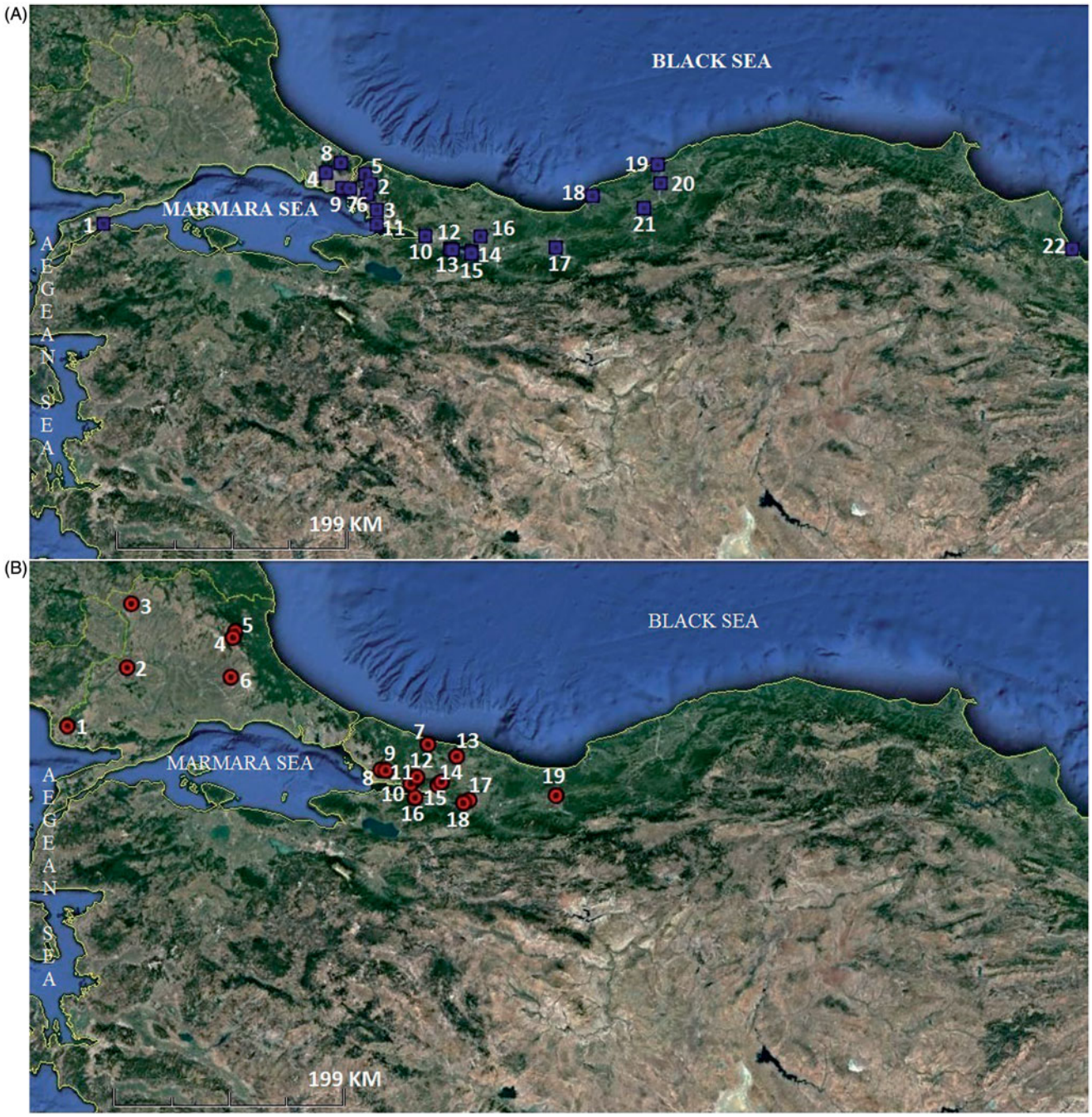

Figure 1. Distribution ranges of the *P. siculus* (A) and *P. tauricus* (B) species in Turkey.

