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A comparative evaluation of cytochrome-*b* diversity of the endemic Anatolian vole species *Microtus dogramacii* (Rodentia: Cricetidae) with the "guentheri" group of voles and *Microtus socialis*

Derya Çetintürk

ABSTRACT. Dogramaci's vole, *Microtus dogramacii*, is an endemic rodent species distributed in the northern region of central Anatolia in Turkey. Existing studies performed at the molecular level have been insufficient to differentiate between *M. dogramacii* and the "guentheri" group of voles (*Microtus hartingi, Microtus guentheri* and *Microtus qazvinensis*). Therefore, *M. dogramacii* was compared with some other *Microtus* species in terms of genetic diversity, mean genetic distance values and phylogenetic approaches for the mitochondrial cytochrome-*b* gene region. The mean genetic distance values between *M. dogramacii* and other species were 3.2–7.0%. In the Median-joining network, Maximum Likelihood and Bayesian MCMC dendrograms, *M. dogramacii* was closer to *M. qazvinensis* and *M. hartingi* than to *M. guentheri* and *M. socialis*. Obtained results, based on the cytochrome-*b* analyses, suggest that *M. dogramacii* may be a recently evolved species.

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KEY WORDS: Dogramaci's vole, cytochrome-*b*, *Microtus hartingi*, *Microtus guentheri*, *Microtus qazvinensis*, *Microtus socialis*

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Сравнительная оценка разнообразия эндемичной анатолийской полевки *Microtus dogramacii* (Rodentia: Cricetidae) по гену цитохрома-*b* с полевками группы "guentheri" и *Microtus socialis*

Д. Четинтюрк

РЕЗЮМЕ. *Microtus dogramacii*, является эндемичным видом грызунов, распространенным в северном регионе центральной Анатолии в Турции. Существующие исследования, проведенные на молекулярном уровне, оказались недостаточными для определения положения *M. dogramacii* среди других полевок группы "guentheri" (*Microtus hartingi, Microtus guentheri* и *Microtus qazvinensis*). В данной работе мы сравнивали генетическое разнообразие по гену цитохрома-*b* (*cytb*) у *M. dogramacii* с другими видами этой группы. Средние значения генетической дистанции между *M. dogramacii* и другими видами составляли 3.2–7.0%. В медианной сети и дендрограммах *M. dogramacii* оказывается ближе к *M. qazvinensis* и *M. hartingi*, чем к *M. guentheri* и *M. socialis*. Результаты, полученные на основе анализа последовательностей цитохрома-*b* в сочетании с кариологическими и морфологическими характеристиками позволяют предположить, что *M. dogramacii* может быть недавно эволюционировавшим видом.

КЛЮЧЕВЫЕ СЛОВА: полевка Дограмачи, цитохром-*b*, *Microtus guentheri*, *Microtus qazvinensis*, *Microtus socialis*

Introduction

Anatolia contains approximately 67 rodent species and is a rich region in terms of species diversity. Fifteen of these rodent species are classified within the genus *Microtus* Shrank, 1798 (Yiğit *et al*., 2006; Kryštufek & Vohralík, 2009; Wilson *et al*., 2017). Dogramaci's vole, *M. dogramacii* Kefelioğlu et Kryštufek, 1999 is one of the endemic vole species found in Anatolia, along with *M. anatolicus* Kefelioğlu et Kryštufek, 1999 and the recently identified *Microtus elbeyli* Yiğit *et al*., 2016. Kefelioğlu & Kryštufek (1999) classified *M. dogramacii* as a new species, with the type locality of Suluova, Amasya, based on karyological and

