



EVALUATING THE TAXONOMIC STATUS OF THE BALKAN WALL LIZARD *PODARCIS TAURICUS* (PALLAS, 1814) FROM THE ANATOLIAN PART OF ÇANAKKALE (TURKEY) USING MITOCHONDRIAL DNA

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ABSTRACT

Background: *Podarcis muralis*, *Podarcis siculus*, and *Podarcis tauricus* are known to extend their range into the territory of Turkey. A new locality of *P. tauricus* was discovered in the Çardak Lagoon, in the Anatolian part of Çanakkale, Turkey. There has been no report on the evaluation of the taxonomic status of newly recorded population in the Anatolian side of Çanakkale. **Objectives:** For this reason, the purpose of this study is to investigate the phylogenetic position of this newly recorded population using partial 16S rRNA and cytochrome b gene sequences. **Methods:** 16S rRNA and *cyt b* gene sequences of the Çardak population were compared with the Gelibolu, Kırklareli (Thrace Region) and Şile populations (Anatolian part of İstanbul). We reconstructed the phylogenetic tree according to Neighbor-Joining tree approach using MEGA v.6. Also we built a haplotype network to examine the haplotype diversity and structure for the concatenated mt DNA dataset. **Results:** Neighbor-Joining tree and the haplotype network have the same topology showing the newly discovered population grouped together with the other *P. tauricus* populations. **Conclusions:** According to our results, Çardak population is genetically almost similar with Gelibolu population, which isolated by Dardanelles strait.

Key Words: 16S rRNA; *cyt b*; Çardak Lagoon; Çanakkale; *Podarcis tauricus*

1. INTRODUCTION

The genus *Podarcis* Wagler, 1830 belong to the family of Lacertidae and including 23 species [1, 2]. The origin of the genus *Podarcis* is western European [3]. Only three *Podarcis* species; *Podarcis muralis* (Laurenti, 1768), *P. siculus* (Rafinesque, 1810) (introduced by humans) and *P. tauricus* (Pallas, 1814) have been recorded in Turkey [4].

Balkan Wall Lizard, *Podarcis tauricus* ranges from Crimean Peninsula and southern Ukraine, through southern Moldova, eastern and southern Romania (excluding the Danube Delta) to Bulgaria, FYROM, eastern and southern Serbia, Albania, mainland Greece, Hungary, and northwestern Turkey (on both sides of the Bosphorus) according to IUCN Red List and Psonis et al., (2018) [5].

Podarcis tauricus is a medium-sized lizard with a total body length of up to 22 cm (Fig 1) and listed as Least Concern (LC) according to IUCN Red List. It inhabits low and sparse vegetation with pebbly substrates and open places with sandy or it lives in a forest and feeds with insects [6] (Fig 2). The dorsum is greenish or bluish and beside the dorsum, there is a longitudinal line with light color [4, 7].



Figure 1: General view of female specimen of *Podarcis tauricus*.

The first records of *P. tauricus* from European part of Turkey were reported from İstanbul [8-10]. There have been many studies on new locality records of this species in Thrace region [6,11-13]. The first Anatolian record (Beykoz, İstanbul) has given by Bird (1936) [14]. A new locality was added from Polenezköy in the Anatolian part of İstanbul [15,16]. The other records of the species were reported in the northwestern Anatolia [7,13,17-22]. A new locality of *P. tauricus* was discovered in the Çardak Lagoon, in the Anatolian part of Çanakkale by Gül and Tosunoğlu (2017) [23]. All samples from the European and Anatolian parts of Turkey belonged to the subspecies *P. t. tauricus* [7,16,24].

There are many phylogeographic and phylogenetic studies about *Podarcis* genus [3,25-27]. Although there have been molecular phylogeny studies on *Podarcis tauricus* from Turkey to date [24,27], there has been no report on the evaluation of the taxonomic status of newly recorded population (Çardak) in the Anatolian side of Çanakkale.

In this study, we used partial ribosomal subunit 16S rRNA and cytochrome *b* gene sequences to investigate the taxonomic status of *P. tauricus* population from Çardak Lagoon (Anatolian part of Çanakkale), in comparison to Gelibolu, Kırklareli (Thrace Region) and Şile populations (Anatolian part of İstanbul) in Turkey.