missing data. In the BI analysis, the following settings were conducted: number of Markov Chain Monte Carlo (MCMC) generations = six millions; sampling frequency =100; burn-in =25%. The burn-in size was determined by checking convergence of –log likelihood (-ln L) using MrBayes 3.2.3 (Ronquist and Huelsenbeck 2003). NJ, MP and ML trees were evaluated using bootstrap analyses with 1000 replicates and statistical support of the resultant BI trees was determined based on Bayesian posterior probability (BPP). Best fit nucleotide substitution models were determined for each gene region with MEGA 6.0 v (Tamura et al. 2013) for NJ, MP, ML and BI analyses based on Akaike's information criteria (AIC). We a priori regarded tree nodes with bootstrap values (BS) 70% or greater as sufficiently resolved (Huelsenbeck and Hillis 1993), and those between 50 and 70% as tendencies. In the BI analysis, we considered nodes with a BPP of 95% or greater as significant (Leaché and Reeder 2002). Uncorrected pairwise sequence divergences for each gene were calculated using MEGA 6.0 v (Tamura et al. 2013). *Podarcis peloponnesiaca* (Gen-Bank accession number AY896179.1 (Poulakakis et al. 2005 b) and *Eremias velox* (Gen-Bank accession number DQ658845.1 (Guo et al. 2011) for 16 S rRNA and *Podarcis peloponnesiaca* (Gen-Bank accession number AY896123.1 (Poulakakis et al. 2005 b) and *Eremias velox* (Gen-Bank accession number JQ690234.1 (Pouyani et al. 2012) for cytb were selected as the outgroups for *P. siculus*. *Podarcis hispanica* (Gen-Bank accession number HQ898057.1 (Kaliontzopoulou et al. 2011) and *Eremias velox* (Gen-Bank accession number

Table 1. List of the samples used for *P. siculus* for 16S rRNA and cytb

		Genbank Accession no	
Sample no	Locality	16S rRNA	cytb
1-1	Çanakkale-Gelibolu	MF187695	MF187722
1-2	Çanakkale-Gelibolu	MF187696	MF187723
2-1	İstanbul-Reşadiye	MF187685	MF187710
2-2	İstanbul-Reşadiye	MF187685	MF187709
3	İstanbul-Tuzla	MF187685	–
4	İstanbul-Başakşehir	MF187686	MF187711
5	İstanbul-Beykoz	MF187685	MF187709
6	İstanbul-Samandıra	MF187686	MF187712
7	İstanbul-Üsküdar	MF187686	MF187711
8	İstanbul-Belgrad Ormanları	–	MF187710
9-1	İstanbul	MF187686	MF187711
9-2	İstanbul	MF187685	–
10-1	Kocaeli-Gebze Orman İşletme	MF187685	MF187707
10-2	Kocaeli-Gebze Orman İşletme	MF187685	MF187708
10-3	Kocaeli-Gebze Orman İşletme	MF187685	MF187708
10	Kocaeli-Gebze	–	MF187709
11-1	Kocaeli-Darıca	MF187692	MF187711
11-2	Kocaeli-Darıca	MF187691	MF187717
12-1	Kocaeli-Maşukiye	MF187688	MF187714
12-2	Kocaeli-Maşukiye	MF187688	MF187720
12-3	Kocaeli-Maşukiye	MF187692	MF187721
13	Kocaeli-Yanıkköy	MF187687	MF187713
14-1	Sakarya-Arifiye	MF187693	MF187718
14-2	Sakarya-Arifiye	MF187691	MF187719
15	Sakarya-Dörtyol	MF187686	MF187712
16-1	Sakarya-Adapazarı	MF187686	MF187711
16-2	Sakarya-Adapazarı	MF187686	MF187711
16-3	Sakarya-Adapazarı	MF187686	MF187711
17-1	Düzce	MF187698	MF187724
17-2	Düzce	MF187699	MF187724
18-1	Zonguldak-Ereğli	MF187689	MF187715
18-2	Zonguldak-Ereğli	MF187686	MF187711
19-1	Zonguldak-Filyos	MF187690	MF187716
19-2	Zonguldak-Filyos	MF187691	MF187717
20-1	Zonguldak-Çaycuma	MF187692	MF187721
20-2	Zonguldak-Çaycuma	MF187694	MF187718
21	Zonguldak-Devrek	MF187691	MF187709
22-1	Samsun-Atakum	MF187697	MF187724
22-2	Samsun-Atakum	MF187693	MF187725

