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Phylogenetic and Phylogeographic Relationships of Populations of *Meriones tristrami* Thomas, 1892 (Rodentia: Gerbillinae) in Turkey as Inferred from Cytochrome-*b* and RFLP Analysis

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Abstract: The present study aimed to reveal the relationship between the genetic diversity of Tristram's jird *Meriones tristrami* subpopulations by using cyt-*b* sequences, the amplified fragments of cyt-*b* produced by restriction endonuclease (RFLP; Msp I, Rsa I, Noc I and Hae III were used) and the distribution on the Anatolian Peninsula. Eighteen haplotypes were identified in the subpopulations of this species, with the highest nucleotide diversity in the Central Anatolia. The haplotype diversity was determined to be 0.970 among subpopulations. The fixation index (*F*st) and the gene flow parameter (Nm) based on cyt-*b* sequences showed the effective gene flow between the western and south-eastern subpopulations. Both cyt-*b* sequences and RFLP analyses produced almost similar topology in the Bayesian and UPGMA trees, indicating a gene flow from subpopulations of the South-east to Central Anatolia and the Western Black Sea coast. The main factor for the genetic diversity is considered to be the intermittent distribution from west to east as a result of the great altitude of the Eastern Anatolian Plateau as well as the sea and lake system fragmenting the territory of Anatolia in the Pliocene – Pleistocene Age.

Key words: speciation, evolution, genetic diversity, Tristam's jird, divergence

Introduction

Mitochondrial genes are widely used in systematic studies to highlight divergences or phylogenetic relationships in many taxonomic groups. The cytochrome-*b* gene is the most useful genetic tool for phylogenetic works, being the most conventionally utilised mitochondrial gene (Meyer & Wilson 1990, Irwin et al. 1991, Adkins et al. 2001). The species of the genus *Meriones* Illıger, 1811 (Gerbillinae) are the dominant rodents in arid and semi-arid steppe ecosystems in Asia and parts of North Africa. The systematic status of the genus is not fully understood and the phylogenetic/phylogeographic relationships

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of the taxa have not been fully established; in addition, the genetic researches comprising the phylogenetic relationship among taxa are also scant (Adkins et al. 2001, Musser & Carleton 2005, Chevret & Dobigny 2005, Naseri et al. 2006, Du et al. 2010, Ito et al. 2010). Cricetid and gerbillid rodents have been reported as diversified during the Miocene (Flynn et al. 1986, Abiadh et al. 2010). The analysis of the divergence times of major rodent groups has revealed the genus *Meriones* is closely related to *Mus-Rattus* clade (Adkins et al. 2001). While studying the phylogeny of the subfamily Gerbillinae using cyt-*b* and COII genes, it has been shown that the genus *Meriones* is not monophyletic (Chevret & Dobigny 2005, Ito et al. 2010).

Five species of the genus *Meriones* occur in Turkey (Yiğit et al. 1997, Musser & Carleton 2005). Of these, *M. tristrami* Thomas, 1892 (of the subgenus *Pallasiomys*) is the most common jird in the semi-arid steppe of Asiatic Turkey; in this country, it is represented by six subspecies (Yiğit et al. 1998): *M. t. blackleri* Thomas, 1903, *M. t. lycaon* Thomas, 1919, *M. t. bogdanovi* Heptner, 1931, *M. t. bodenheimeri* Aharoni, 1932, *M. t. intraponticus* Neuhäuser, 1936 and *M. t. kilisensis* Yiğit & Çolak, 1998. The distribution of Tristram's jird extends from the Iranian border to the Aegean coasts of Turkey westwards, with interruptions in the eastern part of Turkey. Morphologically, these subspecies are often not distinct due to the poor intra-specific variations and they are mostly supported by chromosomal variations (Yiğit et al. 1998, Yiğit & Çolak 1998). Even though the diploid number of chromosomes (2n) has been constantly reported as 72, the chromosomal arms showed variations from 74 to 84 (Qumsiyeh 1986, Yiğit et al. 1998, Yiğit & Çolak 1998, Darvish 2009). All these karyological records provide taxonomic support for the generic systematics but are far from establishing the phylogenetic and phylogeographic relationships of subpopulations, which are undoubtedly related to the Anatolian geological evolution. However, more informative data about the geographical isolation and genetic differentiation have been received from allozyme studies (Yiğit et al. 2016).