morphological differences in comparison with *Microtus socialis* Pallas, 1773 and *M. guentheri* Danford et Alston, 1880. Despite bearing a different chromosomal number (2n = 48), Dogramaci's vole has been classified as a member of the "guentheri" group of voles (2n = 54). The subgenus *Sumeriomys* Argyropoulo, 1933 contains both the "guentheri" group (*M. guentheri, M. hartingi* Barret-Hamilton, 1903*, Microtus mustersi* Hinton, 1926; *M. dogramacii* and *M. qazvinensis* Golenishchev *et al*., 2003), whose species feature 2n = 46, 48, 54 chromosomes (Kryštufek *et al*., 2009; Kryštufek *et al*., 2012; Zima *et al*., 2013; Arslan & Zima, 2014; Kryštufek & Shenbrot, 2022) and "socialis" group (*M. socialis*, *M. paradoxus* Ognev et Heptner, 1928, *M. irani* Thomas, 1921, and *M. anatolicus* and *M. schidlovskii* Argyropulo, 1933), whose species feature chromosomal numbers are 2n = 46, 48, 60, 62, 64 (Akhverdyan, 1989; Golenishchev *et al*., 2002; Zima *et al*., 2013; Mahmoudi *et al*., 2014; 2022). Based on morphological measurements and cytogenetic features, populations of the "guentheri" group of voles found in Thrace and western and south-eastern Anatolia have been described as *Microtus hartingi*, *Microtus lydius* and *M. guentheri*, respectively (Yiğit & Çolak, 2002; Yiğit *et al*., 2012; Zima *et al*., 2013; Markov *et al*., 2014). Yiğit & Çolak (2002) and Yiğit *et al*. (2012) also categorized the western and central Anatolian populations as *M. lydius lydius* and *M. lydius ankaraensis,* respectively*,* and the Thracian population as *M. hartingi,* based on morphometric differences. Jaarola *et al*. (2004) determined that *M. dogramacii* is more closely related to *M. guentheri* than to *M. socialis,* according to cytochrome-*b* (*cytb*) analyses. Kryštufek *et al*. (2009, 2012) indicated that *M. hartingi* and *M. dogramacii* are closely related species and that both Thracian and western Anatolian populations should be classified as *M. hartingi,* based on their studies examining the *cytb* gene region. They also confirmed the separation of the *M. guentheri* and *M. socialis* clades based on *cytb* analysis. Şekeroğlu *et al*. (2011) revealed the existence of substantial chromosomal variation among *M. dogramacii* samples. Similarly, Thanou *et al*. (2012) analyzed the *cytb* region and suggested that the western Anatolian and Thracian populations belong to *M. hartingi*. Mahmoudi *et al*. (2015) and Thanou *et al*. (2020) found that *M. qazvinensis* (Iran) and *M. dogramacii* (Turkey) samples formed closely-related clades separated from *M. hartingi*, *M. guentheri* and *M. socialis* according to the *cytb* data. Accordingly, Pardiñas *et al*. (2017) and Kryštufek & Shenbrot (2022) considered *M. qazvinensis* as a subspecies of *M. dogramacii*. Yiğit *et al*. (2017) studied the *cytb*, *cytochrome c oxidase subunit 1* (*CO1*) and 12S rRNA regions and found that genetic distance values have not supported the observation that *M. hartingi* and *M. lydius* represent separate species, although speciation may have occurred in this genus despite low genetic distance values (Baker & Bradley, 2006) and accepted *M. lydius* as a subspecies of *M. hartingi* as compatible with Golenishchev *et al.* (2022), Zorenko *et al.* (2023) and Kryštufek & Shenbrot (2022). A hybridization study examining Bulgarian and Anatolian specimens of *M. hartingi* performed by Zorenko *et al*. (2016) found that F1 hybrids were viable and prolific; however, backcrossing sterility in males was observed in subsequent hybrid generations, characterized by high mortality, reduced body weights, reduced spermatozoa quantity, and lower levels of spermatogenesis.

The barrier properties of the Dardanelles and Bosphorus Straits, located in the Marmara Sea and thought to have developed during the Late Pliocene epoch (approximately 2 Mya) (Bacescu, 1985; Tortonese, 1985; Çağatay *et al*., 2000; Yaltırak *et al*., 2000) and despite of the fact that these straits were opened and closed during the Pleistocene Period, intermittently (Meriç, 1995; Chepalyga, 1995), have been hypothesized to have caused the diversification of terrestrial animals, such as Thracian and Anatolian populations of *M. hartingi*. In addition, the 2n = 54 chromosome number is ancestral to the genus *Microtus,* which appears to have split between 0.5 and 3.5 million years ago (MYA) (Lemskaya *et al*., 2010); in the "guentheri" group of voles, the formation of new species, such as *M. dogramacii,* is highly likely. Also, speciation events of the subgenus *Sumeriomys* coincided with Middle Pleistocene (0.774–0.129 MYA) (Abramson *et al.,* 2021). In this study, it was attempted to clarify the taxonomical status of *M. dogramacii*, which has a distribution area in the northern region of central Anatolia (Amasya and surrounding provinces), and the controversial phylogenetic relationship between *M. dogramacii* and the other species of "guentheri" group, which feature a 2n = 54 chromosomal number, by examining the genetic diversity of *cytb* sequences among *M. dogramacii*, *M. hartingi*, *M. guentheri, M. qazvinensis* and *M. socialis*.

Materials and methods

Sampling, DNA isolation and Polymerase Chain Reaction (PCR)

DNA isolation of five *M. dogramacii* specimens stored in Ankara University Mammalian Research Collection (AUMAC, http://www.mammalia.ankara.edu.tr) was performed using GeneAll® ExgeneTM Tissue SV mini kit (Atlas Biotechnology, Turkey) from liver tissues. Other sequences were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). *cytb* sequences of *M. dogramacii* (5 AUMAC, 2 GenBank samples), *M. hartingi* Thracian population (6 AUMAC, 2 GenBank samples), *M. hartingi* Anatolian population (30 AUMAC, 3 GenBank samples), *M. guentheri* (5 AUMAC, 3 GenBank samples), *M. qazvinensis* (9 GenBank samples), *M. socialis* (3 GenBank samples) (Fig. 1) and as an outgroup *Microtus arvalis* (2 samples-AUMAC) specimens were analyzed in this study (Appendix 1). Species of the specimens were identified based on the external and skull characteristics (Yiğit *et al*., 2006). The 1100 base-pair *cytb* gene region was amplified using the L14727-SP and H15915-SP prim-

ers (Jaarola & Searle, 2002), and the reaction mixture components and reaction conditions used were those described by Yiğit *et al*. (2017). Polymerase chain reaction (PCR) products were electrophoresed in 0.8% agarose gel for 1 hour at 70 volts in 1 × TAE (Tris-acetate-EDTA) buffer, and the PCR bands were visualized using a SYNGENE Bio Imaging system. Sequencing was performed by MEDSANTEK, Turkey.

Cytb gene analyses

Forward and reverse *cytb* sequences were displayed in Chromas Lite 2.1.1 (www.technelysium.com.au) and aligned using MegaX Software (Kumar *et al*., 2018) and 524 base-pair raw data was obtained. Using DnaSP 6 (Rozas *et al*., 2017), genetic diversity values (nucleotide and haplotype diversity; polymorphic sites, including parsimony-informative and singleton-variable sites; and the total number of mutations) and haplotypes were defined. Mean genetic distance values (*d*) between species were estimated in MegaX Software (Kumar *et al*., 2018) with 10.000 bootstrap replications considering *p*-distance (Hamming, 1950) parameter.