Table 2. List of the samples used for *P. tauricus* for 16S rRNA and cytb

		Genbank Accession no	
Sample no	Locality	16S rRNA	cytb
1	Edirne-Enez	MF187700	MF187684
2	Edirne-Uzunköprü	MF187706	MF187684
3-1	Edirne-Büyükdöllük	MF187700	MF187684
3-2	Edirne-Büyükdöllük	MF187700	MF187684
3-3	Edirne-Büyükdöllük	–	MF187684
4-1	Kırklareli-Vize-Sergen	MF187700	MF187684
4-2	Kırklareli-Vize-Sergen	MF187700	MF187683
4-3	Kırklareli-Vize-Sergen	MF187700	MF187684
4-4	Kırklareli-Vize-Sergen	MF187701	MF187684
4-5	Kırklareli-Vize-Sergen	MF187700	MF187683
4-6	Kırklareli-Vize-Sergen	MF187700	MF187683
5	Kırklareli-Vize-Evrencik	MF187700	MF187684
6-1	Tekirdağ-Saray	MF187700	MF187684
6-2	Tekirdağ-Saray	MF187700	MF187684
7-1	İstanbul-Ağva	MF187700	MF187684
7-2	İstanbul-Ağva	MF187702	MF187684
7-3	İstanbul-Ağva	MF187703	MF187684
8-1	Kocaeli-Gebze-Balçık	MF187703	MF187684
8-2	Kocaeli-Gebze-Balçık	MF187703	MF187684
8-3	Kocaeli-Gebze-Balçık	MF187703	MF187684
8-4	Kocaeli-Gebze-Balçık	MF187700	MF187684
9	Kocaeli-Gebze-Mollafenari	MF187700	MF187684
10-1	Kocaeli-Körfez-Belen	MF187700	MF187684
10-2	Kocaeli-Körfez-Belen	MF187700	MF187684
10-3	Kocaeli-Körfez-Belen	MF187700	MF187684
11	Koceli-Körfez-Dereköy	MF187704	MF187684
12-1	Kocaeli-Körfez-Sipahiler	MF187700	MF187684
12-2	Kocaeli-Körfez-Sipahiler	MF187700	MF187684
12-3	Kocaeli-Körfez-Sipahiler	MF187700	MF187684
13	Kocaeli-Kandıra	MF187700	MF187684
14	Kocaeli-Çubuklu	MF187700	MF187684
15	Kocaeli-Yassıbağ	MF187705	MF187684
16-1	Kocaeli-Gölcük	MF187700	MF187684
16-2	Kocaeli-Gölcük	MF187700	MF187684
17	Sakarya-Serdivan-Esentepe	MF187700	–
18-1	Sakarya-Serdivan-Dereköy	MF187700	MF187684
18-2	Sakarya-Serdivan-Dereköy	MF187700	MF187684
19-1	Düzce-Yörükköy	MF187700	MF187684
19-2	Düzce-Yörükköy	MF187700	MF187684
19-3	Düzce-Yörükköy	MF187700	MF187684
19-4	Düzce-Yörükköy	MF187700	MF187684

DQ658845.1 (Guo et al. 2011) for 16S rRNA and *Podarcis hispanica* (Gen-Bank accession number AY234154.1 (Busack et al. 2005) and *Eremias velox* (Gen-Bank accession number JQ690234.1 (Pouyani et al. 2012) for cytb were selected as the outgroups for *P. tauricus*.

Results

Podarcis siculus

Phylogenetic analyses: sequence variation

A total of 501 homologous base pairs of the 16S rRNA sequences and 469 homologous base pairs of the cytb sequences for 39 individuals were obtained, respectively. There was 2 bp-deletion in 16S rRNA gene, while there was 4 bp-deletion in cytb gene. In total, 15 mitochondrial haplotypes for 16S rRNA gene were identified and 19 haplotypes for cytb gene were recognized. When we combined both the 16S rRNA and cytb sequences, we identified 23 haplotypes. In the NJ, ML and MP the best fit model selected by MEGA 6.0 v (Tamura et al. 2013), HKY + G + I (Kishino and Hasegawa 1989) for 16S rRNA and HKY + G + I (Kishino and Hasegawa 1989) for 1st, 2nd and 3rd codon positions of cytb. Because of the best fit model similarity, the sequences of 16S rRNA and cytb were combined and GTR + G + I (Tavaré 1986; Nei and Kumar 2000) model was selected for the combined sequences. In BI, the likelihood settings for the best-fit model were selected as HKY (Kishino and Hasegawa 1989) in for 16S rRNA and cytb genes. Because of the best fit model similarity, the sequences of 16S rRNA and cytb were combined. GTR + G + I (Tavaré 1986; Nei and Kumar 2000) model was selected for the combined data.

Phylogenetic relationships: genetic distances

NJ, MP, ML and BI phylogenetic analyses of each of the studied genes and the combined dataset gave very similar results and they showed only minor differences, mainly concerning relationships between these groups and their support values. The phylogenetic tree of the BI analysis of the 16S rRNA and cytb is shown Figures 2 and 3. Because the combining tree and the tree belonging to 16S rRNA is almost similar, the combining tee is not shown.

The phylogenetic analyses of 16S rRNA gene employing four different optimality criteria yielded very slightly different topologies, and only the BI tree is shown in Figure 2.