2. MATERIALS AND METHODS

2.1. Collection of Animal Samples

In this study, 16 individuals of *P. tauricus* collected from four different localities in Turkey were analyzed (Fig 2). The four adult specimens of *P. tauricus* were collected from Çardak Lagoon (Çanakkale, Turkey) on March 08, 2016. Çardak Lagoon is located between the coordinates 40° 22' 51" - 40° 23' 36" N and 26° 42' 45" - 26° 44' 18" E and occupies an area of 1.2 square kilometers. Çardak Lagoon is located on the northeastern coast of the Çanakkale Strait (Dardanelles) separating Europe and Asia in the province of Çanakkale, Turkey. Gelibolu peninsula is situated in Europe part of Çanakkale. Four *P. tauricus* specimens were captured from the city centre of Gelibolu (Thrace region) (40° 24'N, 26° 39'E; 8 m. a.s.l.) in October, 2008. Kırklareli is located in the east of Thrace region. Four *P. tauricus* specimens were captured from the Pınarhisar (Kırklareli, Thrace region) (41° 38'N, 27° 30'E; 281 m. a.s.l.) in April, 2009. Şile is located in Anatolian part of İstanbul. Four *P. tauricus* specimens were captured from the Şile (İstanbul) (41°10'N, 29°36'E; 70 m. a.s.l.) in April, 2016. All samples were collected with the guidelines of the local ethics committee; Çanakkale Onsekiz Mart University, 2016/03-01) in Turkey. While the specimens were still alive, color pattern characteristics were recorded and colored photographs were taken.