On the phylogeographic basis, land bridges, climatic phases and the mammalian migrations between Asia and Africa, including those related to the species of the subfamily Gerbillinae, were discussed in details in Vrba (1995), Cox (2000) and Chevret & Dobigny (2005). Görür et al. (1995) suggested that the main mammal migration to Anatolia started probably during the Late Miocene. According to some fossil records on the mammalian fauna of Anatolia, arvicolid rodents were distributed in West Anatolia during the Late Pliocene – Early Pleistocene (Sarica 2000). Şen (1977) reported that *Pseudomeriones abbreviatus* appeared very close to the contemporary *M. tristrami* and was recorded from the Ankara Province from the Pliocene. Suata-Alpaslan (2009) stated that *Pseudomeriones* spp. were very common in fossil deposits of the Anatolian steppe during the Early Pliocene. Apart from these, the Central Anatolian lake system still covered large areas during the Holocene and acted as a natural barrier against the main routes of fauna occupying the Anatolian Peninsula (Kuzucuoğlu et al. 2011). The environmental factors to which Anatolia has been subjected in geological times have defined the actual distribution of this jird through the peninsula. In this frame, cyt-*b* and RFLP analyses are useful techniques for exploring the phylogeny of animal populations.

The present study aims to enlighten the phylogenetic and phylogeographic relationships of *M. tristrami* subpopulations in Turkey by using cyt-*b* and RFLP analyses.

Materials and Methods

Sampling

This study was performed using 22 specimens of Tristram's jird from ten localities (Table 1, Fig. 1). The tissue samples obtained from the previous studies and deposited at the Ankara University Mammalian Research Collection (AUMAC, http://www.mammalia.ankara.edu.tr) were used. The cyt-*b* sequences of *Meriones meridianus*, *Rattus rattus* and *Mus musculus* were obtained from the GenBank database and used as outgroups in the analyses. The specimens from different close locations were assigned to six subspecies and were grouped under seven subpopulations. The abbreviations of subpopulations are as follows: *M. t. blackleri* = Mtb, *M. t. intraponticus* = Mti, *M. tristrami* (Denizli Prov-

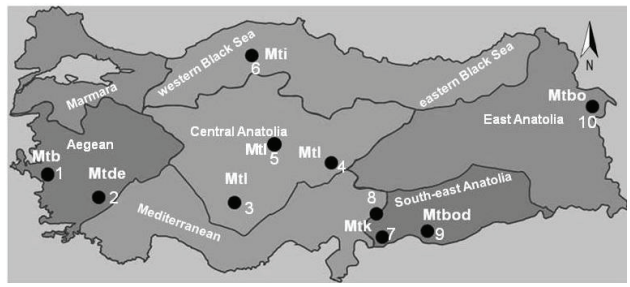

Fig. 1. Sampling localities of Tristram's jirds in Turkey (solid lines show the seven regions conventionally divided in respect to geographic and climatic peculiarities; see Table 1 for abbreviations and numbers).

ince at the transition zone between Mtb and Mtl) = Mtde*, M. t. lycaon* = Mtl, *M. t. kilisensis* = Mtk, *M. t. bodenheimeri* = Mtbod and *M. t. bogdanovi* = Mtbo). Of these subpopulations, Mtb is from the coastal part of Western Anatolia, Mtde – from the inner part of Western Anatolia, Mtl from the Central Anatolian Region, Mti – from the Black Sea Region, Mtk and Mtbod – from South-eastern Anatolia and Mtbo – from Eastern Anatolia (Fig. 1).

DNA extraction and PCR amplification

Total genomic DNA extraction from muscle tissues (stored at -80˚C) was performed following a modified CTAB extraction protocol (Doyle & Doyle 1987). The polymerase chain reaction was carried out using 200-300 ng template DNA. The 25 µL reaction mix consisted of 1.2 µM of each primer (L14841 and H15767 mitochondrial cytochrome-*b* universal primers, see Irwin et al. 1991, Thermo Scientific), 2.5 mM MgCI2 (Fermentas), 2 Unit Taq Polymerase (Fermentas), 4mM dNTP (Fermentas dNTP mix) and 20 mM buffer with (NH4)2SO4 (Fermentas). Cycling conditions (TECHNE TC-3000 Thermal Cycler) were an initial denaturation step at 94˚C for 2 minutes, followed by 35 cycles. After then, denaturation (45 seconds at 94˚C), primer annealing (45 seconds at 50˚C), extension (1 minute 30 seconds at 68˚C) and final extension (10 minutes at 68˚C; Chevret & Dobigny, 2005) steps were carried out. Amplified DNA of the samples was electrophoresed in 1% agarose gel for 2 hours at 70V in 1xTAE. 100 bp DNA was used as a marker. The gel was stained with ethidium bromide and viewed in SYNGENE Bio Imaging. After purification, amplified DNA was sequenced as forward and reverse.