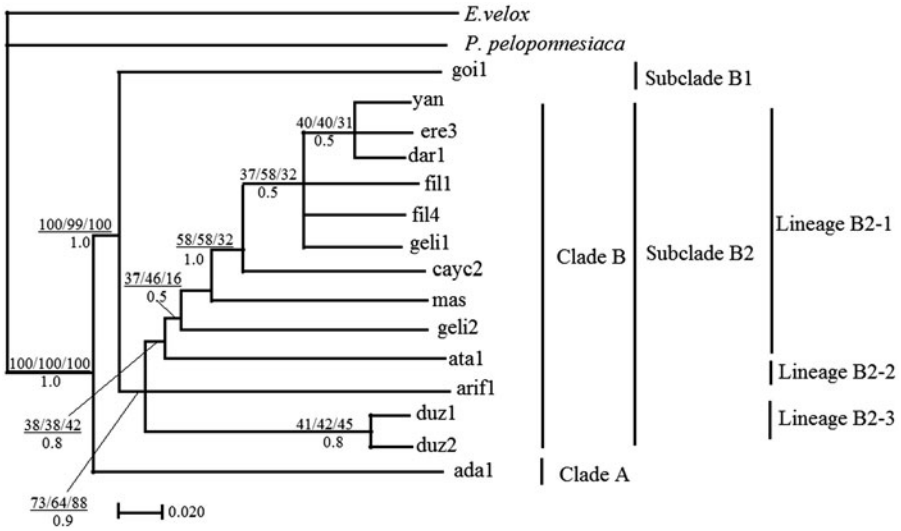

Figure 2. Bayesian tree of a 501-bp sequence of 16 S rRNA for *P. siculus*. Numbers above branches represent bootstrap support for NJ/ML/MP (1000 replicates) inherence, and numbers below branches indicate Bayesian Posterior Probabilities.

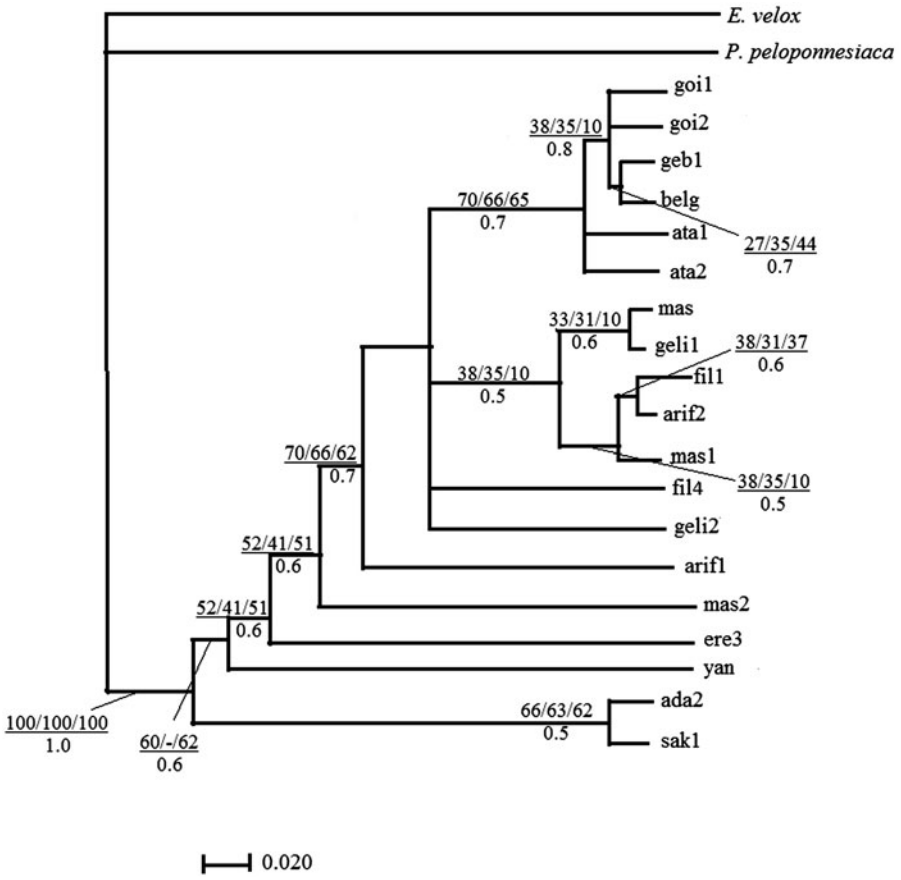

Figure 3. Bayesian tree of a 469-bp sequence of cytb for *P. siculus*. Numbers above branches represent bootstrap support for NJ/ML/MP (1000 replicates) inherence, and numbers below branches indicate Bayesian Posterior Probabilities.

Anatolian populations of *P. siculus* formed three clades (Clades A–C) for 16 S rRNA.

The main relationships were as follows for 16 S rRNA:

Clade A consists of a haplotype (ada1), (NJ, ML and MP BS= 100, 99 and 100, respectively and BPP= 1.0).