Median-joining network was drawn in Pop Art version 1.7 (Leigh & Bryant, 2015) to analyze relationships among species using all haplotypes. Default options were considered and outgroups were discounted.

Hasegawa, Kishino, Yano model with Gamma distribution (Hasegawa *et al*., 1985) as an appropriate evolutionary model according to the Akaike information criterion (AIC) and Bayesian information criterion (BIC) for dendrograms defined in jModelTest 2.1.7 (Posada, 2008; Darriba *et al*., 2012). Maximum Likelihood dendrogram was built in IQ Tree web server (http://iqtree.cibiv.univie.ac.at/, Trifinopoulos *et al*. 2016) with 1000 number of bootstrap alignments. A Bayesian Markov Chain Monte Carlo (MCMC) dendrogram was also constructed in the Beast v1.75 program (Drummond & Rambaut, 2007). Five independent runs were performed using 10.000.000 iterations each, and a total of 5.000 burn-in data points were rejected. Using the program Tracer v.1.5 (http://beast.bio.ed.ac.uk/Tracer), effective sample size (ESS) values were determined to test the accuracy of the Bayesian analyses; the results were accepted if ESS values were 200 or higher. The obtained dendrograms were viewed and edited in the FigTree 1.4 program (http://tree.bio.ed.ac.uk/software/figtree).

Evolutionary divergence times for the *Microtus* species were also calculated based on the mammalian *cytb* divergence rate (3.27×10^{-7} mutations/site/year; Martínková *et al*., 2013), and the calibration point of the Anatolian and European populations of *Microtus arvalis* split (0.295 MYA, Çetintürk *et al*., 2021) was determined in the Beast v1.75 program (Drummond & Rambaut, 2007). "Yule process of speciation" and "Log-normal distribution" models were selected in the Beauti program within the Beast v1.75 Program and the species were grouped before the analysis.

As a species delimitation test, Automatic Barcode Gap Discovery (ABGD; Puillandre *et al*., 2012) was performed (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html). ABGD method is preferred to delimit genetic clusters by detecting a significant gap in the pairwise distance distribution. Kimura-2 parameter (Kimura, 1980) was used and outgroups were not taken into consideration in data analysis. Other parameters were applied as followed: A proxy for the minimum gap width (X): 1.0, pmin (prior minimum distance): 0.001, Pmax (prior maximal distance): 0.08, steps: 10, number of bins: 20.

Results

The genetic diversity values that were determined during the analysis of 524 base-pair sequences from 68 samples yielded 48 haplotypes (Tab. 1, Fig. 1), six of which were specific to *M. dogramacii*, 5 were specific to *M. hartingi* (Thrace), 23 were specific to *M. hartingi* (Anatolia), one was a common haplotype shared between *M. hartingi* Thracian and Anatolian populations, six were specific to *M. guentheri,* four were specific to *M. qazvinensis*, and three were specific to *M. socialis*. Haplotype diversity (Hd) values were found to be high, with the highest value identified for *M. socialis* (1.000) and the lowest value identified for *M. qazvinensis* (0.750). Nucleotide diversity (Pi) value was highest in *M. dogramacii* (5.6%), whereas these values were

Table 1. Genetic diversity values of *Microtus* species analyzed in this study.

	Number of samples (haplotypes)	Haplotype diversity (Hd)	Nucleotide diversity (Pi)	Polymorphic sites	Parsimony informative sites	Singleton variable sites	Total number of mutations
M. dogramacii	7 (6)	0.952±0.096	0.056±0.001	8	3	5	8
M. hartingi (Thrace)	8 (6)	0.893±0.012	0.007±0.002	13	4	9	13
M. hartingi (Anatolia)	33 (24)	0.972±0.000	0.012±0.002	46	21	25	48
M. guentheri	8 (6)	0.929±0.007	0.011±0.002	14	5	9	14
M. qazvinensis	9 (4)	0.750±0.012	0.004±0.001	6	4	2	6
M. socialis	3 (3)	1.000±0.074	0.008±0.003	7	0	7	7
Total	68 (48)	0.986±0.000	0.039±0.003	111	80	31	124