Analyses of cyt-*b* sequences

The sequences of 22 specimens were used in cyt-*b* analyses. The sequences were aligned using CLUSTALX (Thompson et al. 1997). Forward and reverse sequences of each sample were aligned and all their consensus sequences were obtained; 712 bp of approximately 1000 bp were evaluated. Nucleotide diversity, haplotype diversity and polymorphism within subpopulations were calculated with DNASP ver 5.10.01 (Rozas et al. 2003). Genetic distance data with gamma distribution were obtained in MEGA 5.05 program (Tamura et al. 2011) using Kimura-2 Parameter (Kimura 1980). In addition, the nucleotide substitution matrix rate was estimated using the Maximum Composite Likelihood Method with the GTR Model (Tavaré 1986) in MEGA 5.05 program (Tamura et al. 2011). Analyses of molecular variance (AMOVA), the fixation index (*F*st) and the gene flow parameter (Nm) were calculated in Arlequin 3.5 (Excoffier & Lischer 2010).

The phylogenetic relationships among subpopulations were reconstructed using the Bayesian Markov Chain Monte Carlo (Bayesian MCMC) Method. HKY+G Model (Hasegawa 1985) was determined in jmodeltest 0.1 Programme (Posada 2008) and used in performing Bayesian Markov Chain Monte Carlo (Bayesian MCMC) Method implemented in Beast v1.75 program (Drummond & Rambaut 2007). The Bayesian Tree was visualised with FigTree, version 1.4 (http://tree.bio.ed.ac.uk/software/figtree). Accuracy of five independent Bayesian MCMC runs was tested with Tracer v1.5 (http://beast.bio.ed.ac.uk/Tracer).

Analysis of RFLP

The fragments (sites) from amplified cyt-*b* sequences of 22 specimens were used in the RFLP analyses. Four restriction enzymes (Msp I, Rsa I, Noc I and Hae III, FastDigest Fermentas) were used for amplified cyt-*b* (1000 bp) in the RFLP analysis. PCR-RFLP mix consisted of 18 µl distilled water, 2 µl enzyme buffer and 1 µl restriction enzyme. After the incubation stage at 37˚C for 15-30 min, restriction fragments were electrophoresed with 2 % agarose gel for 60-80 min at 100V in 1xTAE. Data were coded by 1 or 0 according to the presence or absence of band profiles, respectively. POPGENE version 1.32 was used in the estimation of the genetic diversity and the differentiation. The average number of alleles per locus (na), average effective number of alleles (ne), Nei's gene diversity value (h) and the Shannon information index (I) were calculated in order to reveal the genetic diversity in populations. The phylogenetic relationships among samples were explored using the Nei 72 distance matrix was calculated using NTSYSpc ver. 2.2 (Rohlf 2000) software. UPGMA dendrograms were constructed to show phylogenetic relationships between samples. hylogenetic relationships among haplotypes were revealed using Network 4.612 Program (Bandelt et al. 1999).

Results

Sequence diversity and descriptive statistics

Cyt-*b* sequences of 22 samples grouped under seven subpopulations revealed 194 mutations resulting in 18 haplotypes (Table 1). The sequence with 712 bp comprised 179 variables, including 68 parsimony informative ones and 111 singleton variable sites. Haplotype diversity was notably high (0.970 ± 0.028). Among the determined 18 haplotypes, haplotype 7 had the highest frequency (recorded in four

specimens from three localities). In the saturation analysis, the ratio of transitions and transversions (R: 1.47) indicated the moderate transversion rate within the subpopulations of *M. tristrami*. In addition, the nucleotide frequencies were 28.44% (A), 31.26% (T/U), 26.56% (C) and 13.73% (G). The overall nucleotide diversity was 0.04572. The nucleotide diversities of the different subpopulations were 0.00139 (Mtb), 0.131 (Mtde), 0.00975 (Mtbo), 0.02538 (Mtl), 0.02696 (Mti) 0.00952 (Mtk) and 0.00418 (Mtbod). The highest value was recorded for the Denizli subpopulation (Mtde; 0.131), which occupied the transition zone between Mtb and Mtl. The observed variation in cyt-*b* region was analysed at three different hierarchical levels Using Molecular Variance Analyses (AMOVA). The highest genetic variation appeared among subpopulations (77.29 %) based on the RFLP tree analyses (Fig. 4), followed by 18.19 % of variation within the subpopulations and 4.52 % among specimens within the subpopulations.