Clade B consists of 14 haplotypes (goi1, yan, ere3, fil1, fil4, dar1, geli1, cayc2, masuk, geli2, ata1, arif1, duz1 and duz2) from Turkey (NJ, ML and MP BS= 100, 99 and 100, respectively and BPP= 1.0). Clade B is divided into two subclades (Subclade B1 and Subclade B2). Subclade B1 consists of goi1 haplotype and Subclade B2 has 13 remain haplotypes (NJ, ML and MP BS= 73, 64 and 88, respectively and BPP= 0.9). The relationships in the B1 clade were partially resolved. Subclade B2 is divided into three lineages (Lineage B2-1, Lineage B2-2 and Lineage B2-3). Lineage B2-1 has two haplotype (duz1 and duz2), Lineage B2-2 has a haplotype

(arif1) and Lineage B2-3 has remaining haplotypes (NJ, ML and MP BS = 73, 64 and 88, respectively, and BPP = 0.9). The relationships in the B2 Clade were unresolved.

The p-distances were as follows for 16 S rRNA:

The p-distances among the populations of *P. siculus* in Turkey were very low and they were ranged from 0.2 (goi1 and ada1; goi1 and arifl; yan and ere3; yan and fil1; mas and geli1; mas and geli2; mas and cayc2; ere3 and dar1; fil1 and geli1; dar1 and fil4; cayc2 and fil4; geli1 and fil4) to 1.6% (goi1 and ere3; dar1 and duz1) in 16 S rRNA (Table 3).

The main relationships were as follows for cytb:

Clade A includes three haplotypes (sak1 and ada2) from Sakarya and Clade B consists of 17 haplotypes (mas2, ere3, belg, goi1, geb1, goi2, duz2, ata1, arif1, fil4, geli1, mas, geli2, mas1, arif2, fil1 and yan) for cytb (NJ, ML and MP BS = -, 100 and -, respectively and BPP = 1.0). Subclade B1 consists of two subclades (Subclades B1 and Subclade B2). Subclade B1 has a haplotype (yan) and Subclade B2 has 16 remain haplotypes (BPP = 0.6). The relationships in the B Clade were unresolved for cytb (Figure 3).

The p-distances were as follows for cytb:

The values of p-distances were ranged from 0.0% (fil4 and arif1; fil1 and araif2) to 3.3% (sak1 and mas) in cytb and the p-distances in cytb among the populations of *P. siculus* in Turkey were relatively higher than the distances in 16 S rRNA (Table 3).

The main relationships were as follows for combining data:

Clade A consists of two haplotypes (ada2 and sak1) and Clade B includes 21 ones (dar1, ere3, mas2, yan, res1, goi1, res3, goi2, duz1, duz2, ata1, ata2, arif1, geli1, geli2, cayc2, mas, fil1, fil4, mas1 and arif2) (NJ, ML and MP BS = 100, - and 100, respectively and BPP = 1.0). Clade B consists of three subclades (Subclade B1, Subclade B2 and Subclade B3). Subclade B1 has a haplotype (res3), Subclade B2 has a haplotype (res1) and Subclade B3 has 19 remaining in NJ and ML (NJ, ML and MP BS = 100, - and 100, respectively, and BPP = 0.9). The relationships in the B Clade were unresolved for combine data.

The interrelationships among these groups are rather ambiguous, showing a monophyly for 16 S rRNA, cytb and combining data according to all phylogenetic analyses (NJ, ML, MP and BI).

Podarcis tauricus

Phylogenetic analyses: sequence variation

A total of 497 homologous base pairs of the 16 S rRNA sequences and 412 homologous base pairs of the cytb sequences for 38 individuals were obtained, respectively.

Table 3. Comparison of uncorrected p-distance (in %) for fragments of 16S rRNA and cytb among haplotypes of *P. siculus* in Turkey