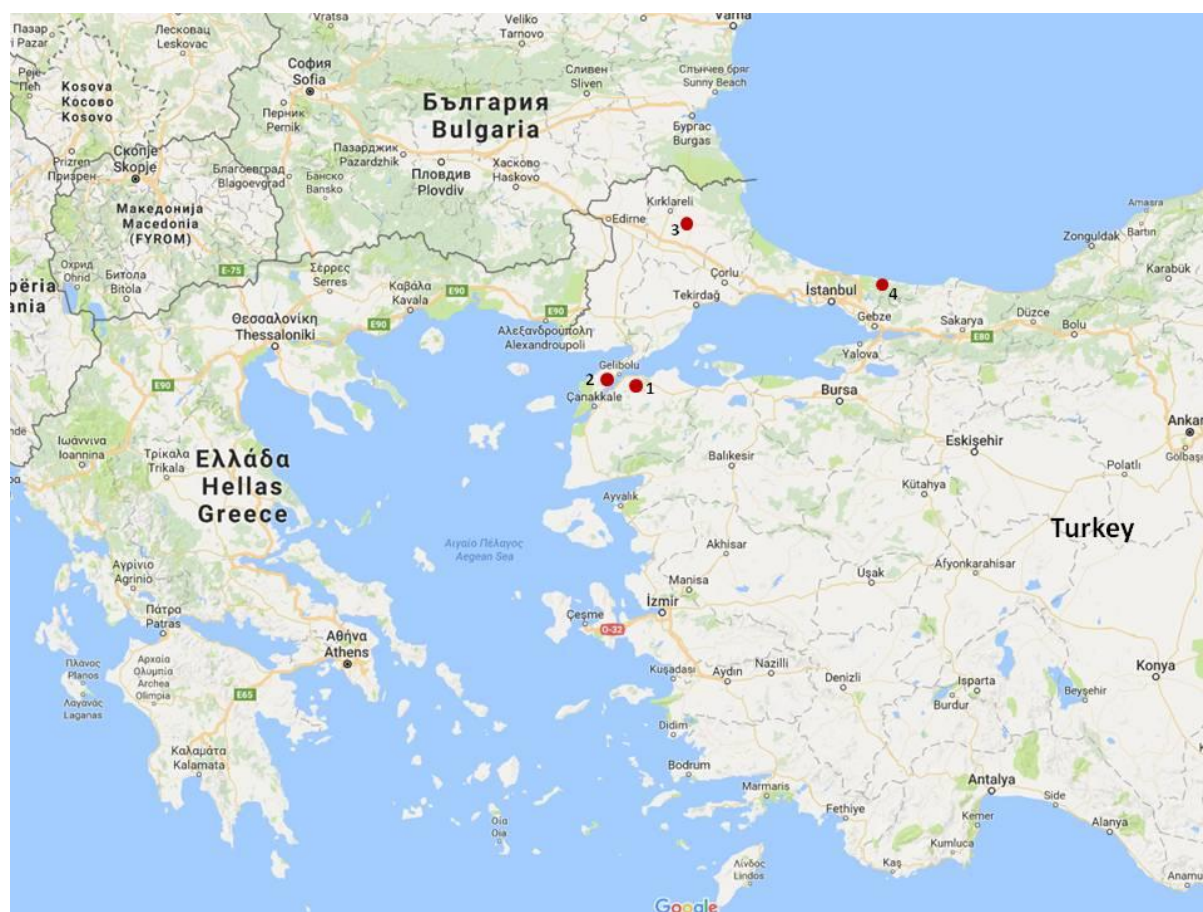


Figure 2: The locations where samples of the *Podarcis tauricus* populations used in the present study were collected. 1. Çardak; 2. Gelibolu; 3. Kırklareli, 4. Şile.

2.2. DNA extraction, amplification and sequencing

Muscle samples were ground to a fine powder in liquid nitrogen using a micro pestle. Total genomic DNA was extracted using a Genomic DNA Mini Kit (Geneaid Biotech) following the manufacturer's instructions. Total DNA concentrations were determined on a 1% agarose gel and estimated spectrophotometrically with Spectroquant Pharo 300 Spectrophotometer. Partial sequences of the mitochondrial genes encoding 16S ribosomal RNA (16S) and cytochrome *b* (cytb) were selected for the molecular phylogenetic analysis. We amplified an approximately 303-bp fragment from the mitochondrial cyt *b* gene using the primer pair L14724 and H15149 primers [28,29]. PCRs were performed using the following cycling conditions in the presence of 3 mM MgCl₂: an initial denaturing step at 94°C for 5 min; 35 cycles of denaturing at 92°C for 45 s, annealing at 49°C for 45 s, and extending at 72°C for 60 s; and a final extending step of 72°C for 5 min. In addition, we amplified an approximately 470-bp fragment from the mitochondrial 16S rRNA gene using the primer pair 16Sar-L and 16Sbr-H [30], following the PCR conditions described in Sotiropoulos et al. (2007) [31]. Successfully amplified products were sequenced by BM Lab Systems (Ankara, Turkey) using the same primers as in PCR.

2.3. Alignment and phylogenetic analysis

We classified the individuals from newly reported localities using with mitochondrial gene regions (16S; 470 bp and cyt *b*; 303 bp). The determined 16S rRNA and cyt *b* sequences have been deposited in GenBank (MF348205-MF348208 for cyt *b* and MF348209-MF348212 for 16S). We analyzed the genetic relation between the newly discovered population and already known subspecies (*P. t. tauricus*, *P. t. thasopulae* and *P. t. ionicus*) in the literature.

All sequences corrected and equalized of their length using with Bioedit [32]. Alignments of both mitochondrial genes were concatenated using Geneious v.7.1 [33] and obtaining in a single combined data matrix. We generated phylogenetic trees for concatenated nucleotide sequence (773 bp) using the *P.t. tauricus*, *P.t. thasopulae* and *P.t. ionicus* sequences obtained from Genebank (Table 1). We reconstructed the phylogenetic tree according to NJ approach using MEGA v.6 [34]. *P. gaigeae* was assigned as outgroup for phylogenetic analyses. Also we built a haplotype network to examine the haplotype diversity and structure for the concatenated mt DNA dataset. A median-joining network was constructed using the software PopArt [35] with the parameter epsilon set to 0.

Table 1: List of specimens *Podarcis* used, with haplotypes, locality, country, the accession numbers, and references.