The genetic distance based on the cyt-*b* sequences of the specimens under-grouped in seven subpopulations (Table 1). The type locations of four subpopulations (Mtb, Mti, Mtl, Mtk) were in Anatolia except for Mtbod and Mtbo. The range of Mtbo was confined to the eastern border of the country and was very restricted, while Mtk and Mtbod were distributed in South-eastern Anatolia near the left and right sides of the Euphrates River. The remaining subspecies were from Central Anatolia (Mtl), north of Central Anatolia (Mti) and from the coastal part of Western Anatolia (Mtb). One subpopulation was registered from the inner part of Western Anatolia (Mtde). In pair-wise comparisons, the genetic distance was found to vary within the range of 0.007 to 0.103 between subpopulations. The greatest genetic distances appeared in Mtb - Mtde (d = 0.103) and Mtb – Mtk (d = 0.098). In contrast to this, the low values for the genetic distance indicated that the genetic contact was more or less uninterruptedly continuous in the Central Anatolian between the south-eastern subpopulations (Mtbod – Mtk, Mtbod – Mtl, Mtbod – Mti, with d = 0.007, 0.018 and 0.018, respectively). Despite the karyological difference, the genetic distance was found to be very low in two adjacent subpopulations in the south-eastern part of the study area (Mtbod, Mtk, with d = 0.007) as expected. The karyological difference was not supported by the genetic distance based on our results for cyt-*b*.

RFLP analysis

The polymorphisms of fragment lengths on the amplified cyt-*b* sequences were assessed through using four different restriction enzymes. We found that the Rsa I and Hae III enzymes digested amplified sequences on two different sites, the Msp I enzyme digested DNA samples on one site and the Nco I enzyme did not digest any cyt-*b* sequences. Consequently, five restriction sites were detected with the four enzymes in all samples. In the Iğdır sample, the Rsa I enzyme digested cyt-*b* sequences by giving different sites from other samples. The Hae III enzyme did not digest cyt-*b* sequences in the Iğdır samples (Mtbo). Mtbo in Kastamonu (Mti), Karaman (Mtl), Denizli (Mtde), Manisa (Mtb) and Kayseri (Mtl) samples differed from Şanlıurfa (Mtbod), Gaziantep and Kilis samples (Mtk) due to different restriction sites. The sites digested by the Msp I separated Gaziantep and Kayseri samples from other samples. In addition, we found that the Nco I enzyme had no restriction site in any sample. The Nei's gene diversity value (h) reflected a relatively low genetic diversity for subpopulations in the study area (h = 0.1749). It was calculated that the average number of alleles per locus (na) = 1.8333, the average effective number of alleles (ne) = 1.2572 and the Shannon information index (I) = 0.2912. According to the Principal Coordinates Analysis, 96.91% of the total variation between populations was explained by the first three axes: 42.39%, 41.85% and 12.67% of the total variation were explained by the first, second and third axis, respectively. Six haplotypes determined by four different restriction enzyme models were based and evolutionary relationship network was constructed to explain phylogenetic relationships (Fig. 2). According to this network, the samples from the Central and Western Anatolia (Karaman, Denizli, Kastamonu and Manisa) shared the same haplotype and formed the main haplotype group. Samples from other localities formed the other five haplotypes. The values of the fixation index (Fst) and the gene flow parameter (Nm) among the haplogroups of *M. tristrami* were also calculated from the RFLP data. The highest Fst values indicated great genetic differentiation and were recorded when comparing the south-eastern – eastern (Fst = 0.8; Nm = 0.12) and the western – eastern (Fst = 0.46; Nm = 0.57) subpopulations. The Fst value (0.15) and Nm (2.82) between the western and south-eastern subpopulations also indicated the effective gene flow in these subpopulations.

Phylogenetic assessments

A clear genetic relationships among the subpopulations was obvious from the Bayesian tree (Fig. 3), confirming our results on the genetic distance. For the subpopulations of *M. tristrami* in Central and South-eastern Anatolia, we established very close sub-clusters as suggested also by the Fst and the Nm values. The Central Anatolian clade comprised of

the subspecies *M. t. lycaon* and *M. t. intraponticus*, while *M. t. bodenheimeri* and *M. t. kilisensis* were established in the south-eastern subpopulations. Regardless of the NF differences in the karyotypes of these subspecies, they appeared closely related in BI tree. *Meriones t. blackleri* (Mtb) was morphologically quite different from the other populations by the white tip of the tail. Its subpopulations from two distant locations (Manisa and Iğdır) controversially formed a common clade, which might originate from the sequence homoplasy or from a recent divergence within the basal groups. This clade was connected to other subpopulation, though with low probability (0.53). Considering all subpopulations belonging to the same species, specimens from adjacent regions were grouped in the same clade, as expected (Fig. 3).