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
16S rRNA																		
goi1																		
ada1	0.2																	
yan	1.4	1.2																
mas	0.8	1.0	0.6															
ere3	1.6	1.4	0.2	0.8														
fil1	1.2	1.4	0.2	0.4	0.4													
fil4	1.2	1.4	0.6	0.4	0.4	0.4												
dar1	1.4	1.2	0.4	0.6	0.2	0.6	0.2											
arif1	0.2	0.4	1.2	0.6	1.4	1.0	1.0	1.2										
cayc2	1.0	1.2	0.8	0.2	0.6	0.6	0.2	0.4	0.8									
geli1	1.0	1.2	0.4	0.2	0.6	0.2	0.2	0.4	0.8	0.4								
geli2	0.6	0.8	0.8	0.2	1.0	0.6	0.6	0.8	0.4	0.4	0.4							
ata1	0.6	0.8	0.8	0.6	1.0	0.6	1.0	1.2	0.4	0.8	0.8	0.4						
duz1	0.6	0.8	1.2	0.6	1.4	1.0	1.0	1.2	0.4	0.8	0.8	0.4	0.8					
duz2	0.6	0.8	1.2	1.0	1.4	1.0	1.4	1.6	0.4	1.2	1.2	0.8	0.4	0.4				
cytb																		
goi1																		
goi2	0.2																	
geb1	0.4	0.2																
belg	0.2	0.4	0.2															
ada2	1.5	1.3	1.1	1.3														
sak1	1.3	1.5	1.3	1.1	0.2													
yan	1.3	1.1	0.9	1.1	0.2	0.4												
mas	0.6	0.4	0.6	0.9	1.7	1.9	1.5											
ere3	1.3	1.1	1.3	1.5	0.6	0.9	0.4	1.5										
fil1	0.9	0.6	0.9	1.1	1.9	2.1	1.7	0.6	1.3									
fil4	0.6	0.4	0.6	0.9	1.7	1.9	1.5	0.4	1.1	0.6								
arif1	0.9	0.6	0.9	1.1	1.5	1.7	1.3	0.6	0.9	0.9	0.2							
arif2	1.1	0.9	1.1	1.3	1.7	1.9	1.5	0.9	1.1	0.2	0.9	0.6						
mas1	1.3	1.1	1.3	1.5	1.9	2.1	1.7	0.6	1.3	0.4	0.6	0.4	0.2					
mas2	1.5	1.3	1.5	1.7	0.9	1.1	0.6	1.3	0.2	1.5	0.9	0.6	1.3	1.1				
geli1	0.9	0.6	0.9	1.1	1.9	2.1	1.7	0.2	1.3	0.4	0.2	0.4	0.6	0.4	1.1			
geli2	0.9	0.6	0.9	1.1	1.9	2.1	1.7	0.6	1.3	0.4	0.2	0.4	0.6	0.4	1.1	0.4		
ata1	0.4	0.2	0.4	0.6	1.5	1.7	1.3	0.6	0.9	0.4	0.2	0.4	0.6	0.9	1.1	0.4	0.4	
ata2	0.4	0.2	0.4	0.6	1.5	1.7	1.3	0.6	0.9	0.4	0.2	0.4	0.6	0.9	1.1	0.4	0.4	0.0

There was 3 bp-deletion in 16 S rRNA gene, while there was 4 bp-deletion in cytb gene. In total, 7 mitochondrial haplotypes for 16 S rRNA gene were identified and 2 haplotypes for cytb gene were recognized. When we combined both the 16 S rRNA and cytb sequences, we identified 9 haplotypes. In the NJ, ML and MP the best fit model selected by MEGA 6.0 v (Tamura et al. 2013), HKY + G + I (Kishino and Hasegawa 1989) for 16 S rRNA and HKY + G (Kishino and Hasegawa 1989) for 1st, 2nd and 3rd codon positions of cytb. Because of the best fit model similarity, the sequences of 16 S rRNA and cytb were combined and HKY + G + I (Kishino and Hasegawa 1989) model was selected for the combined sequences. In BI, the likelihood settings for the best-fit model were selected as HKY (Kishino and Hasegawa 1989) in for 16 S rRNA and cytb genes. Because of the best fit model similarity, the sequences of 16 S rRNA and cytb were combined. HKY + G + I (Kishino and Hasegawa 1989) model was selected for the combined data.

Phylogenetic relationships: genetic distances

NJ, MP, ML and BI phylogenetic analyses of each of the studied genes and the combined dataset gave very similar results and they showed only minor differences, mainly concerning relationships between these groups and their support values. Combining tree and the tree belonging to 16 S rRNA were almost similar. Because of this reason, only BI trees for 16 S rRNA and cytb are shown in Figures 4 and 5.

The main relationships were as follows for 16 S rRNA, cytb and combine data:

All analyses shown that *P. tauricus* populations in Turkey have only one clade (Clade A). Clade A consists of seven haplotypes (ser2, ser6, agva2, bal1, kor, uzun and yas2) (NJ, ML and MP BS = 100, 100 and 100, respectively, and BPP = 1.0) for 16 S rRNA while it consists of two haplotypes (ser2 and ser6), (NJ, ML and MP BS = 100, 100 and 100, respectively, and BPP = 1.0). For combine data, Clade A consists of nine haplotypes (ser2, ser3, ser6, agva2, bal1, kor, uzun, gol1 and yas2) (NJ, ML and MP BS = 100, 100 and 100, respectively, and BPP = 1.0).

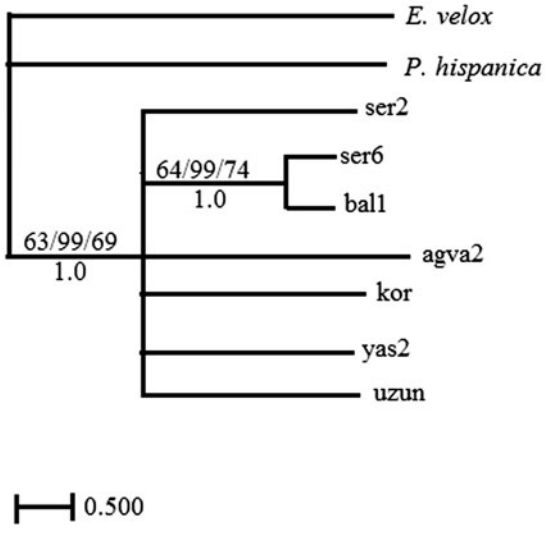

Figure 4. Bayesian tree of a 497-bp sequence of 16 S rRNA for *P. tauricus*. Numbers above branches represent bootstrap support for NJ/ML/MP (1000 replicates) inherence, and numbers below branches indicate Bayesian Posterior Probabilities.