Haplotypes	Locality	Country	Accession Number (16S rRNA)	Accession Number (cyt <i>b</i>)	References
Ptau_Cardak <i>P. t. tauricus</i>	Cardak (Çanakkale)	Turkey	MF348210	MF348205	Present Study
Ptau_Sile <i>P. t. tauricus</i>	Sile (İstanbul)	Turkey	MF348212	MF348206	Present Study
Ptau_Gelibolu <i>P. t. tauricus</i>	Gelibolu (Çanakkale)	Turkey	MF348211	MF348207	Present Study
Ptau_Kirklareli <i>P. t. tauricus</i>	Pınarhisar (Kırklareli)	Turkey	MF348209	MF348208	Present Study
Ptau660 <i>P. t. tauricus</i>	Keşan	Turkey	KX658353	KX658051	Psonis et al (2017)
Ptau454 <i>P. t. tauricus</i>	Azov Sea, coast of Kerch Peninsula	Crimea	KX658243	KX657939	Psonis et al (2017)
Ptau510 <i>P. t. tauricus</i>	Larisa – Kalyvia, 3.5km NE	Greece	KX658286	KX657982	Psonis et al (2017)
Ptau516 <i>P. t. tauricus</i>	Grevena – Voio Mt., Aidona	Greece	KX658291	KX657988	Psonis et al (2017)
Ptau523 <i>P. t. tauricus</i>	Trikala – Antichasia Mt., Korydalos	Greece	KX658296	KX657994	Psonis et al (2017)
Ptau661 <i>P. t. tauricus</i>	Paragem II pedras berma estrada	Turkey	KX658354	KX658052	Psonis et al (2017)
Ptau747 <i>P. t. tauricus</i>	Florina – Prespes lakes	Greece	KX658383	KX658081	Psonis et al (2017)

Ptau865 <i>P.t. tauricus</i>	Nestos - Keramoti	Greece	KX658421	KX658122	Psonis et al (2017)
Ptau872 <i>P. t. tauricus</i>	Pella – Agra lake	Greece	KX658426	KX658127	Psonis et al (2017)
Ptau857 <i>P. t. thasopulae</i>	Kavala, Thasos Isl.	Greece	KX658414	KX658114	Psonis et al (2017)
Ptau858 <i>P. t. thasopulae</i>	Kavala, Thasos Isl.	Greece	KX658415	KX658115	Psonis et al (2017)
Ptau643 <i>P.t. ionicus</i>	Golemaj	Albania	KX658340	KX658038	Psonis et al (2017)
Ptau713 <i>P.t. ionicus</i>	Aitoloakarnania- Trichonida lake	Greece	KX658359	KX658057	Psonis et al (2017)
Ptau718 <i>P.t. ionicus</i>	Korinthia- Ancient Feneos	Greece	KX658364	KX658062	Psonis et al (2017)
Ptau770 <i>P.t. ionicus</i>	Eptanisa Islands- Zakynthos	Greece	KX658397	KX658097	Psonis et al (2017)
Ptau885 <i>P.t. ionicus</i>	Arkadia - Kosmas	Greece	KX658439	KX658140	Psonis et al (2017)
<i>P. gaigeae</i>	-	Greece	AY768731	AY768775	Poulakakis et al (2005a)

3. RESULTS

The resulting tree topology (Fig 3) has two main clades: (1) *P. t. tauricus* and *P. t. thasopulae* are grouped together in the same clade as in the study of Psonis et al. (2017) [27], and this clade is containing also the newly discovered population of *P. tauricus tauricus*. (2) This clade consists of *P. t. ionicus* subspecies from Greece and Albania (Table 1).