The UPGMA dendrogram produced using the RPLP data only partly confirmed the BI topology of the cyt-*b* sequences. According to the UPGMA dendrogram based on the Nei 72 distance matrix, the subpopulations from South-eastern, Central and Western Anatolia grouped in a different clade. The Iğdır specimens (eastern) appeared to be the most divergent branch. The UPGMA dendrogram confirmed that the early branching of the eastern from the south-eastern subpopulations (Fig. 4). The major difference among these topologies originated from the basal groups.

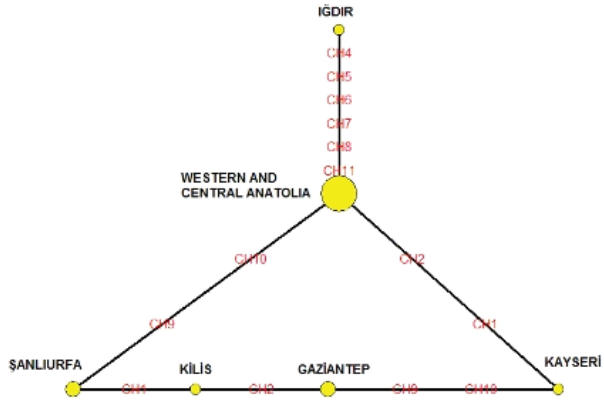

Fig. 2. A median-joining network based on six mitochondrial DNA cytochrome b RFLP haplotypes determined in populations of *M. tristrami*. Circles size is in proportion to haplotypes frequencies.