The p-distances were as follows for 16 S rRNA and cytb:

The p-distances among the populations of *P. tauricus* in Turkey were very low and they were ranged from 0.0 (ser2 and agva2; ser2 and bal1; ser2 and yas2; agva2 and bal1; agva2 and yas2 and bal1 and yas2) to 0.6% (ser6 and uzun) in 16 S rRNA. On the other hand, the p-distances in cytb among the populations of *P. siculus* were 0.2% in Turkey (Table 4).

The basic topology of the trees derived from NJ, ML, MP and BI of the 16 S rRNA, cytb and combined data set shows a monophyletic relationship for both *P. siculus* and *P. tauricus* specimens in Turkey.

Discussion

The results of the present study identified a number of haplotype clades which based on the observed levels of sequence divergence representing long-separated lineages and diverse evolutionary histories within P. siculus and P. tauricus.

According to current literature, there are many contradictory hypotheses on the taxonomic relationships for *Podarcis* genus. Oliverio et al. (2009) reported morphologically recognized three groups of the species: the first group consisted *muralis, tiliguerta, filfolensis, wagleriana* and *milensis* (Bedriaga 1882); the second one consisted the Iberian and Madeiran

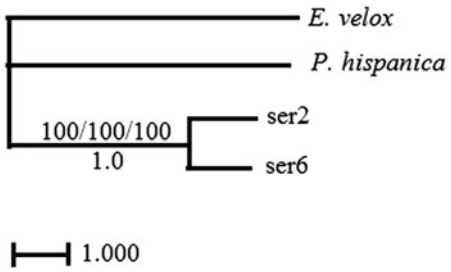

Figure 5. Bayesian tree of a 412-bp sequence of cytb for *P. tauricus*. Numbers above branches represent bootstrap support for NJ/ML/MP (1000 replicates) inherence, and numbers below branches indicate Bayesian Posterior Probabilities.

Table 4. Comparison of uncorrected p-distance (in %) for fragments of 16S rRNA and cytb among haplotypes of *P. tauricus* in Turkey

	1	2	3	4	5	6
16S rRNA						
ser2						
ser6	0.4					
agva2	0.2	0.6				
bal1	0.2	0.2	0.4			
kor	0.2	0.6	0.4	0.4		
yas2	0.2	0.6	0.0	0.4	0.4	
uzun	0.6	1.0	0.4	0.8	0.4	0.4
	1	2	3	4	5	6
cytb						
ser2						
ser6	0.2					

species and the last one consisted *siculus, melisellensis* and two eastern Mediterranean species (Lanza and Cei 1977). Based on the phylogenetic relationships, the *Podarcis* genus was separated into four geographic groups [the Western island group, the Balkan group (including *P. tauricus*), the Italian group (including *P. siculus*) and the Southwestern group] but relationships were mainly unresolved (Harris and Arnold 1999; Oliverio et al. 2000). On the other hand, Oliverio et al. (2009) reported that the Italian group of the genus was split into three groups: the first comprised *P. filfolensis, P. melisellensis. P. wagleriaria, P. muralis,* and *P. raffonei,* the second group was *P. siculus* with its various subspecies and the third one was composed of *P. tiliguerta*.

In order to estimate the times of lineage splitting from sequence divergence data, a known rate belonging to cold-blooded vertebrates is used. In particular, mitochondrial ribosomal genes are considered as more rate-homogeneous (Oliverio et al. 2009). As it was explained in the study of Oliverio et al. (2009), the rate of 0.38% sequence divergence per MY for mitochondrial ribosomal genes derived for European newts by Caccone et al. (1997) and the splitting of the *Podarcis* from other groups would date to ca. 35 MY BP. Furthermore, *P. siculus* would have diverged ca 17-15 MY BP.

The specimens of *P. siculus* in Turkey are mainly distributed from Marmara Region to Central Black Sea Region. Although the mountain ranges in the Western Black Sea Region, which lies between the Marmara Region and the Central Black Sea Region, may consitute a barrier, the p-distances among the specimens from these regions were very low (ranged from 0.2 to 1.6%) for 16 S rRNA. Similarly, the p-distances among these populations were not high (maximum 3.3%) for cytb. These low genetic distances and tree topologies belonging to two genes showed that *P. siculus* had only one lineage in Turkey.