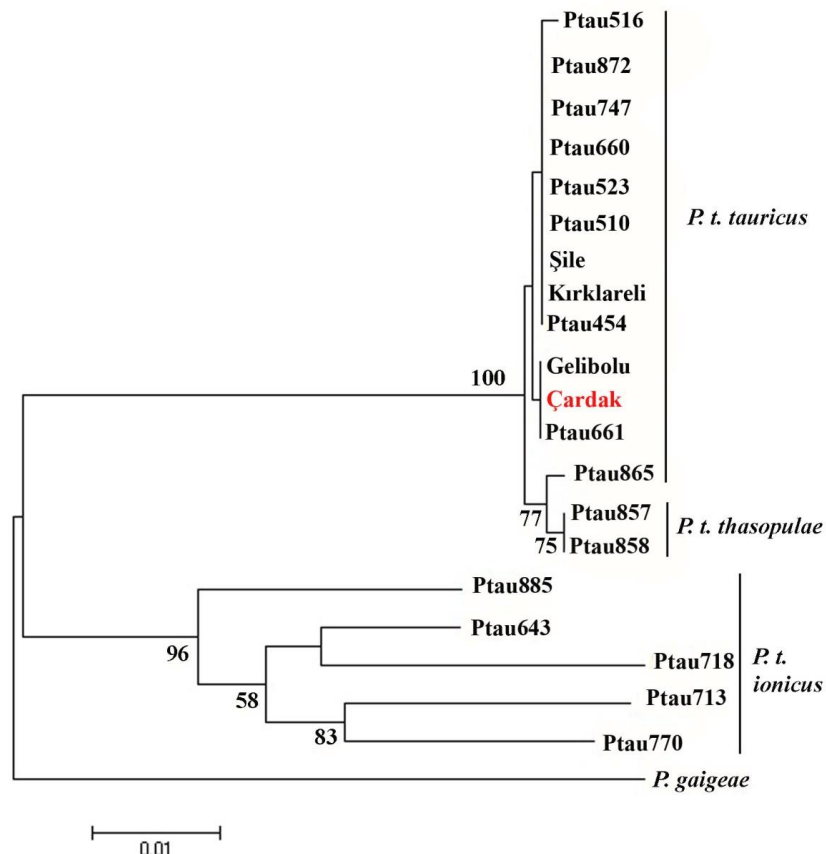


Figure 3: Phylogenetic tree based on Neighbour-Joining (NJ).

This NJ tree topology also confirmed the suggestion of Psonis et al. (2017) [27] that *P. tauricus* should be revised by splitting the species into two separate taxa (subspecies *P. t. tauricus* and *P. t. thasopulae*, together as *Podarcis tauricus* and *Podarcis ionicus*). The haplotype network has the same topology with the NJ tree that *P. t. tauricus* haplotypes are grouped together (Fig 4). As it can be seen from the network, Çardak specimens grouped together with Gelibolu and Ptau661 haplotypes with only a base difference. Geographically Gelibolu situated opposite side of Çardak in Thrace region, so the absence of genetic difference is expected. According to Psonis et al., (2017) [27] the locality name of the Ptau661 haplotype is paragem II pedras berma Estrada. Unfortunately, there is no such a locality in Turkish/ Turkey. But according to the map in the study of Psonis et al., (2017) [27], it is a locality between the cities İzmit and Adapazari in Anatolian part of Turkey.