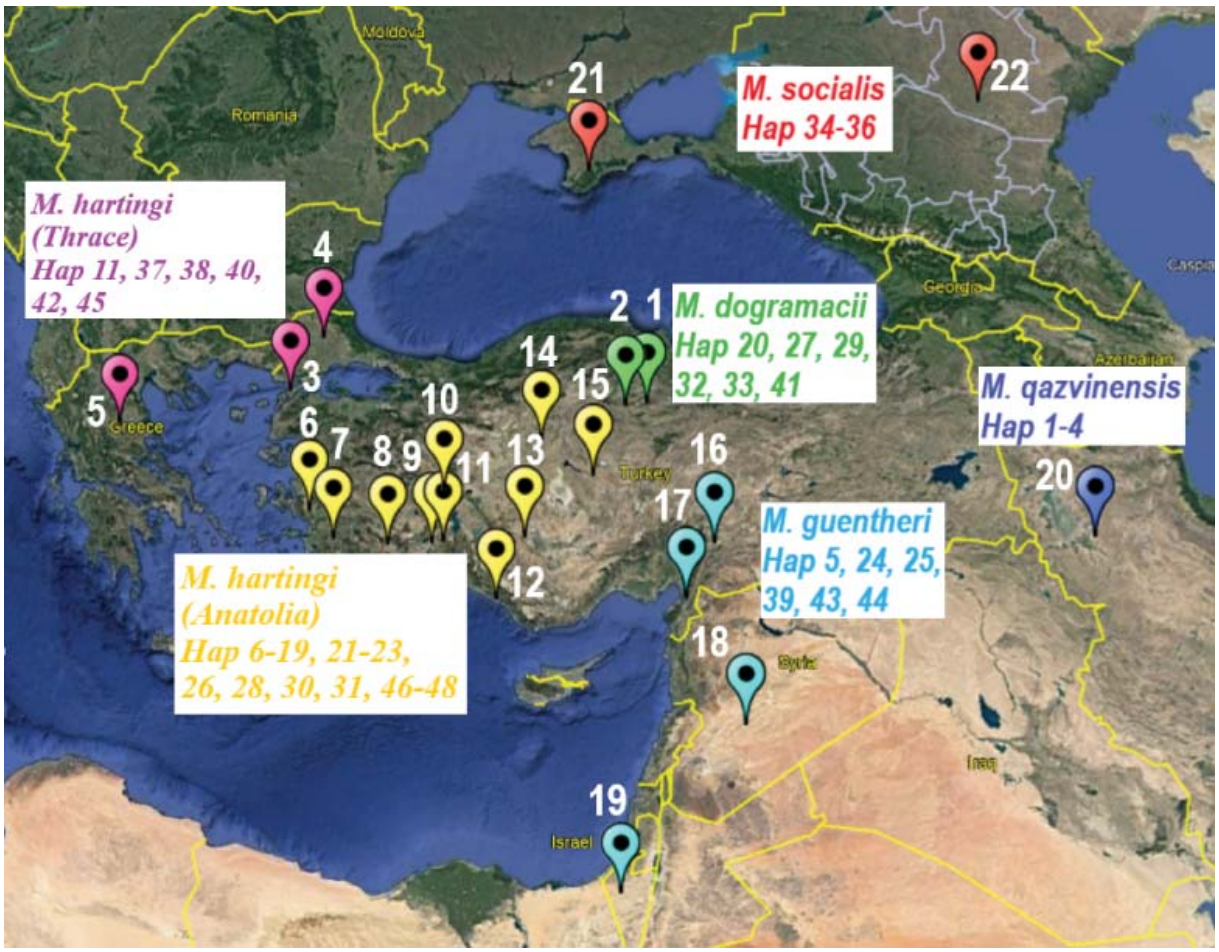

Fig. 1. Localities and haplotype distribution of *Microtus* samples used in analyses. (Localities showed with numbers were given in Appendix 1). The map was taken from Google Earth Pro (Accession date: 10.02.2023).

below 1.5% in the other species. Other genetic diversity values (the numbers of parsimony-informative and singleton-variable sites; and the total number of mutations) were the highest in *M. hartingi* (Anatolia) and the lowest in *M. qazvinensis* and *M. socialis* (Tab. 1).

The mean genetic distance values (*d*) shown in Tab. 2. Whereas the distances between *M. hartingi* Thracian and Anatolian populations provided the lowest values (1.1%), the distance values between *M. guentheri* and *M. socialis* (7.8%) were the highest values and *p*-distance values between *M. dogramacii* and other species varied between 3.2–7.0%.

Median-joining network, Bayesian MCMC and Maximum Likelihood dendrograms, which indicate phylogenetic relationships, are provided in Figs 2–4. In Median-joining network (Fig. 2), *M. hartingi* Thracian and Anatolian populations haplotypes clustered together and although *M. dogramacii* haplotypes established a separate group, they split from *M. qazvinensis* haplotypes with a low number of mutations. *M. dogramacii* and *M. qazvinensis* were closer to *M. hartingi* haplotypes than *M. guentheri* and *M. socialis* which were clearly separated from other species. *M. dogramacii* was separated with high posterior probability value as closer to *M. qazvinensis* in the Bayesian MCMC dendrogram (Fig. 3). *M. hartingi* Thracian and Anatolian populations did not differ with polytomy observed in clades. *M. socialis* split from other species, and *M. guentheri* was located in a different clade. Similar tree topology was acquired in Maximum Likelihood dendrogram (Fig. 4). Furthermore, the Syria–Israel and Anatolian populations of *M. guentheri* were also separated in Bayesian MCMC Dendrogram (pp = 1.0), Maximum Likelihood dendrogram (bootstrap value = 96%) and Median-joining network (Anatolian haplotypes: Hap_5, 24, 25, and 39; Syria–Israel haplotypes: Hap_43 and 44).

Approximate evolutionary divergence times were calculated as follows: 0.189 Mya for *M. hartingi* Thracian and Anatolian populations; 0.250 Mya for *M. dogramacii* and *M. qazvinensis;* 0.384 Mya for *M. dogramacii/M. qazvinensis* and *M. hartingi*; 0.531 Mya for *M. guentheri* and *M. dogramacii/ M. qazvinensis*/*M. hartingi;* and 0.632 Mya for *M. socialis* and all species in the guentheri" group of voles.