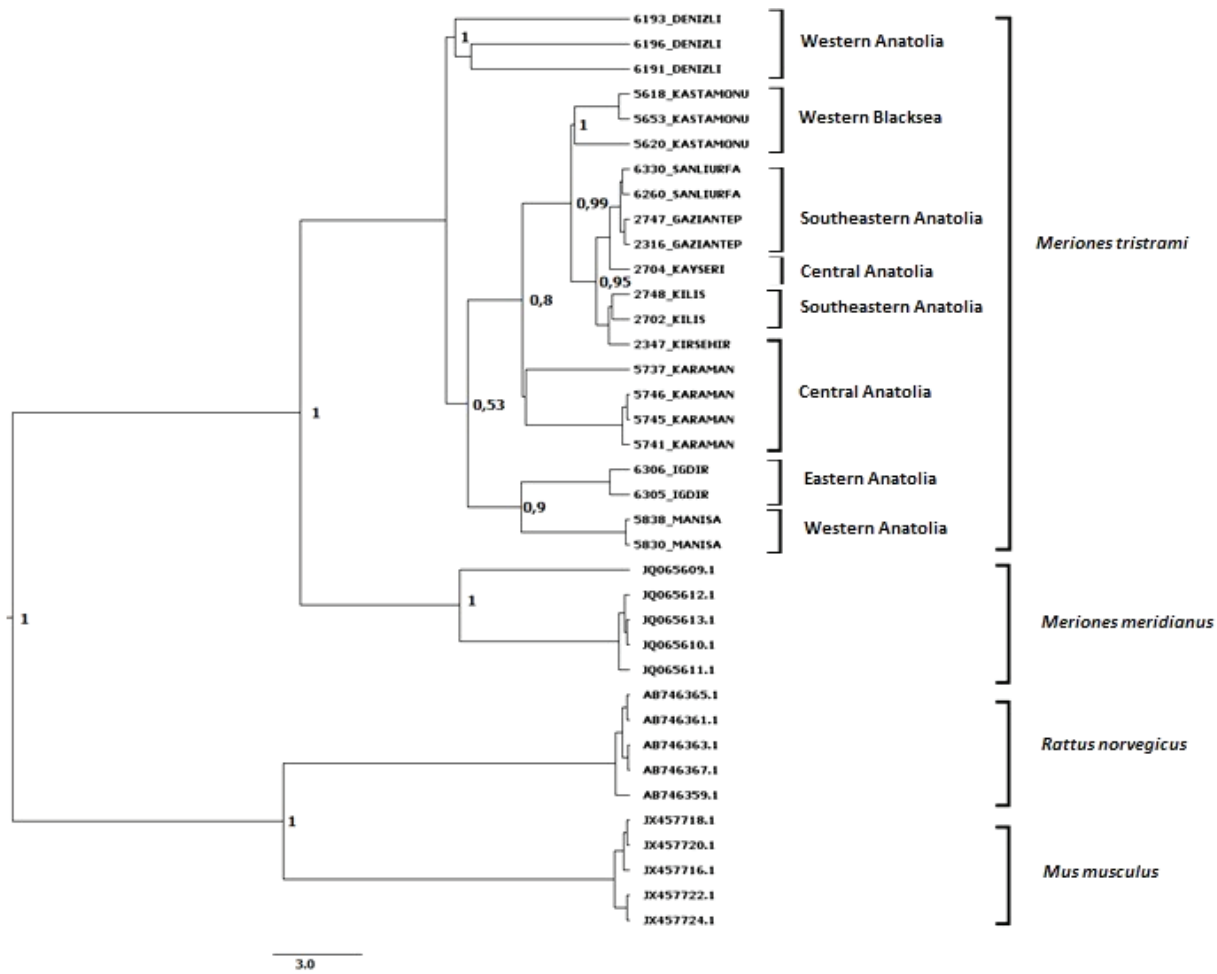

Fig. 3. Bayesian Tree showing the evolutionary relationships among *Meriones tristrami* populations as revealed by the cyt-*b* dataset. Numbers on nodes shows consequent probability values.

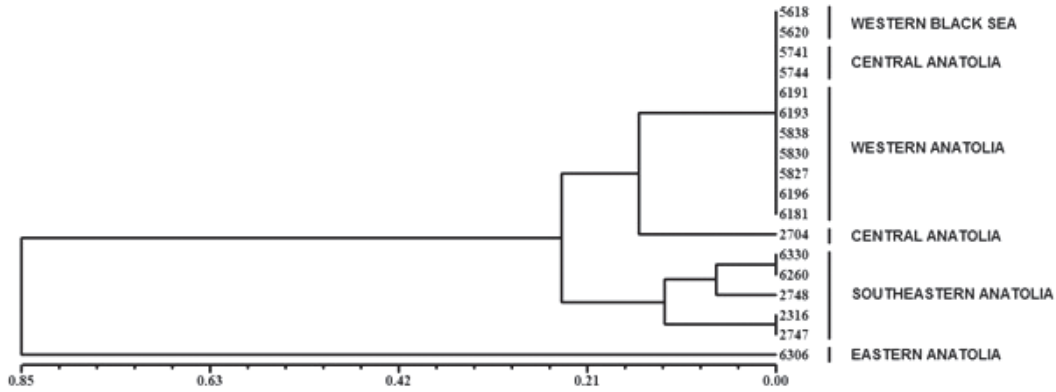

Fig. 4. UPGMA dendrogram based on the Nei 72 genetic distance from the RFLP data.

Table 1. Regions, localities, collection numbers of samples and number of haplotypes

Geographical regions of localities in Turkey	Localities and their numbers on the map	Subpopulations and specimens assigned to subspecies of *M. tristrami*	Collection numbers and abbreviations		Number of haplotypes
Western Anatolia: costal part	Turgutlu-Manisa (1)	*M. t. blackleri*	5838	Mtb. 1	13
	Turgutlu-Manisa (1)		5830	Mtb. 2	12
Western Anatolia: inner part	Cardak-Denizli (2)	No subspecies status assigned	6191	Mtde. 1	13
	Cardak-Denizli (2)		6193	Mtde. 2	14
	Cardak-Denizli (2)		6196	Mtde. 3	7
Central Anatolia	Karadag-Karaman (3)	*M. t. lycaon*	5745	Mtl. 1	7
	Karadag-Karaman (3)		5746	Mtl. 2	7
	Karadag-Karaman (3)		5737	Mtl. 3	10
	Karadag-Karaman (3)		5741	Mtl. 4	11
	Pinarbasi-Kayseri (4)		2704	Mtl. 5	5
	Malya, Kırşehir (5)		2347	Mtl. 6	3
Western Black Sea	Tosya-Kastamonu (6)	*M. t. intraponticus*	5618	Mti. 1	7
	Tosya-Kastamonu (6)		5620	Mti. 2	8
	Tosya-Kastamonu (6)		5653	Mti. 3	9
South-eastern Anatolia	Elbeyli-Kilis (7)	*M. t. kilisensis*	2748	Mtk. 1	4
	Elbeyli-Kilis (7)		2702	Mtk. 2	3
	Nizip-Gaziantep (8)		2747	Mtk. 3	2
	Nizip-Gaziantep (8)		2316	Mtk. 4	1
	Ceylanpinar-Sanliurfa (9)	*M. t. bodenheimeri*	6260	Mtbod. 1	15
	Ceylanpinar-Sanliurfa (9)		6330	Mtbod. 2	16
Eastern Anatolia	Aralik-Iğdır (10)	*M. t. bogdanovi*	6305	Mtbo. 1	17
	Aralik-Iğdır (10)		6306	Mtbo. 2	18