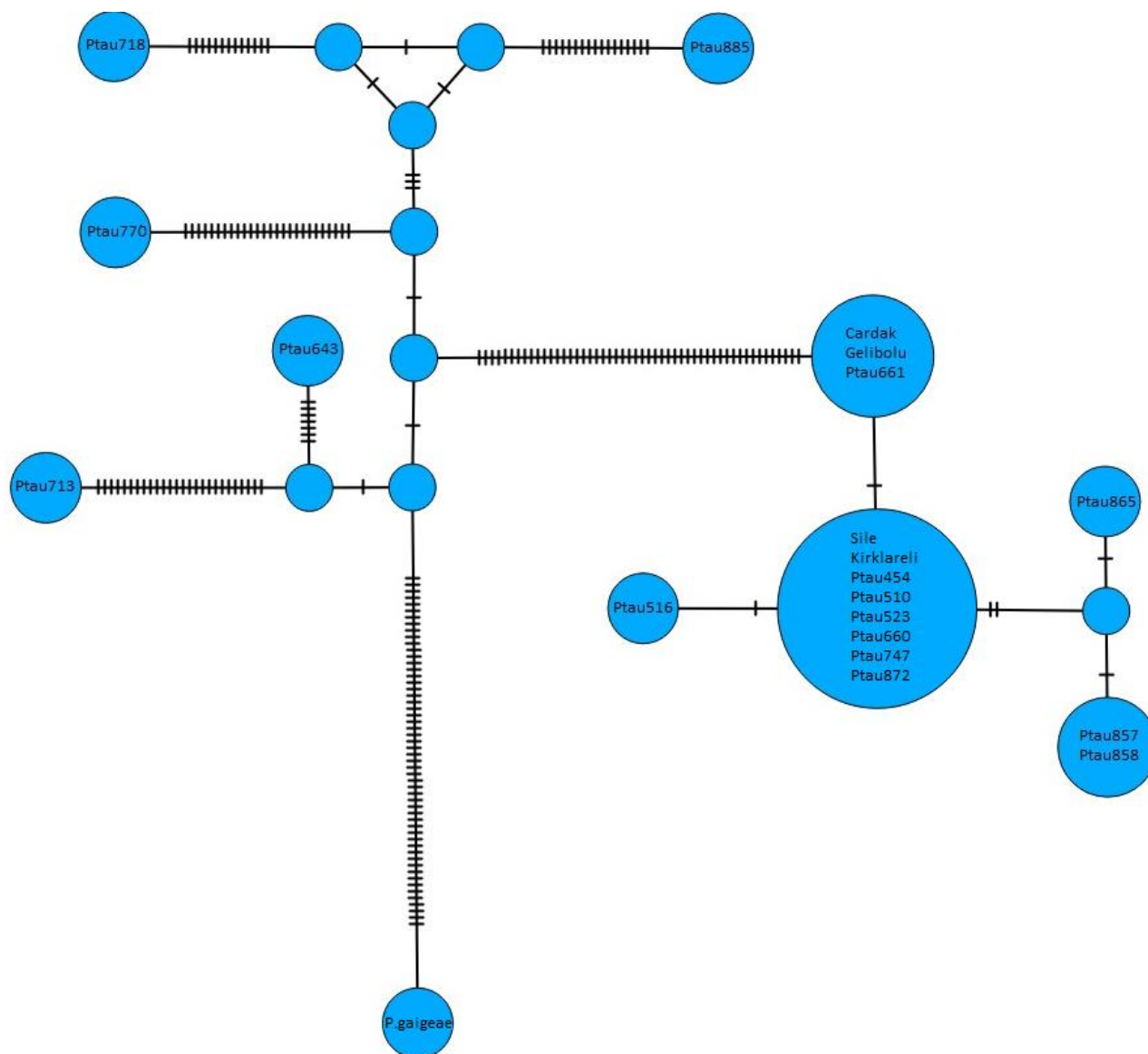


Figure 4: The haplotype network of the concatenated mitochondrial genes.

4. DISCUSSION

Our analyses indicate that four examined populations belong to the *P. tauricus tauricus* subspecies in the literature. Also, the specimens from four populations used in this study were similar to *P. t. tauricus* in terms of coloration and pholidosis [7, 12]. *P. tauricus* specimens taken from the same region were evaluated morphologically (coloration and pholidosis) and found to be similar to the subspecies of *P. tauricus tauricus* by Gül and Tosunoglu (2017) [23]. Phylogenetic and morphologic studies with populations showing the distribution of Thrace and Anatolia are available; and in these studies, it has been reported that nominate subspecies distributes in Turkey [7,24,27]. Until the last study [5], *P. tauricus* is divided into three recognized subspecies [36]. The first one is *P. t. tauricus* (Pallas, 1814); the second one is *P. t. ionicus* (Lehrs, 1902) and the last one is *P. t. thasopulae* (Kattinger, 1942). Psonis et al., (2018) carried out that *Podarcis tauricus* has no subspecies. The two subspecies (*P. t. tauricus* and *P. t. thasopulae*) which appear to be phylogenetically

indistinguishable are grouped together in *P. tauricus*. Besides this, the subspecies *P. tauricus ionicus* is defined as a species and hereafter named *Podarcis ionicus* [5,27].

5. CONCLUSION

Concluding, in our molecular study, when we compare the newly reported population from Anatolian part of Çanakkale with the other *P. tauricus* populations, Çardak population is genetically almost similar with Gelibolu population, which isolated by Dardanelles strait.

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