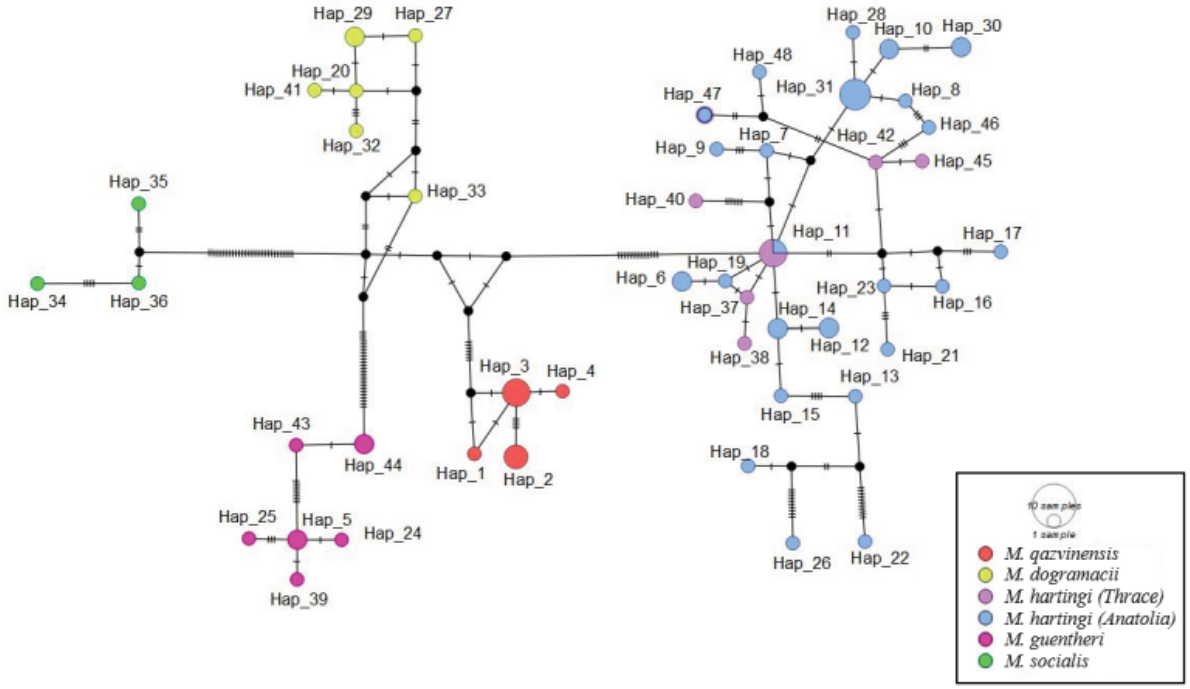

Fig. 2. Median-joining network constructed with haplotypes of *Microtus* species. Black lines on branches show number of mutations and black circles represent median vectors.

Table 2. Mean genetic distance values (*d*) with standard errors between *Microtus* species in this study based on *p*-distance parameter (Hamming, 1950). (Values under and above the diagonal show genetic distance values and standard errors, respectively).

	M. dogramacii	***M. hartingi (Thrace)***	***M. hartingi (Anatolia)***	***M. guentheri***	***M. socialis***	***M. qazvinensis***
M. dogramacii		0.008	0.008	0.009	0.010	0.007
***M. hartingi* (Thrace)**	0.042		0.011	0.011	0.011	0.008
***M. hartingi* (Anatolia)**	0.044	0.011		0.010	0.009	0.008
M. guentheri	0.060	0.067	0.068		0.011	0.010
M. socialis	0.070	0.075	0.077	0.078		0.010
M. qazvinensis	0.032	0.044	0.045	0.066	0.073	

The ABGD results yielded 4 lineages for initial partitions as *M. dogramacii/M. qazvinensis*, *M. guentheri*, *M. hartingi* and *M. socialis* groups with prior maximal distance P=1.00e-02. These lineages were supported by phylogenetic approaches except that *M. dogramacii* and *M. qazvinensis* were clearly separated Median-joining network, Bayesian MCMC and Maximum Likelihood dendrograms unlike ABGD results.

Discussion

Molecular phylogenetic studies examining *M. dogramacii* are scarce among currently published studies on voles. In recent molecular studies regarding the results of *cytb* analyses, Jaarola *et al*. (2004) found that *M. dogramacii* is more closely related to *M. guentheri* than *M. socialis*. Kryštufek *et al*. (2009, 2012) suggested that *M. hartingi* and *M. dogramacii* are closely related species. Besides, Mahmoudi *et al*. (2015) and Thanou *et al*. (2020) reported that genetic distance value between *M. qazvinensis* (Iran) and *M. dogramacii* (Turkey) populations were considerably low (2.8%) and they split from *M. hartingi*, *M. guentheri* and *M. socialis*.

Recent studies examining *M. guentheri*, *M. hartingi* and *M. lydius*, performed by Yiğit & Çolak (2002), Yiğit *et al*. (2012), Zima *et al*. (2013) and Markov *et al*. (2014), proposed that *M. guentheri* populations from