Phylogeographic evaluation

In Western Anatolia, the distribution of the Tristram's jird (Mtb) was confined only to coastal parts. There are mountains and forested zones between the western cost and the Central Anatolia steppe. The mountains that extended from the East to the West established deep valley routes that probably guided *M. tristrami* to reach the western coastal parts. The haplotypic differentiation of Mtb supported the idea that an invasion of Mtb to the Aegean coast of Anatolia occurred during the Late Pleistocene, while glacial fragmentation, mountainous and forested zones could have prevented gene flow between the Mtb and other subpopulations. By considering the genetic proximity Central and South-eastern Anatolia, the invasion to west and east occurred from these regions Anatolia with discontinuous gene flow, this is because the nucleotide diversity in haplotypes ap-

peared high in Mtde, Mtl and Mti, respectively. The contemporary distribution of *M. tristrami* throughout the Anatolian steppe is partly interrupted and the gene flow between Mtb and other subpopulations is probably interrupted by forested and mountainous zone. Other fragmentation in the distribution occurred in the East Anatolian Plateau (Fig. 5) which probably separated Mtbo from other subpopulations. That is why Mtb and Mtbo have high genetic diversities compared to other subpopulations. However, it could be expected that the gene flow in any routes after the last glaciation could be the reason for homoplasies in a tree topology. It might be thought that the lake systems might be the major factor for formation of the subpopulations *M. tristrami* during its expansion into Anatolia.

Discussion

Chevret & Dobigny (2005) performed a complete alignment of cyt-*b* for 35 taxa, including *Meriones* spp., and revealed that transitions and transversions were less saturated at second-codon position, followed by first- and third-codon positions. Our findings were consistent with these reports. In addition, the high C-T at the third-codon positions and the lower A-G saturations were recorded in *M. tristrami*. The analyses of the pair-wise distance of some *Meriones* spp. based on the cyt-*b* sequences found values for the distance of 0.186 for the clade of *M. libycus* and *M. rex – M. crassus*, and 0.086 between *M. rex* and *M. crassus* (Ito et al. 2010), while the distances ranges from 0.007 to 0.103 in subpopulations of *M. tristrami*. These values (K2P) based on the cyt-*b* sequences were calculated to be 0.116–0.171 among species of the genus *Gerbillus* (Abiadh et al. 2010). Bradley & Baker (2001) stated that the genetic distance values < 2% from the cyt-*b* indicated intraspecific variation, 2-11% for conspecific population or valid species and > 11% for species recognition. The values for subpopulations of *M. tristrami* suggested intraspecific variation and supported the close genetic relationships. The distance data have proven that the subpopulations of Tristram's jird are in the early stage of speciation. Bradley & Baker (2001) recorded an inter-specific divergence for cyt-*b* from 2.7% to 19.23%.

The median-joining network tree constructed based on the RFLP data and the BI tree based on the cyt-*b* sequences support the idea that the Anatolian subpopulations (Mti, Mtl) are genetically quite similar to the south-eastern subpopulations (Mtk, Mtbod). Yiğit et al. (2016) also reported that the genetic distance based on the allozyme data was found to be similarly low between the Anatolian and south-eastern subpopulations of *M. tristrami*. Mtk has been first described in the Kilis Province in South-eastern Anatolia based on the karyological difference (number of fundamental arms) from Mtbod; these two south-eastern subpopulations also have different numbers of fundamental arms from the specimens inhabiting the Aegean Region and Central Anatolia (Yiğit et al. 1998, Yiğit & Çolak 1998). According to the cyt-*b* analyses, Mtb and Mtbo appeared to be the most diverged ones among other subpopulations. Even though there were karyological differences in Mtb, Mtl, Mtk, and Mtbod, the haplotype diversity of Mtk and Mtbod appeared low due to the geographical proximity of these two subpopulations. Indeed, the geographical races (subspecies) of *M. tristrami* were described to have very low morphological and biometrical differences except for Mtb, which had a distinctive white tip on the tail and occupied the coastal parts of Western Anatolia.