If we roughly apply the rate of 0.38% sequence divergence per MY for mitochondrial ribosomal genes, the splitting of our haplotypes and Italian populations (AY770920.1 and AY770919.1 haplotypes in Genbank) of the *P. s. siculus* would date to ca 5.2 MY. In addition, the maximum divergence between our haplotypes and Spain populations (HM746963.1 and HM746964.1 haplotypes in Genbank) of the same subspecies was 3.4%. They would have been divergenced each other for ca 8.9 MY. When we compared our specimens to populations of Croatia (EU362073.6 and EU362074.1 haplotypes in Genbank) representing other subspecies (*P. s. campestris*), we found that the maximum divergence was 4.4%. They would have been separated from each other for ca 11.5 MY. Our findings are consistent with the results of Oliverio et al. (2009).

On the other hand, Oliverio et al. (2009) did not state the cytb p-distance values on their study. However, cytb sequence data were also utilized for estimating the genetic variability of the sampled populations, population genetic structure and genetic differentiation among populations (Giovannotti et al. 2010). In the present study, the p-distance in the cytb gene was 12.1% between our samples and a haplotype (JX072953.1 in Genbank) from a Portugal population of the *P. s. siculus*. In addition, the p-distances between our haplotypes and Italy (KF372034.1 and AY770895.1) and Spain (JX072939.1 and JX072941.1) haplotypes of the same subspecies in Genbank were 11.8% and 9.2%, respectively. On the other hand, the genetic distance between our haplotypes and two haplotypes (AY770882.1 and AY770892.1 in Genbank) of *P. s. campestris* from Crotia was 9.8%.

When we compared our specimens with the haplotypes in Genbank (based on the phylogenetic trees and p-distances for 16 S rRNA and cytb genes), we considered that Turkish populations of the Italian wall lizard represent the *P. s. siculus* (Supplemental Figures 1 and 2).

In the present study, the basic intraspecific phylogeographical pattern of *tauricus*'s populations is characterized by the existence of only one main lineage. This lineage, which forms a monophyletic unit, corresponds to the populations of *P. tauricus* in Turkey. Our mtDNA data and results previous studies based on mtDNA (Harris and Arnold 1999; Oliverio et al. 2000, Poulakakis et al. 2005 b) are consistent with the morphological classification of the species.

Considering the distribution of *P. tauricus* in Turkey it appears that there is no significant geographical barrier among populations of the species. Conformable, we found very low p-distances (maximum 0.6% for 16 S rRNA and 0.2% for cytb).

The Balkan species are divided into two subgroups, the first subgroup is *P. tauricus* and the second one is *P. erhardii*. The subgroup of *P. tauricus* consists of *P. tauricus, P. milensis, P. gaigeae* and perhaps *P. melisellensis*) and the subgroup of *P. erhardii* consists of *P. erhardii* and *P. peloponnesiaca* (Poulakakis et al. 2005 b). The distribution of the *P. tauricus* subgroup (*P. tauricus, P. milensis, P. gaigeae* and perhaps *P. melisellensis*) mainly on the Balkan Peninsula and its absence from the rest of Europe, suggest that the ancestral species of this group originated somewhere in the Balkan Peninsula and expanded in this area. This information fits well with the divergence time estimated in study of Poulakakis et al. (2005 b) for the beginning of the diversification of *P. tauricus* subgroup. According to date estimation of divergence events (0.46% sequence divergence per MY for mitochondrial ribosomal genes and 1.55% for cytb), Poulakakis et al. (2005 b) reported the separation time of *P. tauricus* subgroup as 10.8 and 10.5 million years for 16 S rRNA and cytb, respectively.

As in the case of *P. siculus*, if we apply the rate of 0.38% sequence divergence per MY by Caccone et al. (1997) for *P. tauricus*, the splitting of our haplotypes and Greece populations (AY768728.1 and AY768727.1 haplotypes in Genbank) of the *P. t. tauricus* would date to ca 14.2 MY for 16 S rRNA gene (Table 3). On the other hand, the p-distance between our samples and Albanian specimens (KX658351.1 in Genbank) of the other subspecies, *P. t. ionicus* was 4.8%. They would have divergenced each other for ca 12.6 MY.

Because we had only two haplotypes for cytb gene, we did not perform a comparison between our haplotypes and the Genbank haplotypes. Based on our phylogenetic analyses and haplotypes in Genbank belonging to the specimens from European populations of the species, we considered that Turkish populations of the Crimean wall lizard represent the *P. t. tauricus* (Supplemental Figure 3).

Our phylogenetic analyses of the two genes employing four different optimality criteria and low p-distances in these

genes could be explained either by high levels of gene flow among the respective populations of both species (*P. siculus* and *P. tauricus*) in Turkey, as implied in the study of Kornilios et al. (2011).

In conclusion, the monophyly of *P. siculus* and *P. tauricus* was strongly supported by the cladistic analysis of Turkish populations.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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