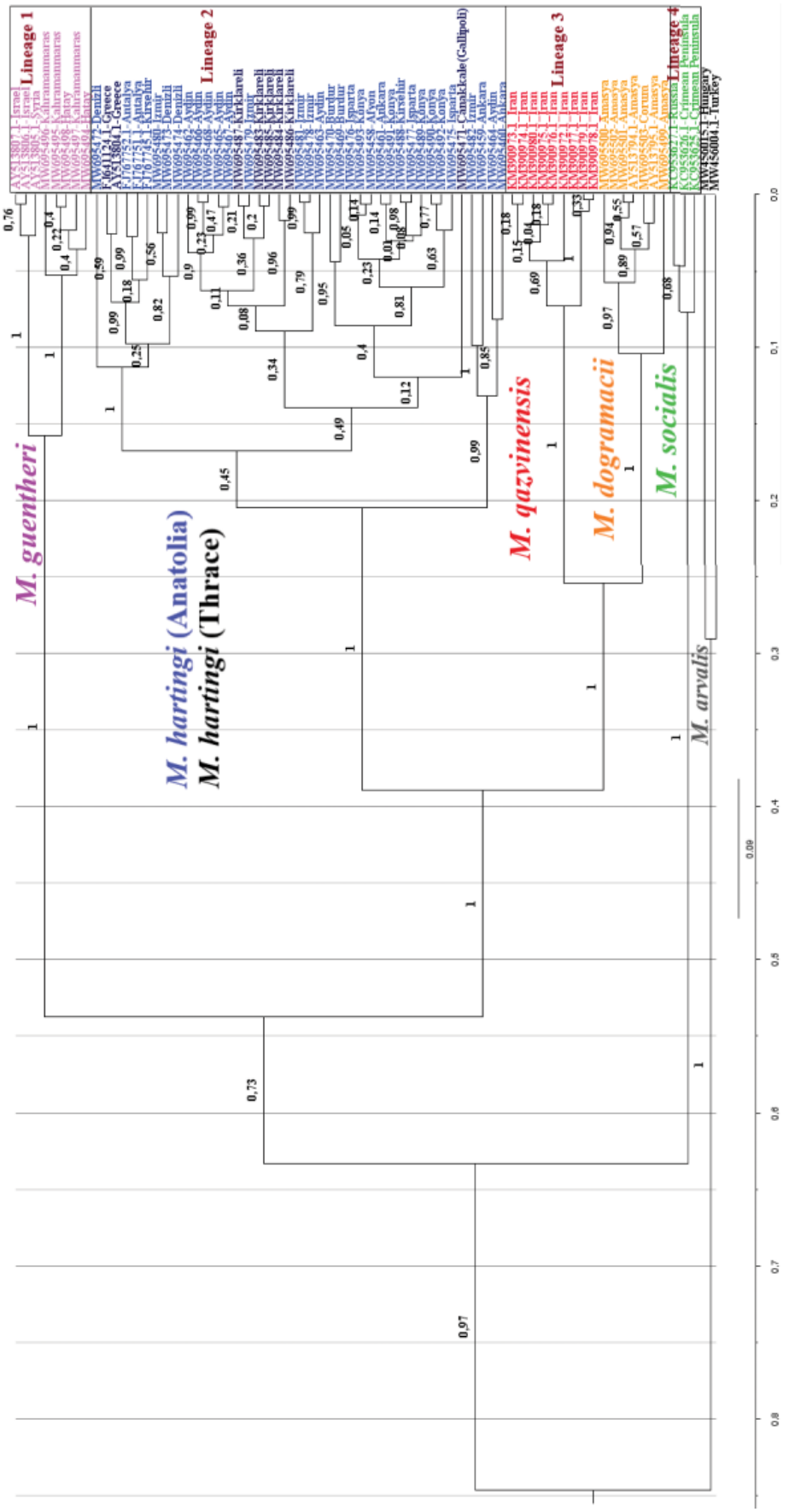

Fig. 3. Bayesian MCMC dendrogram constructed using sequences based on Hasegawa, Kishino, Yano parameter with Gamma distribution (Hasegawa *et al.*, 1985). Boxes shown with rectangles indicate lineages determined according to automatic barcode gap discovery (ABGD, Puillandre *et al.*, 2012) analysis. (Bold numbers on branches show posterior probability values; scale axis is proportional to the divergence times).