Adkins et al. (2001) estimated the divergence times of major rodent groups, including the genus *Meriones as* closely related in the neighbour-joining tree to the *Mus – Rattus* clade. The divergence *Mus – Rattus* was calculated as 23 MYA. This divergence time corresponds to the Miocene, suggested as the time when cricetid and gerbillid rodents have diverged (Flynn et al. 1986). Strömberg et al. (2007) pointed out that the Late Miocene ecosystems of the Eastern Mediterranean were likely dry woodland and forest, while relatively open habitat had become common in Turkey inferring the scarcity of grass macrofossil and pollen. In this evolutionary scene, the allozyme variations of the genus *Meriones* (represented by five species in Turkey) indicate that *M. tristrami* is genetically more similar to *M. crassus* and *M. dahli* than to *M. persicus* and *M. vinogradovi*, while *M. tristrami* is a widespread species in Anatolian steppe with six subspecies (Yiğit et al. 1998, Yiğit & Çolak 1998, Yiğit et al. 2013). In contrast to the allozyme results (Yiğit et al. 2013, 2016), *M. tristrami* shared the same clade with *M. libycus*, *M. vinogradovi* and *M. hurianae* with respect to morphometric peculiarities (Darvish 2009).

Görür et al. (1995) suggested that the main mammal migration to Anatolia started probably during the Miocene when the Bitlis Ocean in SE Turkey had already been closed. Consequently, arvicolids are the dominant rodent in the Plio-Pleistocene deposits of Western Anatolia (Ünay et al. 1995, Ünay & de Bruijn 1998, Sarica 2000). Considering the concept that the Gerbillinae originated in Africa (Chevret & Dobigny 2005), it could be suggested

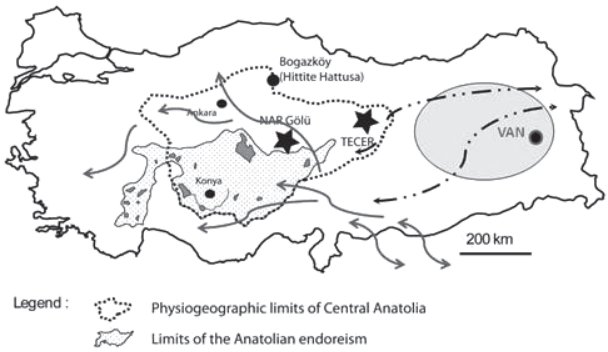

Fig. 5. Pliocene–Pleistocene lakes in Central Anatolia and postulated invasion routes of *M. tristrami* to the West (solid arrows). The dotted line indicates probable contact route between the south and east subpopulations in ancient times and the shaded circle shows the contemporary distributional gap between the east and other subpopulations (modified after Kuzucuoğlu et al. 2011).

that the origin of *M. tristrami* was probably from South-eastern Anatolia during the Plio-Pleistocene. The invasion of the species to Western Anatolia might be interpreted to have occurred through a different route (over the western Black Sea "Mti" rather than over the Central Anatolia "Mtl" route). Apart from these, the limit of the Anatolian endorheism (Kuzucuoğlu et al. 2011) was thought to have formed the natural barriers during the Holocene for rodents, which preferred to live in arid ecosystems (Fig. 5). In support of this finding, during the Pliocene *Pseudomeriones* spp. were recorded from the periphery fossil beds of higher elevations around Central Anatolia rather than in its central parts (Şen 1977, Suata-Alpaslan 2009). The haplotype differentiation of Mtb provides evidence for an interruption in gene flow during glacial periods after the *M. tristrami* occupation of Anatolia. A similar finding was also supported by allozyme analyses of subpopulations of *M. tristrami* in Anatolia (Yiğit et al. 2016). This might be also an evidence to prove the recent differentiation or ongoing isolation of Mtb since the Holocene. The haplotype divergences have been also explained for two sympatric steppe rodents: *Mesocicetus brandti* (see Neumann et al. 2017) and *Cricetulus migratorius* (see İbiş et al. 2017). Neumann et al. (2017) state that *M. brandti* has two haplotype lineages in Turkey and they do not form a clear spatial pattern. Similarly, the haplotypes of *M. tristrami* do not show marked spatial pattern and the eastern haplotypes established a cluster close to the western haplotypes in the Bayesian tree (Fig. 3). İbiş et al. (2017) have studied the cyt-*b* and 12S rRNA of *Cricetulus migratorius*, reporting that the haplotypes clustered together with some exceptions that were clustered in different sublineages. They have also emphasised that the high genetic variability of *C. migratorius* has been associated with the palaeographic and palaeoclimatic history of Anatolia, as reported in the present study for *M. tristrami*. For more precise results, especially to reveal the precise distribution routes of Tristram's jird, the sampling locations should be extended eastwards and south-eastwards and more genetic markers should be employed.

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