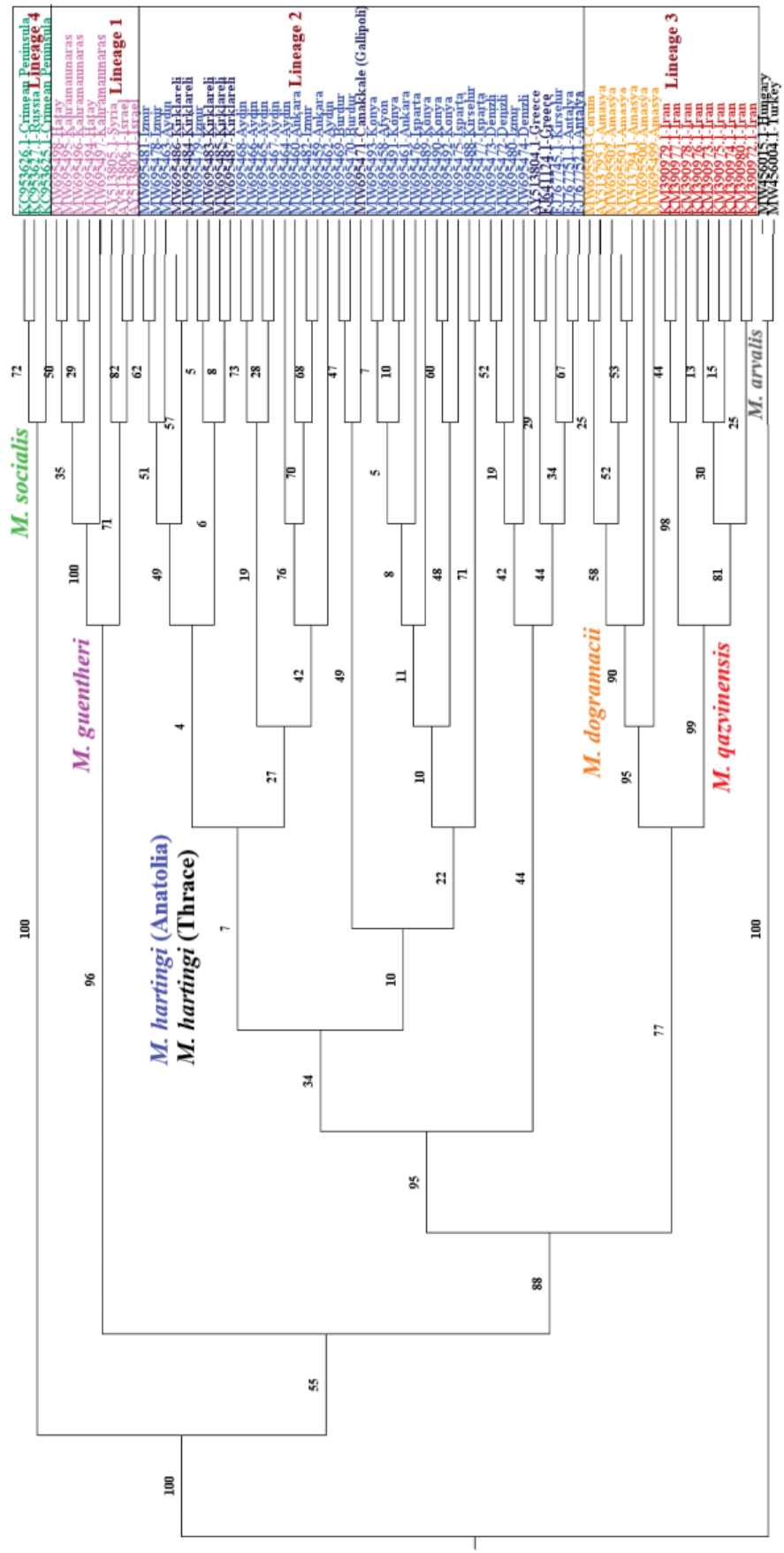

Fig. 4. Maximum likelihood dendrogram constructed using sequences based on Hasegawa, Kishino, Yano parameter with Gamma distribution (Hasegawa *et al.*, 1985). Boxes shown with rectangles indicate lineages determined according to automatic barcode gap discovery (ABGD, Puillandre *et al.*, 2012) analysis. (Bold numbers on branches show bootstrap values).

south-eastern Anatolia have differentiated from western and central Anatolian Guentheri group populations because the Anatolian diagonal has prevented gene flow through the western parts of Anatolia. Based on morphometric differences, Yiğit & Çolak (2002) and Yiğit *et al*. (2012) considered the Thracian population to be *M. hartingi* and the western Anatolian populations to be the subspecies *M. lydius lydius* and *M. lydius ankaraensis*, respectively. In contrast, molecular studies conducted by Jaarola *et al*. (2004), Kryštufek *et al*. (2009, 2012) and Thanou *et al*. (2012) revealed that the Thracian and western Anatolian populations were both *M. hartingi,* according to *cytb* gene analyses. The findings of Yiğit & Çolak (2002) and Yiğit *et al*. (2012) were further supported by the study performed by Zorenko *et al*. (2016), who found that mating *M. h. strandzensis* with *M. h. lydius* resulted in fertile F1 offsprings but the subsequent cross breeding showed backcrossing sterility of males and high mortality of posterity. The low genetic variation in the *M. hartingi* Thracian and Anatolian populations and the 1.62–0.200 Mya divergence time, which may be associated with the development of the Bosphorus and Dardanelles straits, do not support these two taxa representing valid species based on *cytb*, *cytochrome c oxidase subunit 1* (*mt-CO1*) and 12S rRNA sequences (Yiğit *et al*., 2017).

The genetic findings obtained in this study are consistent with the previously reported molecular studies in the literature. According to Bradley & Baker (2001), genetic distance values among either conspecific populations or valid species can range from 2% to 11%, in general. The average intraspecific genetic distances are 1.5% (0.0–4.7%) for rodents and 2.0% (0.2–4.4%) for *Microtus* species (Baker & Bradley, 2006). In this study, the mean genetic distance between *M. hartingi* Thracian and Anatolian populations was 1.1% (Tab. 2) and these species were not split into different lineages in the phylogenetic approaches (Figs 2–4). The Thracian and western Anatolian populations were found to have separated, evolutionarily 0.189 Mya, which coincides with the timing of the formation of Bosphorus and Dardanelles Straits (2 Mya) in spite of the fact that these straits remained open, discontinuously during the Pleistocene Period (Chepalyga, 1995; Meriç, 1995). However, the low mean genetic distance values suggested that the differentiation between these two populations was not sufficient to form a new species.

The mean genetic distances (4.2–6.7%, Tab. 2) between *M. dogramacii* and Anatolian "guentheri" group populations and *M. socialis,* respectively, and the Median-joining network, Bayesian MCMC and Maximum Likelihood dendrograms (Figs 2–4) indicated genetically valid speciation for *M. dogramacii*. After *M. socialis* split, as a basal group (0.632 Mya), *M. guentheri* separated from the remaining "guentheri" group of voles (0.531 Mya). The *Microtus* species originated in south-west Asia, and most of these species are found in Anatolia, Iran and the Caucasus (Shenbrot & Krasnov, 2005; Aulagnier, 2009). The Messinian salinity crisis, which occurred during the late Miocene period (16.8–5.1 Mya) (Strömberg *et al*., 2007), caused the formation of open habitats due to the drying of the Paratethys Ocean. Therefore, social voles likely moved to eastern Anatolia from south-west Asia and spread to other parts of Anatolia. Moreover, the identification of two *M. guentheri* lineages (Anatolia and Syria–Israel) (Figs 2–4) might also indicate diversification within the species. The phylogenetic approaches and divergence times also indicated that *M. dogramacii* represents a distinct species that is more closely related to *M. hartingi* than to *M. guentheri* and *M. socialis*. On the other hand, low genetic distance value between *M. dogramacii* and Iranian *M. qazvinensis* populations (3.2%) were defined and they were located closer in Median-joining network, Maximum Likelihood and Bayesian MCMC dendrograms as well as formed a common lineage in ABGD results. Considering the morphological and karyological differences as well as geographical distance (minimum 1700 km) between these populations, *M. dogramacii* and *M. qazvinensis* could be accepted as valid and closely-related species correspond to Mahmoudi *et al*. (2015) and Thanou *et al*. (2020). Based on these data, and in the light of the reported chromosomal and morphological differences, *M. dogramacii* and *M. qazvinensis* most likely first separated from *M. guentheri* and then originated from *M. hartingi* lineage.

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Appendix 1. GenBank Accession numbers and localities of *Microtus* species analyzed in this study.

Species	Locality and Locality Number in Fig. 1	Accession Number	Haplotype Numbers	References
M. dogramacii	Amasya Province, Turkey (Locality 1)	MW695499–MW695502	Hap27, Hap29, Hap32, Hap33	This study
M. dogramacii	Çorum Province, Turkey (Locality 2)	MW695503	Hap20	This study
M. dogramacii	Amasya Province, Turkey (Locality 1)	AY513794.1	Hap29	Jaarola *et al*. (2004)
M. dogramacii	Amasya Province, Turkey (Locality 1)	AY513795.1	Hap41	Jaarola *et al*. (2004)
M. hartingi (Thrace)	Çanakkale (Gallipoli) Province, Turkey (Locality 3)	MW695471	Hap40	Yiğit *et al*. (2017)
M. hartingi (Thrace)	Kırklareli Province, Turkey (Locality 4)	MW695483–MW695487	Hap11, Hap37, Hap38	Yiğit *et al*. (2017)
M. hartingi (Thrace)	Greece (Locality 5)	FJ641124.1	Hap45	Thanou *et al*. (unpublished)
M. hartingi (Thrace)	Greece (Locality 5)	AY513804.1	Hap42	Jaarola *et al*. (2004)
M. hartingi (Anatolia)	Izmir Province, Turkey (Locality 6)	MW695478–MW695482	Hap6, Hap11, Hap22, Hap23	Yiğit *et al*. (2017)
M. hartingi (Anatolia)	Aydın Province, Turkey (Locality 7)	MW695462–MW695468	Hap12–15, Hap19	Yiğit *et al*. (2017)
M. hartingi (Anatolia)	Denizli Province, Turkey (Locality 8)	MW695472–MW695474	Hap16, Hap17, Hap21	Yiğit *et al*. (2017)
M. hartingi (Anatolia)	Burdur Province, Turkey (Locality 9)	MW695469–MW695470	Hap7, Hap9	Yiğit *et al*. (2017)
M. hartingi (Anatolia)	Afyon Province, Turkey (Locality 10)	MW695458	Hap8	Yiğit *et al*. (2017)
M. hartingi (Anatolia)	Isparta Province, Turkey (Locality 11)	MW695475–MW695477	Hap8, Hap30, Hap31	Yiğit *et al*. (2017)
M. hartingi (Anatolia)	Antalya Province, Turkey (Locality 12)	FJ767751.1, FJ767752.1	Hap47, Hap48	Kryštufek *et al*. (2009)
M. hartingi (Anatolia)	Konya Province, Turkey (Locality 13)	MW695489–MW695493	Hap8, Hap10	Yiğit *et al*. (2017)
M. hartingi (Anatolia)	Ankara Province, Turkey (Locality 14)	MW695459–MW695461	Hap8, Hap18, Hap26	Yiğit *et al*. (2017)
M. hartingi (Anatolia)	Kırşehir Province, Turkey (Locality 15)	MW695488	Hap31	Yiğit *et al*. (2017)
M. hartingi (Anatolia)	Kırşehir Province, Turkey (Locality 15)	FJ767745.1	Hap46	Kryštufek *et al*. (2009)
M. guentheri	Kahramanmaraş Province, Turkey (Locality 16)	MW695495–MW695497	Hap5, Hap24, Hap25	Yiğit *et al*. (2017)
M. guentheri	Hatay Province, Turkey (Locality 17)	MW695494, MW695498	Hap5, Hap39	Yiğit *et al*. (2017)
M. guentheri	Syria (Locality 18)	AY513805.1	Hap43	Jaarola *et al*. (2004)
M. guentheri	Israel (Locality 19)	AY513806.1–AY513807.1	Hap44	Jaarola *et al*. (2004)
M. qazvinensis	Iran (Locality 20)	KM390972.1–KM390981.1	Hap1–4	Mahmoudi *et al*. (2015)
M. socialis	Crimean Peninsula (Locality 21)	KC953625.1–KC953626.1	Hap34, Hap35	Kryštufek *et al*. (2012)
M. socialis	Russia (Locality 22)	KC953627.1	Hap36	Kryštufek *et al*. (2012)
M. arvalis	Turkey	MW456004.1	–	Çetintürk *et al*. (2021)
M. arvalis	Hungary	MW456015.1	–	Çetintürk *et al*. (2021)